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2-DEOXYGLUCOSE UPTAKE IN THE RAT VISUAL SYSTEM UNDER DIFFERENT LEVELS OF AROUSAL

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "2-Deoxyglucose Uptake in the Rat Visual System Under Different Levels of Arousal," submitted by Anthony Randal McIntosh in partial fulfillment of the requirements for the degree of Master of Science.

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Abstract

The autoradiographic 2-deoxyglucose (2-DG) technique was utilized in two experiments designed to examine the influence of arousal on the metabolic, and therefore the functional activity of the rat visual system. A footshock was used to increase arousal level. In the first experiment two visual conditions were employed. One group of rats was exposed to a square-wave display and the other to darkness. The square-wave and darkness groups were subdivided such that half of the subjects were given a 1 mA footshock for 0.5s immediately after being placed into the display chamber. Both shock and exposure to the square-wave display each increased metabolic activity in the superior colliculus, lateral geniculate, and lateral posterior nuclei. The two effects combined in a simple additive fashion and there was no evidence for a true interaction. Compared to subcortex, the effects of shock were less obvious in somatosensory, auditory, and visual cortex. As well, heightened metabolic activity in visual cortex for groups presented with the square-wave display only became obvious when activity in visual cortex was examined relative to activity in auditory or somatosensory cortex. Within groups correlations of cortical and subcortical regional activity indicated that the shock resulted in lower correlations between cortical and subcortical metabolic activity suggesting that arousal may affect cortex and subcortex differently.

In the second experiment the effects of arousal were compared on visually stimulated and unstimulated portions of the visual system in the same animal by testing the rat with one eye occluded. Three groups of rats were tested while wearing

headgear which occluded one eye. The first group was presented with the square-wave display. The second group was also presented with the square-wave display and was, in addition, given a footshock after placement into the stimulus chamber. The third group was given six prior exposures (habituated) to the square-wave display before the test session in an attempt to minimize arousal level. Subcortical results were similar to those of the first experiment in that the shock group showed higher activity in general than the other two groups. Difference scores between corresponding stimulated and unstimulated subcortical visual structures were the same for all three groups. In visual cortex, however, difference scores were highest for animals receiving shock, and lowest for the habituated animals. Larger inter-hemispheric differences in cortex for the animals that received shock appeared to be a function of lower metabolic activity in the hemisphere served by the occluded eye. Within groups correlation matrices again showed a decrease in the correlation between cortical and subcortical activity in the shock condition. Moreover, animals receiving prior exposure to the stimulus display showed different patterns among the correlations than did unshocked animals given 2-DG at initial exposure.

Results of the two experiments suggest that arousal does not affect visual system metabolic activity in a unitary fashion. At the subcortical level, arousal appears to modulate the general activity of structures nonspecifically. The effects in cortex, however, appear to be both a modulation of general activity as well as local effects in specific cortical areas. The changes in the correlations of cortical and subcortical metabolic activity may suggest intervention of system(s) that have different effects at cortical and subcortical levels.

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Chapter 1

Introduction

A growing body of research has focused on the modification of the functional activity of primary sensory systems arising from "nonsensory" factors. These studies have examined such things as the effect of arousal, stimulus novelty, and the acquired significance of the stimulus. It appears from this research that the context in which the presentation of a stimulus occurs can be as important in modulating activity in sensory systems as the stimulus itself. Of particular interest in this study is the relationship between activity in sensory systems and different levels of arousal.

The concept of arousal arose from the well-known observation that most stimuli - particularly weak or familiar ones - have a restricted influence and affect only a limited range of behaviors; however some stimuli, such as novel, aversive, or appetitive ones have a more general effect (Kandel, 1980). In addition to their direct effects, these stimuli appear to modulate a widespread range of responses by altering an animal's receptivity to other stimuli. Most early theorists who invoked the concept of arousal considered it to represent a single continuum ranging from sleep through wakefulness to high excitement. It was also believed that behavioral efficiency is optimal at some intermediate level of arousal between the polar extremes of the continuum, describing the familiar "U"-shaped function between the two variables (Malmo, 1959). This relationship was demonstrated in numerous

behavioral studies (see for example Duffy, 1951, 1957) and seemed consistent with the action of a single system that mediates behavioral activation.

1.1 Physiology of Activation – History and Anatomy

Physiological confirmation of an activating system was first provided by Bremer (1937; cited in Lindsley, 1982) who demonstrated different effects on both cortical electrical activity and behavior through transection at the junction of the spinal cord and medulla (encéphale isolé) or the junction of the midbrain and hypothalamus (cerveau isolé) of the cat. While the cerveau isolé preparation produced a permanently synchronized cortical EEG and pupillary constriction indicating a comatose animal, the encéphale isolé preparation produced an animal with normal sleep and waking cycles and corresponding variations in the cortical EEG from desynchrony (wakefulness) to synchrony (sleep). Results such as these lead some to postulate that the brainstem may contain structures that are functionally tied to different states of consciousness (see, for example, Penfield, 1938). The idea of a subcortical *arousal* system seemed initially supported by the findings of Moruzzi and Magoun (1949) who identified what appeared to be a single, diffusely acting system in the reticular formation of the brainstem (BSRF). From their observation that stimulation of the formation produced cortical EEG desynchrony (considered a correlate of arousal), they assumed that this neural system mediated behavioral arousal.

As research on the BSRF progressed it became obvious that different parts of

the formation were linked to activation of different regions of the central nervous system. Neuroanatomical studies found that the reticular formation is not diffusely organized and can be subdivided into several regions which differ both in cytoarchitecture and connectivity (Brodal, 1958). Single cell research in the brainstem showed that cells responsive to auditory stimuli could be found in abundance in the mesencephalic portion of the reticular formation (Amassian & de Vito, 1955), while few, if any, could be found in the medulla (Scheibel, Scheibel, Mollica, & Moruzzi, 1955). Units responding to spinal stimulation were most easily found in the medulla (Hernández-Peón & Hagbarth, 1955). From the aforementioned stimulation studies as well as others (French, 1958; O'Leary, Kerr, & Goldring, 1958) there appeared to be good agreement between anatomical findings and the effects of stimulation. On the basis of such findings, areas of the reticular formation have been loosely defined as part of either the ascending (midbrain reticular formation (MBRF)) or descending (medullary reticular formation (MRF)) system.

Segundo, Arana, and French (1955) quantified the behavior of monkeys during and following stimulation of the MBRF. They noted that stimulation at low levels seemed to produce an orienting or alerting response. At higher levels, the stimulation produced cowering and at the highest level fear and flight behavior. The accompanying cortical EEG record showed the typical desynchronous pattern. While the behaviors observed from high intensity stimulation could have been a result of the stimulation of "pain" pathways (in the nearby central gray), the study did suggest areas of MBRF were somehow related to arousal.

Cellular confirmation of the involvement of the rostral reticular formation in behavioral and EEG activation was provided by Steriade and colleagues (Steriade,

Ropert, Kitskis, & Oakson, 1980). On the basis of firing patterns of cells in the MBRF it was speculated that this region gives rise to ascending excitatory inputs which are responsible for the activation seen in thalamocortical and corticosubcortical cells throughout states ranging from sleep to wakefulness, and the generation of processes leading to EEG desynchronization. These authors were able to typify neurons in the MBRF as reciprocally linked with many of the forebrain structures involved in sychronization-desynchronization processes such as the medial thalamus and the thalamic reticular nucleus. Furthermore, they were able to characterize cells which appeared related to the tonic level of activity in thalamic and cortical regions. As well, other cells were found that would increase firing rates prior to the shift from sleep to waking, and between REM and slow-wave sleep (Steriade, Oakson, & Ropert, 1982). This research provided the critical link between behavioral states and the activity of the MBRF, and added support to the involvement of the MBRF in states of arousal.

From pharmacological studies it appeared that the desynchrony produced by the ascending reticular formation could be the result of activation of cholinergic inputs (Rinaldi & Himwich, 1955). Early histochemical studies noted that cells in the MBRF showed strong labelling for cholinesterase. Systemic or intraventricular applications of acetylcholine or anticholinesterase drugs lead to low voltage fast activity from the cortical EEG, while cholinergic antagonists (eg. atropine) produced slow-wave patterns (Bradley, 1958). Moreover, atropine can raise the threshold for EEG activation produced both by peripheral stimuli (eg. acoustic startle) and by direct stimulation (Killam & Killam, 1958).

Shute and Lewis (1967), using both lesions and acetylcholinesterase histochem-

ical stains provided evidence for two major sources of cholinergic afferents to the forebrain from reticular and tegmental nuclei. One source originates in the cuneiform nucleus (sometimes referred to as the pedunculopontine tegmental nucleus (PPTg)), and innervates most thalamic and tectal regions via the dorsal tegmental tract. The second originates in the ventral tegmental area and the substantia nigra (the nigral portion of this tract has since been identified as dopaminergic and not cholinergic (Fibiger, 1982)). This area innervates structures in the basal forebrain.

Further research on cholinergic afferents from the MBRF has demonstrated that the PPTg also appears to innervate areas of the basal forebrain (Paxinos & Butcher, 1981). The significance of this finding lies in the fact that nuclei in the basal forebrain are a primary source of cholinergic innervation of cortex (Fibiger, 1982). Two areas in particular, the nucleus basalis of Meynert (nbM) and the diagonal band of Broca (db), send fibers to different cortical areas. Fibers from the nbM terminate primarily in frontal, parietal and temporal cortex, whereas projections from the vertical limb of the db terminate in cingulate and occipital areas. It therefore appeared that part of the effect observed from stimulation of the MBRF could be attributed to activation of these cholinergic systems; direct activation of thalamic structures by stimulation of the PPTg and indirect cortical activation through the basal forebrain via the influence of the PPTg.

Demonstration of the effects of MBRF stimulation on global cerebral activity was provided by Gonzalez-Lima and Scheich (1984; 1985) in a series of autoradiographic 2-deoxyglucose (2-DG) studies. They noted that electrical stimulation of the MBRF could produce different effects at different brain sites. Increases

were observed in auditory and some thalamic structures while metabolic decreases were found in some cortical (frontal, anterior parietal and occipital), limbic and extrapyramidal structures. Gonzalez-Lima and Scheich (1985) accounted for these results by suggesting that the pattern of 2-DG uptake reflected the activation of different parts of the ascending cholinergic system. The site of the stimulation corresponded to the PPTg and thus would have resulted in activation of the dorsal tegmental tract. This tract projects to the thalamic areas which showed the greatest metabolic increases (Hallanger, Levey, Lee, Rye & Wainer, 1987).

Contrary to expectation was the finding that stimulation of the MBRF produced a metabolic decrease in basal forebrain structures. Given the projection patterns of the basal forebrain to cortical and limbic areas, it seems likely that the suppression observed in the basal forebrain may have contributed to the metabolic decreases seen in cortical and limbic structures. Whether the metabolic suppression in the basal forebrain is suggestive of inhibition by the MBRF is open to speculation. Nevertheless, the studies of Gonzalez-Lima and Scheich imply that stimulation of the MBRF leads to much more than simple excitation or inhibition.

1.2 Relation of Activation and Sensory Systems

Some studies have noted differing influences of MBRF stimulation on activity within sensory systems. Lindsley (1958) speculated that stimulation of the MBRF may facilitate perception of stimuli. It was noted that prior to reticular stimulation, visual cortex would show only one evoked response to paired light flashes

50 msec apart. However, after a 5 sec period of reticular stimulation, cortex responded with two evoked potentials corresponding to the two flashes for up to 12 sec after MBRF stimulation. The same stimulation also reduced both the reaction time and the number of trials to criterion in a two-choice discrimination task. Chi and Flynn (1967) examined the effect of stimulation of the hypothalamus and the MBRF on evoked responses induced by shock to the optic tract in the cat. They found that the evoked responses in the lateral geniculate nucleus and superior colliculus were facilitated by stimulation of both the hypothalamus and the reticular formation. Stimulation of MBRF augmented the evoked response in visual cortex while hypothalamic stimulation resulted in suppression of the response. Singer (1977) noted that stimulation of the MBRF resulted in both an increase in spontaneous activity and the responsiveness of cat visual cortex cells. Doty, Wilson, Bartlett and Pecci-Saavedra (1973), however, were unable to detect changes in cortical evoked potentials in the monkey, but obtained similar results for the lateral geniculate nucleus. A possible source of this discrepancy is that the sites of MBRF stimulation were different between the studies. It has been noted that stimulation of different areas within the MBRF has different effects on synaptic transmission in the lateral geniculate nucleus (Wilson, Pecci-Saavedra, & Doty, 1973). This could manifest itself in visual cortex through the projections from the geniculate. Stimulation of the central area of the MBRF seems to produce the most consistent effect, while stimulation of areas lying rostral or caudal to this produce little or no effect.

The effectiveness of MBRF stimulation seems to vary according to the state of the animal. Bartlett, Doty, Pecci-Saavedra, and Wilson (1973) found that a "mod-

erately inattentive" monkey would show large evoked responses in visual cortex and the lateral geniculate nucleus to optic tract stimulation if suddenly alerted by auditory or tactile stimulation, or brief stimulation to the MBRF. However, if the monkey was fully alert, stimulation produced little or no facilitation. Fourment, Hirsch, and Mrac (1988) noted that MBRF stimulation excited cells in the cat lateral geniculate nucleus in states of slow-wave sleep and to a lesser degree during REM sleep. When the cat was in the waking state the effect was least obvious, with some cells showing no change from baseline activity.

Bartlett and Doty (1974) examined the effects on the discharge rates of single cells in the monkey visual cortex in conjunction with MBRF stimulation. About half of the cells tested showed an alteration in baseline activity, or an augmented response to visual stimuli, while the remainder either showed inconsistent effects or none at all. From this study, as well as those mentioned previously, Bartlett and Doty speculated that "the apparent absence of such control (from the MBRF) merely reflects existing maximal operation of the (visual) system." Such a " ceiling" effect has also been noted in the cat (Singer & Drager, 1972).

1.3 Conclusion

The influence of arousal on the activity of sensory structures processing sensory information is still not well understood. While it is generally agreed that higher levels of arousal will increase activity in the brain, it is less clear how these changes affect sensory structures. Even for a limited range of the arousal continuum, in-

consistent information exists. For instance, while some studies suggest that the baseline activity of sensory structures may increase with higher arousal, others suggest that there may be little or no effect. It should be noted, however, that research on the neural mechanisms of arousal typically employ electrical stimulation of the MBRF. The efficacy of such stimulation on modifying sensory system activity seems highly dependent on the ongoing activity in that system, as well as the state of the organism at the time of stimulation. If an organism is already in an aroused state, and is engaged in active processing of the environment, MBRF stimulation appears less able to modulate the activity of sensory systems. Moreover, it seems that the effects of stimulation are more easily detected in subcortical structures than in cortex. This may be a result of differential influences of stimulation on cortical versus subcortical structures (as suggested by Gonzalez-Lima and Scheich, 1985). Finally, and perhaps most importantly, it appears that the effectiveness of MBRF stimulation can vary depending on the site of stimulation (Wilson, Pecci-Saavedra, & Doty, 1973). This poses interpretational problems as the stimulation may activate more than just the cuneiform nucleus/PPTg (typically the target of stimulation). The MBRF is not a single nuclear complex and contains many fibers of passage that are likely activated by the stimulation, thus making it difficult to attribute effects solely to stimulation of a single area. This problem has lead some to speculate that stimulation of the MBRF may not be entirely compatible with the changes in cerebral activity that accompany behavioral arousal (Sillito, Salt, & Kemp, 1985).

Nevertheless, the finding that stimulation of the MBRF (at least in some cases) enhances stimulus receptivity of sensory nuclei appears consistent with studies that

have used external stimuli to change arousal level. Swadlow and Weyand (1985) found that cells of the rabbit LGNd would show enhanced responses to optimal stimuli when the animal was aroused by an auditory stimulus. There was no alteration of baseline activity of the cells when the auditory stimulus was presented without concomitant visual stimulation. Livingston and Hubel (1981) noted that cells in the cat LGNd showed augmentation of their characteristic responses to visual stimuli in states of wakefulness as compared to sleep. In visual cortex, however, the results were less consistent. Some cells showed a reduction in spontaneous activity, or no change. Visual responses were either enhanced or not changed during the waking state. This is consistent with the findings of many MBRF studies where effects are more easily detected in thalamic structures than in cortex. Furthermore, if the animal was alerted with a loud noise, the responses of some cells to their optimal stimulus was enhanced while background activity was decreased, suggesting an increase in the signal-to-noise ratio. These results were essentially confirmed through the use of the double-label 2-deoxyglucose technique. This entailed giving the animal two injections of 2-DG, one labelled with ^{3}H and the other with ^{14}C. The injections took place at different times during the experiment and brain images were processed with two types of film such that 2-DG uptake could be assessed in two different periods by comparing the images from the ^{14}C 2-DG with the image produced by both types of 2-DG. Visual stimulation was presented to only one visual field and the differences in 2-DG uptake between homologous visual structures compared during sleep and wakefulness. For the LGNd, differences were stronger in the waking state. In visual cortex, the 2-DG label extended further through the cortical layers in the waking state.

1.4 Foreward

Classically, investigations of functional plasticity within the brain have used the evoked potential recording technique both at the structural and cellular level. However, the inherent sampling problems associated with evoked potential studies have prevented a thorough analysis of changes in activity at all levels of a sensory system within a given animal. The number of regions make it difficult to map these changes in a single experiment. Although limited in its resolution compared to microelectrode recordings the 2-DG technique allows for an examination of the activity of the entire brain in a single animal. The autoradiographic images produced by the radiolabelled 2-DG allows for easier determination of the pattern of activity within a given structure or system. The technique was implemented in the current study to examine the activity of the rat visual system under different arousal levels.

As stated previously, there are interpretational difficulties surrounding the use of brain stimulation to manipulate arousal level. To circumvent these problems, environmental salience was manipulated to change arousal level (Rescorla, 1988). A footshock was used as another method to modify arousal level. Presumably, the shock would result in an increase in the salience of the environment thereby increasing arousal level. Conversely, habituation to the environment would lower its salience resulting in a lower level of arousal.

The purpose of this thesis was to investigate the relationship between high and low levels of arousal in the waking state and metabolic activity in the visual

system. Two experiments were conducted. The first examined the effect of a footshock on visual system activity under conditions of patterned light and low visual stimulation. The second experiment examined whether arousal changes receptivity to visual stimuli. For this experiment, in addition to the high arousal condition (ie. footshock), a condition of low arousal was added where subjects were habituated to the stimulus display prior to testing.

Chapter 2

Experiment 1

As an organism goes through various stages of arousal there appears to be changes in the activity of the central nervous system. For example, it has been established that the shift from sleep to wakefulness is accompanied by a change in the cortical EEG pattern from synchrony to desynchrony (Steriade, Ropert, Kitsikis, & Oakson, 1980). With these changes in arousal, there also appears to be a change in the response of sensory systems to sensory stimuli. Livingston and Hubel (1981) noted that cells in the cat LGNd showed augmentation of their characteristic responses to visual stimuli in states of wakefulness as compared to sleep, which was suggestive of an increase in the signal-to-noise ratio. Cortical results, however, were less clear. Some cells showed a reduction in spontaneous activity, or no change. These results were confirmed through the use of the 2-DG technique.

Even within the waking state, changes in arousal seem to affect operations of sensory systems. Bartlett et al. (1973) noted an enhanced response of cells in visual cortex and the LGNd to visual stimuli when a monkey was suddenly alerted by an auditory stimulus. These results are similar to Swadlow and Weyand (1985) who found that if an arousing auditory stimulus was presented along with a visual stimulus, the responsiveness of cells in the LGNd of the rabbit was enhanced. Moreover, if the auditory stimulus was presented without visual stimulation, no

changes in the firing rates of the cells were noted.

Experiment 1 was designed to determine how arousal affects metabolic activity in the rat visual system. The aforementioned studies suggest that the effects of arousal may depend on the types of sensory stimuli that are present. With this in mind, a two-by-two factorial design was implemented. Two visual conditions were used: stripes (square-wave gratings) and black box. The stripes condition presumably represented potent visual stimulation, whereas the black box condition represented a low visual stimulation condition. Arousal level was increased through the use of footshock thus creating two levels: shock and no shock, which was completely crossed with visual condition.

2.1 Procedure

Subjects. Sixteen naive male Long-Evans, black-hooded rats served as subjects. They were obtained from a colony at the University of Calgary. The parent stock were obtained from Canadian Breeding Farms, Quebec, Canada. During the experiment rats were raised in a colony and housed in groups of two to four in clear plexiglass wire-top cages. All rats were handled by the experimenter at least every third day for approximately one month prior to the beginning of the experiment. The weights of the rats ranged from 250-350 g at the time of 2-DG injection.

There were four groups of four rats each: stripes-no shock; black box-no shock; stripes-shock; and black box-shock.

Apparatus. Two visual display chambers were used. One was a 33x29x38 cm black box, and the other was a white translucent drum (43 cm high, 36 cm diameter)

with horizontal and vertical square-wave gratings of various spatial frequencies showing on the inner surface, ceiling and floor similar to the display used by Rooney and Cooper (1988; see Figure 2.1 for square-wave display). Within the display chambers, the animals were confined to a clear plastic cylinder (18 cm high, 21 cm diameter) which was fixed atop a 24x24x5 cm grid platform. The grid was wired to a Grason-Stadler shock generator (model E1064GS) for the administration of scrambled shock. Background illumination for the square-wave display was provided by the ambient room light such that the average intensity within the display chamber was 0.5 log foot-lamberts.

Surgery and 2-DG Injection. Twenty-fours hours prior to injection of 2-DG, each animal, under sodium pentobarbital anaesthesia (Somnotol, 65 mg/kg), had a right external jugular catheter filled with a heparin solution (100 USP/ml) implanted. The catheter was plugged, looped underneath the skin, coiled between the shoulders, and held in place by silk thread anchored to wound clips overlying the incision. Topical anaesthesia (Xylocaine) was applied to all wound sites during the surgical procedure.

The following day the animal was weighed and a 1.1 mCi/kg dose of 2-deoxy-d-glucose-1[^{14}C] (American Radiolabelled Chemicals, specific activity 55 mCi/mmol) was withdrawn from its vial. A steady stream of nitrogen was used to evaporate the alcohol vehicle from the 2-DG. After evaporation the 2-DG was reconstituted in approximately 0.6 cc of saline and loaded into a 1 cc syringe. Following this, the animal was transferred to a holding cage, the anchor stitches holding the catheter cut, and the 2-DG injected through the catheter followed by a 0.1 cc flush of saline. The catheter was then trimmed and plugged close to the animal's body.

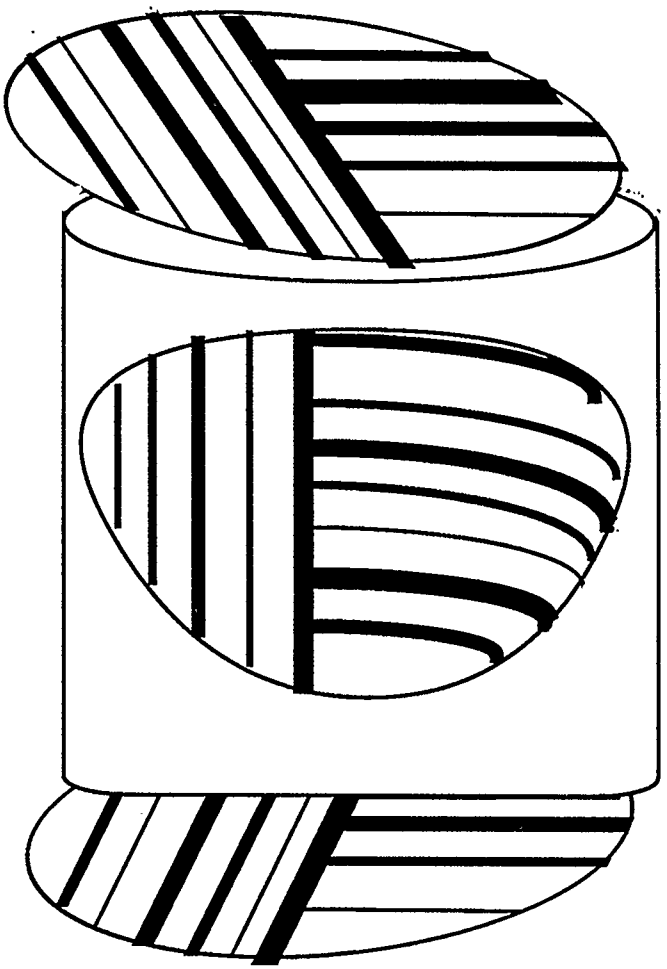

Figure 2.1: Schematic diagram of square-wave display chamber. Roof and floor are shown. Cut away shows pattern on walls of chamber.

Rats to be tested in the black box were dark-adapted for 20 min prior to 2-DG injection. The injection took place under dim illumination.

After injection of 2-DG the rat was placed in the test chamber. For the two shock groups, a 1 mA footshock was delivered for 0.5s immediately after the rat had been placed in the chamber. For the black box groups, all room lights remained off during testing to ensure complete darkness.

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 animal was then perfused through the left ventricle of the heart with 40 ml of saline followed by 80 ml of modified Hand's fixative (Hand, 1981). The brain was removed and frozen in 2-methylbutane cooled to -50°C. Coronal sections 30μm thick were cut in a cryostat (American Optical, Cryo-cut II) at -15°C, and quickly dried onto warmed microscope slides on a hot plate. The slide-mounted sections were then apposed to X-ray film (DuPont Cronex) along with a set of Amersham [^{14}C]methacrylate standards of known ^{14}C tissue concentration for 14 to 21 days before the film was developed.

To assess the ^{14}C uptake in structures of interest, the autoradiographs were placed on an illuminated panel and the light transmittance through the image measured. Light readings were taken through a 260μm reticule centered within the field of a Canon 50 mm macro lens mounted on a Spectra brightness spot-meter (model UB 1/4). Light meter readings from the brain areas of interest, and from the seven methacrylate standards were digitized and stored in a Cromemco System Three microcomputer. These readings were then converted to ^{14}C tissue equivalents by a Fortran program which used a spline subroutine that describes the

relationship between light meter readings and ^{14}C values (International Mathematics and Statistical Library, version 9.1). The methacrylate standards established the spline function and the light readings from the brain areas were converted to ^{14}C values by placement along the function. This conversion took account of the non-linear relationship which exists between autoradiographic light density and ^{14}C tissue equivalents.

Light meter readings were taken through the dorsal lateral geniculate nucleus (LGNd), the lateral posterior nucleus (LPN), and stratum griseum superficiale (SGS), and stratum griseum mediale (SGM) of the superior colliculus (SC). Readings for these structures were taken through at least five adjacent sections. Cortical readings were taken from somatosensory cortex, monocular area 17 and primary auditory cortex. The sections chosen were determined from Zilles's cortical atlas for the rat (1985). Three adjacent sections were used for analysis of somatosensory cortex. For visual and auditory cortex six sections were used; three located rostrally and three caudally. Only layer IV of cortex was analyzed. In addition, readings were taken from the corpus callosum anterior to the splenium, and from three non-visual subcortical structures (ventral posterior nucleus of the thalamus (VTN), substantia nigra reticular (SNr), and the posterior mammillary nucleus (PMN)). A ratio value for each brain locus was calculated by dividing the ^{14}C value for each locus by the average ^{14}C value for the corpus callosum. Ratio values were used to minimize the effects of possible variation in effective isotope uptake across subjects.

Statistical Analyses. For statistical analyses, ratio values from all subcortical regions were averaged within and across sections thus creating means for the left and right hemisphere for each structure. Similarily, for cortex, average ratio values were taken from the readings lying within each area of interest.

To determine if the experimental manipulations produced any significant effects repeated measures analyses of variance were carried out. Visual condition and shock served as the between subjects factors. Three separate analyses were carried out, one each for thalamus (LGNd and LPN), for the superior colliculus (SGS and SGM), and for cortex (somatosensory, visual, and auditory). Within each of these, *area* (eg. LGNd versus LPN) operated as a within subjects factor. Since ratio values were obtained from both hemispheres for all subjects, *hemisphere* also operated as a within subjects factor. Following this, multiple comparisons were carried out (assuming a significant result was obtained from the initial analysis) with the Tukey B procedure.

2.2 Results and Discussion

Behavioral Observations

The behavior of rats in the stripes-no shock and stripes-shock groups were monitored for the first 20 min after placement into the stimulus chamber. Because of the experimental condition for the two groups presented with the black box such observations were not possible. Animals presented with stripes only explored the environment for the first 5 to 8 min. After this period the animals sat quietly or began grooming. For the stripes-shock group, after receiving shock animals were

hypoactive for periods ranging from 1 to 5 min. After this the animals would explore up to at least 10 min after receiving shock, then typically would sit quietly for the remaining interval with the occasional movement.

Subcortical Structures.

Figures 2.2 and 2.3 represent the average ratio values obtained from SGS, SGM, LGNd, and LPN for each group. There was no main effect of hemisphere or any significant interaction involving hemisphere, therefore the ratio values for the two hemispheres were averaged together for the figures. As well, the average ratio values for the three non-visual subcortical structures are shown. What is apparent from Figures 2.2 and 2.3 is that both shock and visual condition strongly elevate metabolic activity. The effects seem only additive and thus no interaction is evident.

Statistical analysis substantiated what can be deduced from Figures 2.2 and 2.3. For thalamus, there were main effects of both shock ($\underline{F}(1,12) = 19.22$, $\underline{p}<.01$) and visual display ($\underline{F}(1,12) = 5.89$, $\underline{p}<.05$) and a nonsignificant interaction. There was also a strong thalamic area by visual display interaction ($\underline{F}(1,12) = 43.65$, $\underline{p}<.01$) suggesting that the effect produced by the visual condition was different for the two thalamic nuclei (LGNd and LPN). This was demonstrated by post-hoc comparisons in that the differences between the stripes-no shock and black box-no shock groups were significant for the LGNd but not for the LPN. For the superior colliculus, there was a main effect of shock ($\underline{F}(1,12) = 14.88$, $\underline{p}<.01$), and a collicular layer by visual display interaction ($\underline{F}(1,12) = 67.01$, $\underline{p}<.01$). Differences between the visual conditions were greater for SGS than for SGM explaining the presence of the interaction (summary tables and the results of the post-hoc analyses are shown

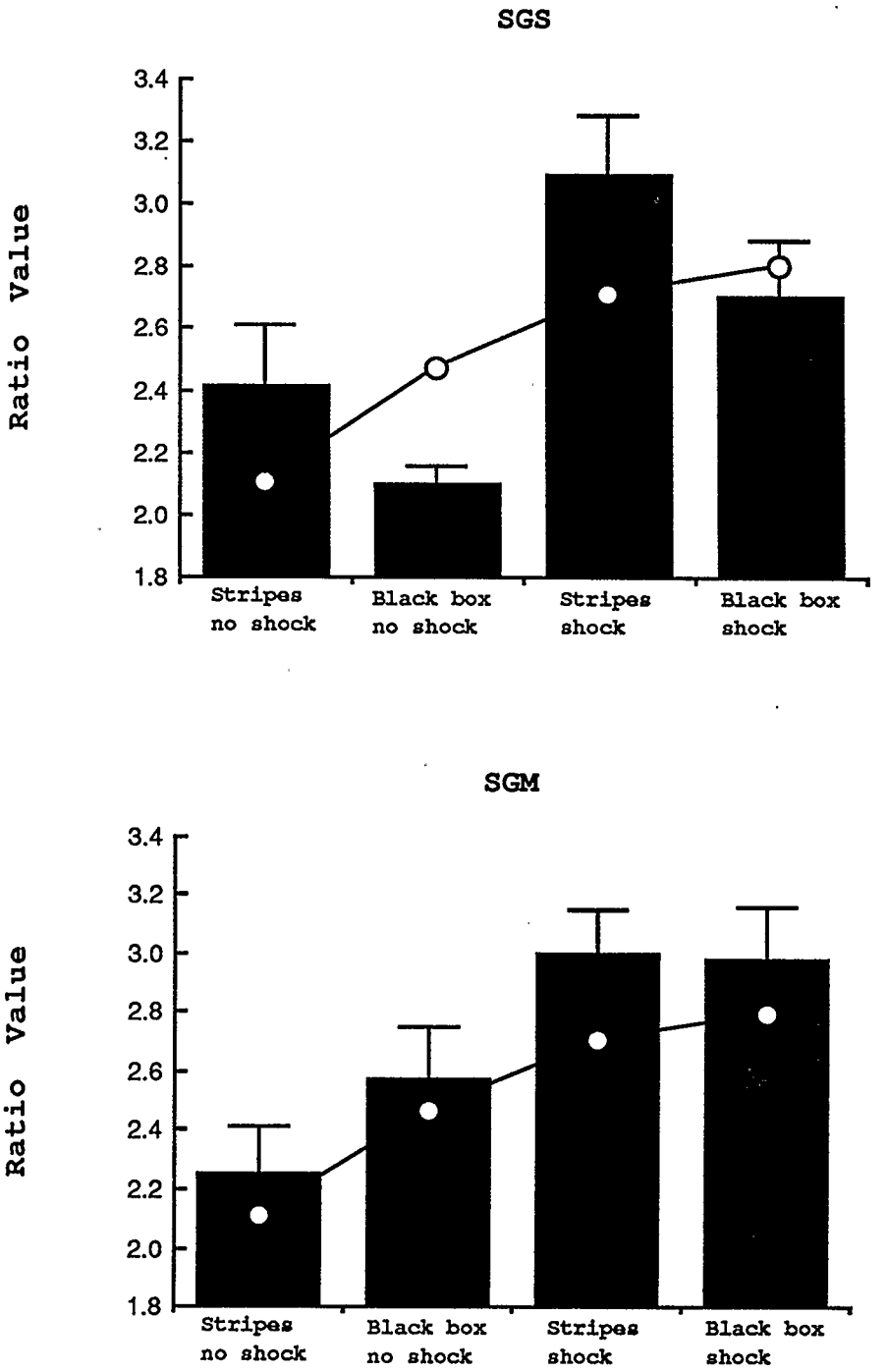

Figure 2.2: Group means with standard error bars for SGS and SGM of the SC. Open circles indicate average ratio value of the VTN, PMN, and SNr.

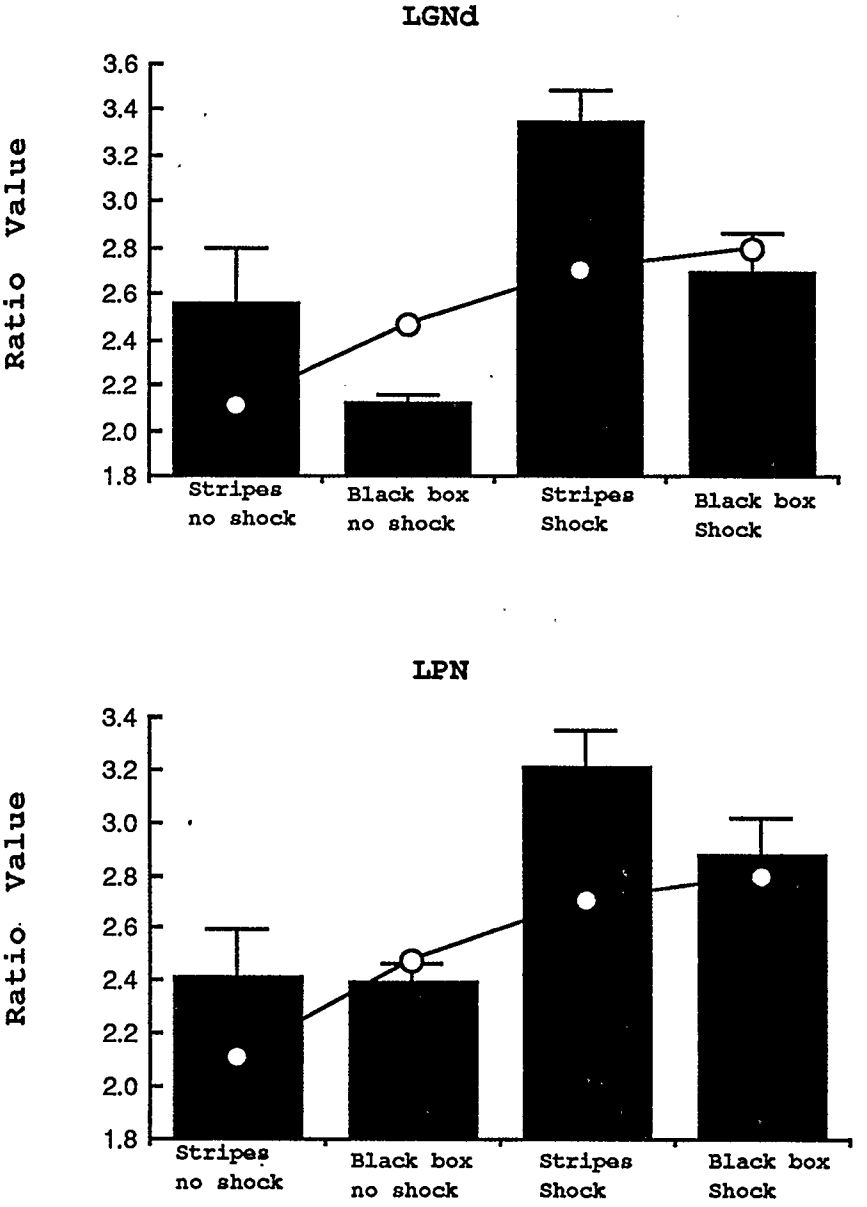

Figure 2.3: Group means for the LGNd and LPN of the thalamus. Open circles indicate average ratio value of the VTN, PMN, and SNr.

in Tables 2.1 and 2.2).

The effect of shock was also noted in a oneway analysis of variance on the average of the three non-visual subcortical structures (VTN, SNr, and PMN) The two groups which received shock had significantly higher ratio values than the two groups which had not ($\underline{F}(1,12) = 12.38$, $\underline{p}<.01$, $\underline{\eta^2} = .436$).

Cortical Areas.

Effects were much less obvious in cortical areas than in subcortical areas. Figure 2.4 shows the average ratio values for each cortical area by group. It should be noted that readings from somatosensory cortex were not obtained for one animal tested in the black box-shock condition because of sectioning problems. While trends which mimic the effects of shock in subcortical structures are apparent, they are less strong, and in somatosensory cortex are seemingly absent in the case of the comparison of the two groups presented with the black box.

Statistical analysis yielded main effects of both shock ($\underline{F}(1,11) = 12.37$, $\underline{p}<.01$) and visual condition ($\underline{F}(1,11) = 13.73$, $\underline{p}<.01$), and an interaction of visual condition and cortical area ($\underline{F}(2,22) = 6.83$, $\underline{p}<.01$). These results suggest that shock produced higher ratio values for cortex on average, but that the magnitude of the effect was smaller than for subcortical structures. As well, the significant interaction was a result of higher ratio values in somatosensory cortex for animals presented with the black box than for those presented with the striped display (see Table 2.3 for summary tables).

The cortical results are especially puzzling with respect to area 17 given numerous other studies which suggest that patterned light is a more effective stimulus

Table 2.1
Summary Table For Analysis of Superior Colliculus

Source	SS	df	MS	F	η^2
Condition	6.19	1	6.19	14.88**	.553
Visual	0.20	1	0 20	<1	
C x V	0.14	1	0.14	<1	
Error	5.00	12	0.42		
Area	0.21	1	0.21	16.45**	.578
C x A	0.00	1	0.00	<1	
V x A	0.86	1	0.86	67.08**	.848
C x V x A	0.04	1	0.04	3.25	
Error	0.15	12	0.01		

**p<.01

Post-Hoc Results (Tukey B, *=p<.01)

SGS

Mean	Group	Str	Blk	Str-S	Blk-S
2.407	Str				
2.050	Blk	*			
3.088	Str-S	*	*		
2.702	Blk-S	*	*	*	

SGM

Mean	Group	Str	Blk	Str-S	Blk-S
2.407	Str				
2.050	Blk	*			
3.088	Str-S	*	*		
2.702	Blk-S	*	*		

Abbreviations:Str=stripes-no shock; Blk=black box-no shock; Str-S=stripes-shock Blk-S=black box-shock

Table 2.2
Summary Table For Analysis of Thalamus

Source	SS	df	MS	F	η^2
Condition	7.41	1	7.41	19.23**	.615
Visual	2.27	1	2.27	5.90**	.329
C x V	0.22	1	0.22	<1	
Error	4.63	12	0.39		
Area	0.01	1	0.02	<1	
C x A	0.00	1	0.00	<1	
V x A	0.43	1	0.43	43.65**	.784
C x V x A	0.00	1	0.00	<1	
Error	0.12	12	0.01		

**p<.01

Post-Hoc Results (Tukey B, *=p<.01)

LGNd

Mean	Group	Str	Blk	Str-S	Blk-S
2.557	Str				
2.280	Blk	*			
3.350	Str-S	*	*		
2.690	Blk-S	*	*	*	

LPN

Mean	Group	Str	Blk	Str-S	Blk-S
2.420	Str				
2.327	Blk				
3.220	Str-S	*	*		
2.880	Blk-S	*	*	*	

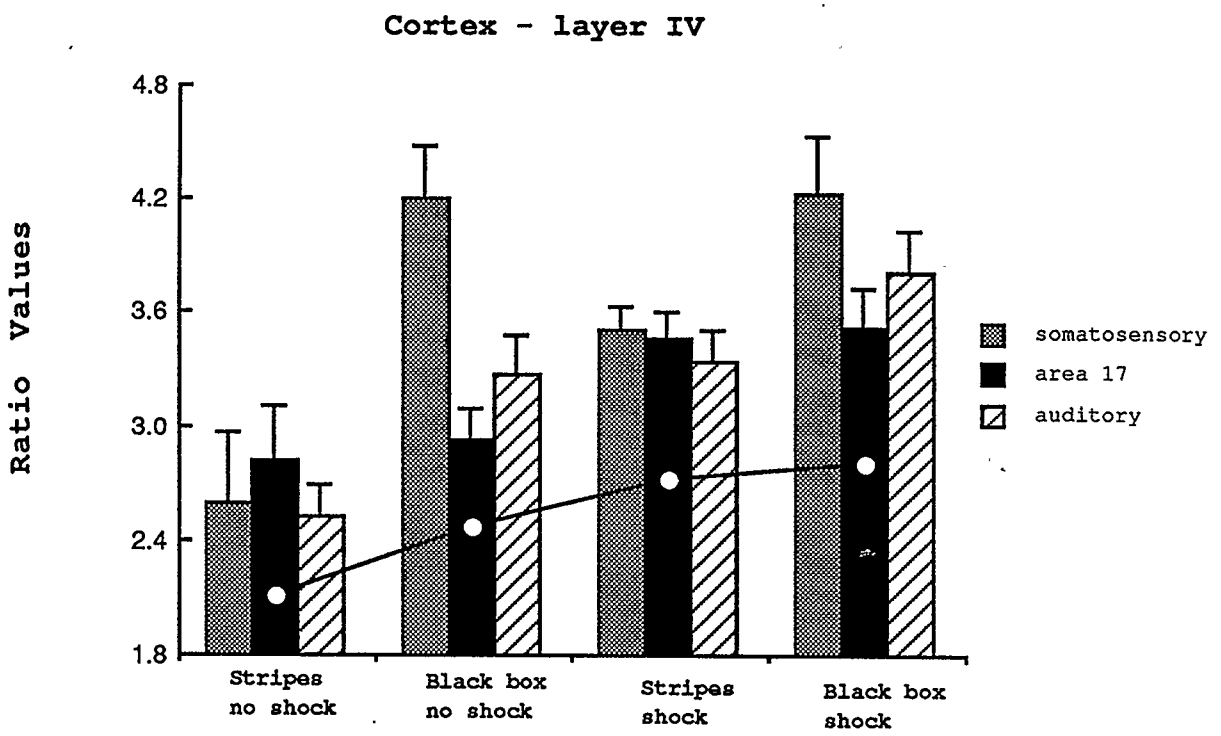

Figure 2.4: Average ratio values for layer IV of somatosensory cortex, area 17, and auditory cortex by group. Open circles represent the average ratio value for the VTN, PMN, and SNr.

Table 2.3
Summary Table For Analysis of Cortex

Source	SS	df	MS	F	η^2
Condition	8.83	1	8.83	12.37**	.529
Visual	9.80	1	9.80	13.73**	.555
C x V	0.57	1	0.57	<1	
Error	7.85	11	0.71		
Area	3.05	2	1.05	5.69*	.341
C x A	0.28	2	0.14	<1	
V x A	3.67	2	1.84	6.86**	.384
C x V x A	0.92	2	0.46	1.71	
Error	5.89	22	0.27		

*p<.05; **p<.01

for visual cortex than darkness (McIntosh & Cooper, 1989; Rooney & Cooper, 1988). From inspection of Figure 2.4 and the statistical analysis it appears that activity in area 17 is not higher in animals presented with stripes when compared to animals presented with the black box. However, it is interesting to note that, if activity in area 17 is examined relative to the other cortical areas (ie. as a ratio over auditory cortex for example) then differences between stripes and black box are more congruent with expectation. In other words, the two groups presented with the square-wave display show higher *relative* activity in area 17 than do groups presented with the black box (see Table 2.4).

Relation of Cortical and Subcortical Metabolic Activity

Two points emerge from the cortical and subcortical results. First, the effect of shock at the subcortical level is to elevate the metabolic activity of all structures. In other words, the effects of shock are general and additive. Second, the observed activity in subcortical structures is not necessarily indicative of cortical activity. This raises the possibility that the relationships between cortical and subcortical metabolic activity are different among the four groups.

To address this possibility, correlations between the brain areas were computed for each group. A graphic representation of these correlations is presented in Figure 2.5. What is immediately apparent from Figure 2.5 is that the two shock groups show a distinctly different pattern in the correlations as compared to the two no shock groups, most consistently between cortical and subcortical structures.

In order to quantify the differences in the cortical-subcortical correlations between the groups, a fourway univariate analysis of variance was carried out using

Table 2.4
Summary Tables for Analysis of Area 17 Relative to Other Cortical Areas

Relative to Somatosensory Cortex

Source	SS	df	MS	F	η^2
Condition	0.00	1	0.00	<1	
Visual	0.28	1	0.28	8.42**	.382
C x V	0.08	1	0.08	2.63	
Error	0.371	11	0.034		

Group Means & Standard Error

Str	1.13	.151
Blk	0.71	.051
Str-S	0.99	.045
Blk-S	0.88	.081

Relative to Auditory Cortex

Source	SS	df	MS	F	η^2
Condition	0.00	1	0.00	<1	
Visual	0.84	1	0.84	7.58**	.378
C x V	0.02	1	0.02	1.47	
Error	0.122	11	0.011		

Group Means & Standard Error

Str	1.11	.062
Blk	0.90	.024
Str-S	1.04	.020
Blk-S	0.96	.078

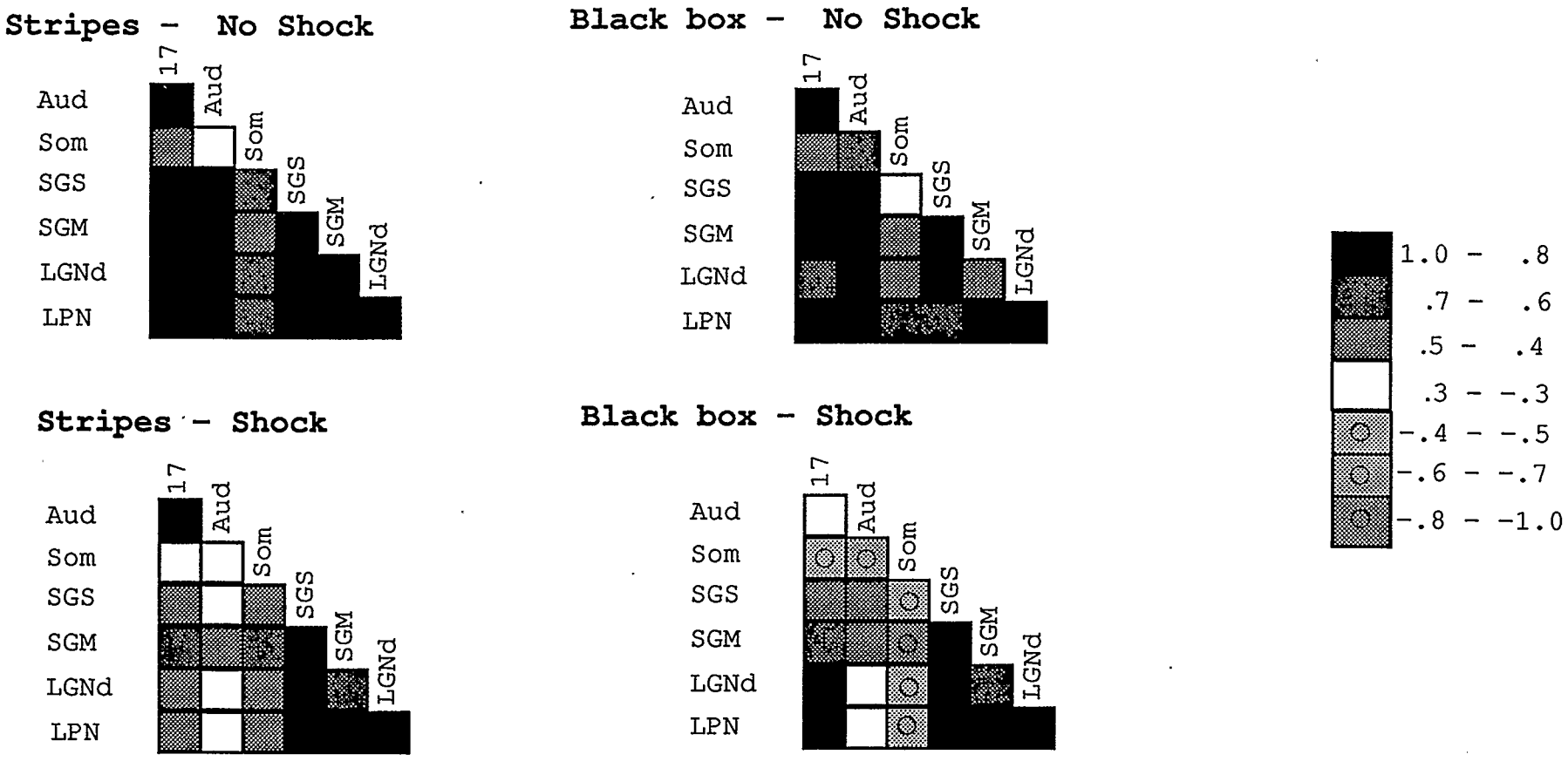

Figure 2.5: Within groups correlation matrices for cortical and subcortical structures. Legend gives range of values for shading scale. Negative correlations are indicated by a circle within each shaded square. Abbreviations: 17=area 17; Aud=auditory cortex; Som=somatosensory cortex.

the correlations as the dependent variable. The factors were: visual condition; shock; cortical area (visual, auditory, somatosensory); and subcortical area (LGNd, LPN, SGS, and SGM). As the interest was in the differences between cortical and subcortical relations, only these correlations were used (for summary tables and the specifics of this analysis the reader is referred to Appendix A).

This analysis indicated that the differences between the correlation matrices were significant as there were both main effects of shock, visual condition, and a significant interaction of the two variables. When collapsed into shock versus no shock, the greatest difference appeared to be in the correlations of area 17 with SGS, area 17 with LPN, and auditory cortex with LPN. In all cases the correlations were significantly higher for the no shock conditions.

When broken down into the four groups, the average correlation of all subcortical areas with visual and auditory cortex showed the greatest difference between groups. With somatosensory cortex, however, the two stripes groups did not show different correlations; with the two black box groups, the shock seemed to increase the average correlation in the negative direction. As well, for cortical areas in general, all correlations with subcortical areas were significantly different. In most instances this entailed a significant reduction in the correlation with shock except for the LGNd; the stripes-shock group showed a significant decrease, while the black box-shock group showed a significant increase (both of these relative to their no shock counter-parts).

Summary

The results stemming from Experiment 1 suggest that the effect of shock-induced arousal is different for cortical and subcortical areas. All subcortical areas examined showed higher metabolic activity in the two shock groups compared to their no-shock counterparts. This effect seemed to combine in a simple additive fashion with the changes in 2-DG uptake produced by the two visual conditions.

While a similar trend was observable in cortex, it was not as strong as the subcortical effect. As well, cortical areas seemed to respond differently to higher levels of arousal depending on the visual condition. The expected response of visual cortex to the stripes display was only evident when activity in area 17 was examined relative to the other cortical areas. The effect of shock on somatosensory cortex was more pronounced for the two stripes groups than for the two black box groups. That shock does not affect cortical and subcortical areas in a similar fashion was further substantiated by examination of the within groups correlation matrices. If the effect of heightened arousal was the same at both cortical and subcortical levels, then the correlations between areas should have been similar for all groups. However, the two shock groups showed distinctly different patterns in these correlations.

Chapter 3

Experiment 2

Experiment 1 suggests that shock-induced arousal does not have the same effects on cortical and subcortical structures. Such heterogeneous effects have been noted in other 2-DG studies. Hand (1987), for example, observed a decrease in rat C3 cortical barrel organ glucose uptake after 5-10 daily stroking sessions of the corresponding vibrissa which was not accompanied by changes at the thalamic level of the whisker system. McIntosh and Cooper (1989) also found that rats which received six prior exposures to a square-wave display showed lower metabolic activity in visual cortex as compared to rats presented with the display for the first time and that this "habituation" effect did not occur in subcortical visual structures. It is possible that the effects obtained in the above mentioned studies were, in part, the result of a minimal level of arousal. Animals habituated to the stimulus situation might have had a lower level of arousal than animals placed in the situation for the first time.

Species such as the rat, in which sensory systems are highly crossed, allows the experimenter to differentially stimulate sensory pathways in the two hemispheres, and thus compare homologous sensory structures from the two sides in the same animal. In a sense, an animal can serve as its own control. For instance, the results of the McIntosh and Cooper study were based on the differences between the activity in structures fed by an occluded eye as compared to the activity in structures

fed by the eye presented with the stimulus (ie. interhemispheric difference scores).

In an effort to further examine the effects of arousal, experiment 2 determined how footshock would affect difference scores in the visual system of the monocularly occluded rat. In addition, to aid in the interpretation, a condition with a lower level of arousal was added by placing rats in the visual display six times prior to testing in effect habituating the rats to the stimulus chamber.

If the effects produced by changes in arousal are simply additive, as experiment 1 suggests, then the difference between the stimulated and unstimulated hemispheres should be the same for all groups. Moreover, habituation of one group of rats represented an attempt to replicate the findings of McIntosh and Cooper (1989).

3.1 Procedure

Subjects. Nine naive male Long-Evans, black-hooded rats served as subjects. All rats were handled at least every third day for one month prior to 2-DG injection. The weights of the rats ranged from 300-350 g at the time of injection.

The rats were divided into three groups of three rats each: stripes-shock, stripes, and stripes-habituated. Subjects serving in the stripes-habituated group received six 45 min exposures to the striped display prior to being tested.

Apparatus. The square-wave display used in experiment 1 was used in experiment 2.

Surgery and 2-DG injection. Each subject underwent implantation of an external jugular catheter under sodium pentobarbital anaesthesia 24h prior to testing. In addition, an aluminum post was cemented to the surface of the skull anterior to

bregma. The post served as an anchor for plastic goggles which occluded vision in one eye (see Figure 3.1).

Approximately 20 min prior to injection of 2-DG the plastic goggles were placed on the rat and held on the post with an alligator clip. This period allowed the rat to adapt to the goggles. After the adaptation period, the catheter was freed and 2-DG injected through it. Immediately after 2-DG injection the animal was placed into the stimulus chamber. Subjects in the stripes-shock group received footshock after placement into the test chamber.

Histology and Autoradiography. Forty-five minutes after injection the animal was given a lethal dose of sodium pentobarbital through the catheter and perfused as in experiment 1. Preparation and quantification of the autoradiographs were as in the previous experiment. In addition to ratio values for the brain areas of interest, ratio value interhemispheric difference scores were also computed by subtracting loci in the hemisphere innervated primarily by the occluded eye (occluded) from the corresponding loci in the adjacent hemisphere (exposed).

Procedures for statistical analyses were as in experiment 1. For the comparison of the within groups correlations two additional factors were added: cortical hemisphere (exposed and occluded), and subcortical hemisphere. This resulted in a sixway analysis of variance (see Appendix A).

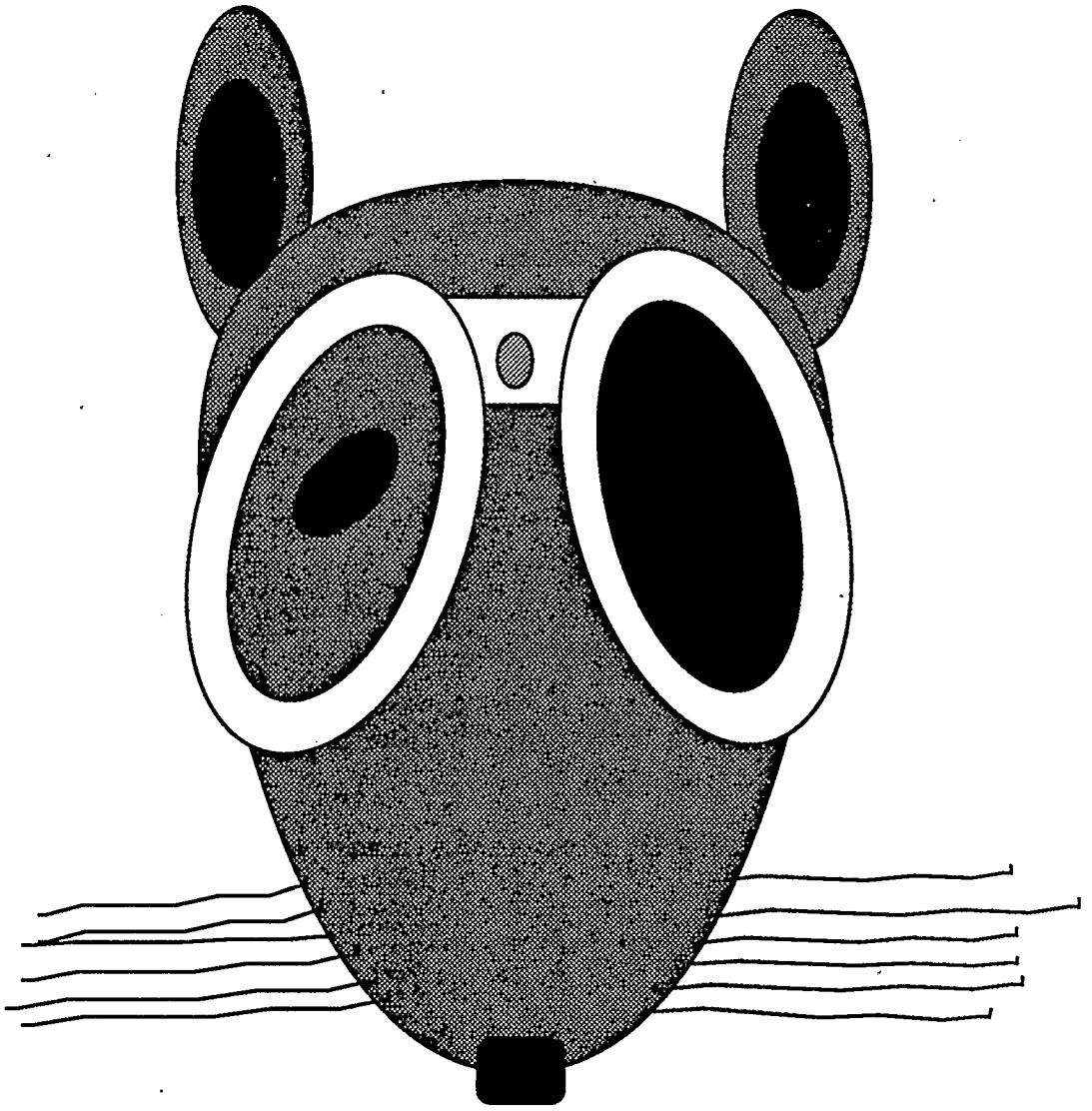

Figure 3.1: Schematic diagram of a rat with monocularly occluding goggles. Left eye is covered by an opaque occulder.

3.2 Results and Discussion

Behavioral Observations

The behavior of all rats was monitored for the first twenty minutes after injection with 2-DG. The stripes-shock group, after receiving shock, were generally hypoactive for periods of 2 to 5 min. The remaining time was usually spent exploring and/or grooming. The stripes group showed active exploration of the chamber for anywhere from 10 to 15 min after which animals typically began grooming. Rats that underwent habituation explored for only 1 to 2 min followed by periods of grooming and hypoactivity.

Subcortical Structures

Figures 3.2 and 3.3 shows the ratio values for the LGNd, LPN, SGS and SGM for the three groups. Values for each hemisphere are also given. The difference between hemispheres is shown in Figures 3.4 and 3.5. Figures 3.2 and 3.3 indicate that animals given shock again showed higher metabolic activity in both hemispheres while the difference between hemispheres (Figures 3.4 and 3.5) did not appear to vary as a function of either shock or habituation.

Summary tables for the statistical analyses for the SC and thalamus are presented in Tables 3.1 and 3.2. For the SC there was a group main effect ($F(2,6) = 21.05$, $p<.01$) but no interaction of group with either SC layer or hemisphere suggesting that the effect of shock was additive. Post-hoc comparison indicated that the stripes-shock group showed higher ratio values on average as compared to the other two groups. For thalamus, the group main effect did not exceed conventional significance levels ($F(2,6) = 4.86$, $p<.06$), which was most likely the result

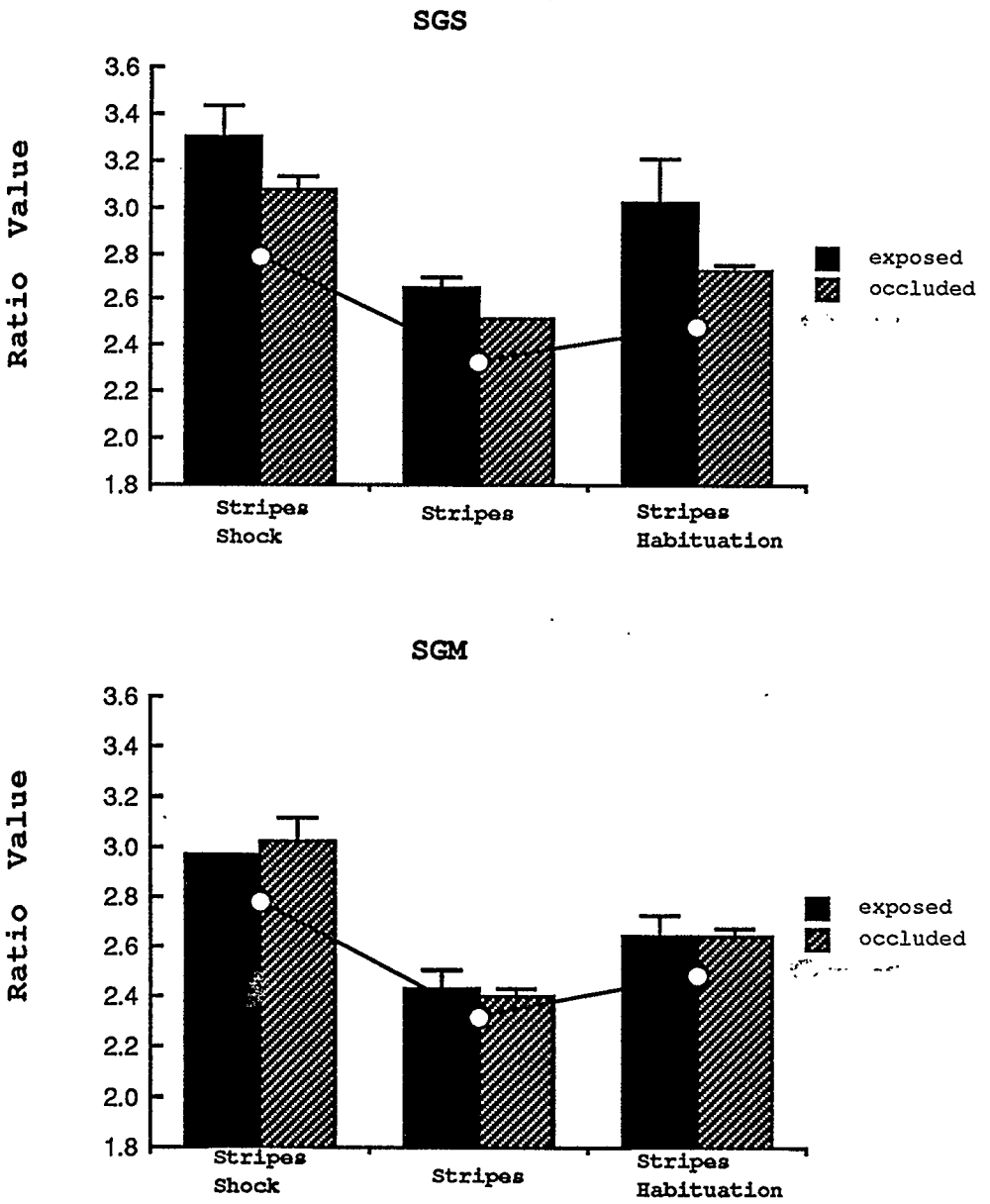

Figure 3.2: Average ratio values by group for SGS and SGM of the SC. Means for both hemispheres are shown. Open circles represent the average of the VTN, PMN, and SNr.

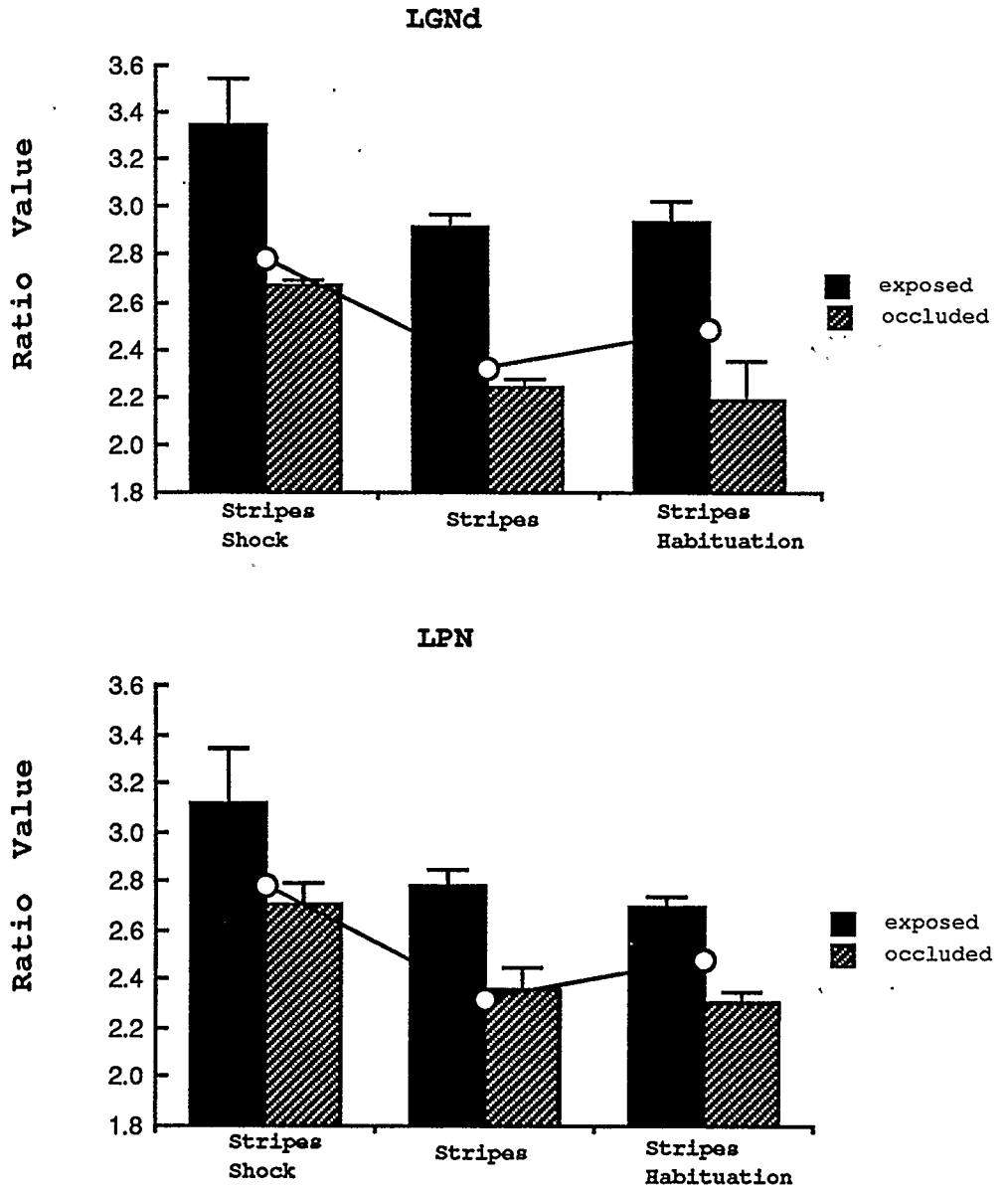

Figure 3.3: Average ratio values by group for the LGNd and LPN of the thalamus. Means for both hemispheres are shown. Open circles represent the average of the VTN, PMN, and SNr

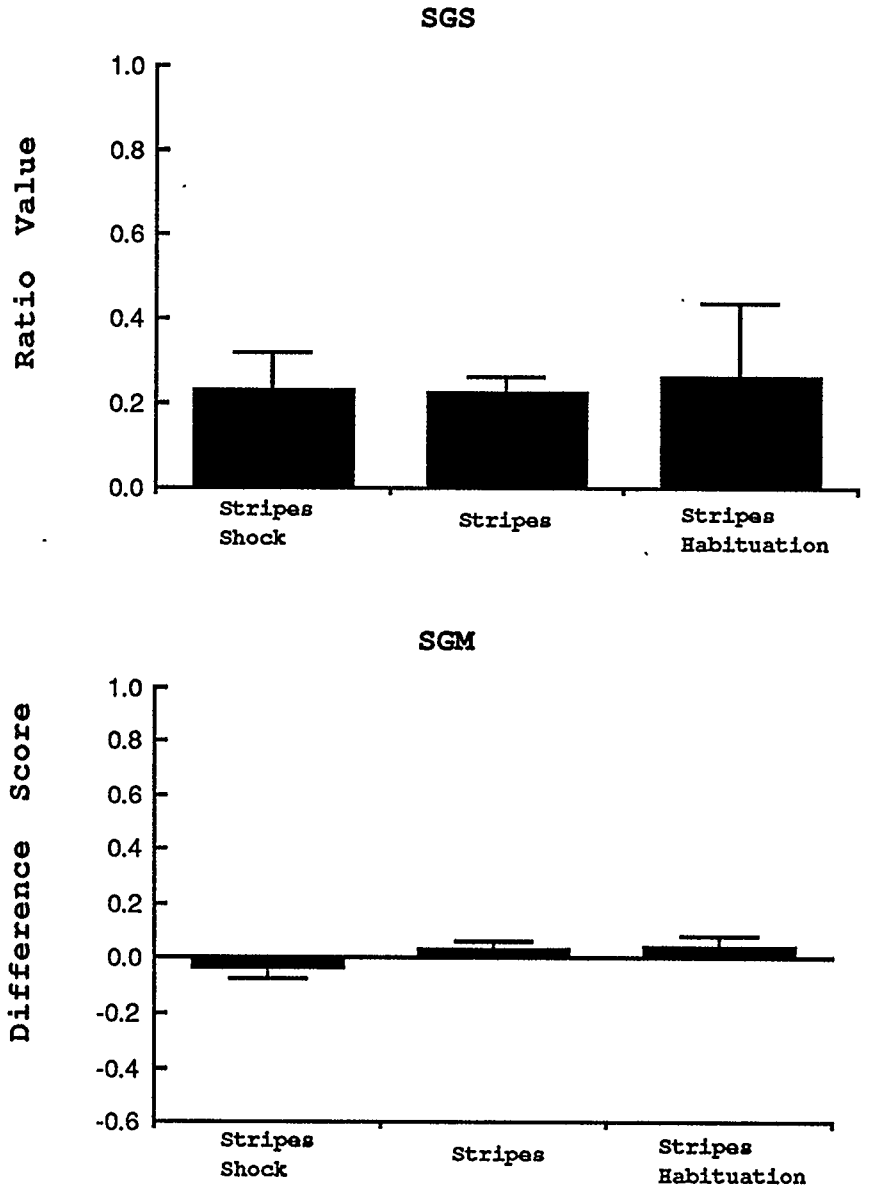

Figure 3.4: Interhemispheric difference scores for SGS and SGM of the SC by group. Positive scores indicate lower metabolic activity contralateral to the occluded eye

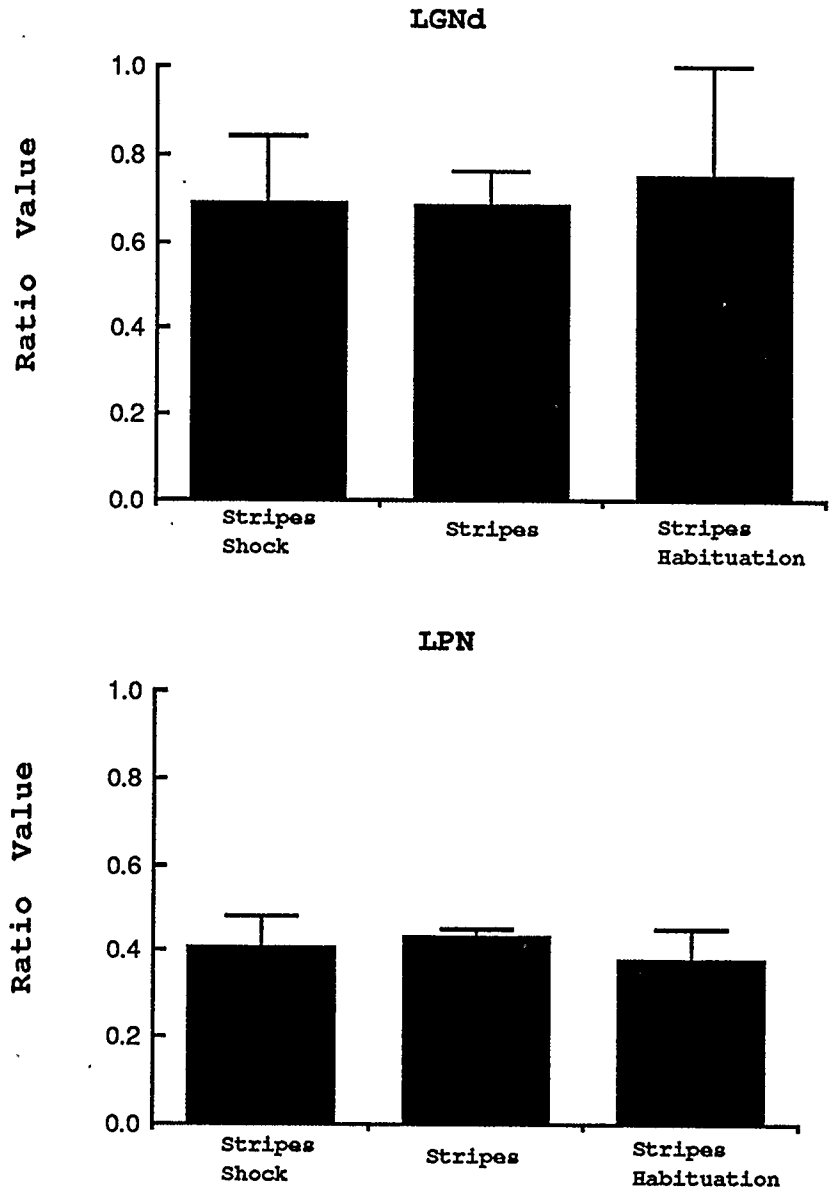

Figure 3.5: Interhemispheric difference scores for the LGNd and LPN of the thalamus by group. Positive scores indicate lower metabolic activity contralateral to the occluded eye

Table 3.1
Summary Tables For Analysis of Superior Colliculus

Source	SS	df	MS	F	η^2
Group	1.29	2	0.99	21.05**	.875
Error	0.28	6	0.05		
Area	0.33	1	0.33	39.38**	.867
G x A	0.03	2	0.01	1.52	
Error	0.04	6	0.01		
Hemi	0.11	1	0.11	15.58**	.772
G x H	0.01	2	0.01	<1	
Error	0.04	6	0.01		
A x H	0.09	1	0.09	16.28**	.731
G x A x H	0.01	2	0.01	<1	
Error	0.03	6	0.01		

**p<.01

Post-Hoc Results (Tukey B, *=p<.01)

	Colliculus Overall			
Mean	Group			
		Str-S	Str	Str-Hab
3.090	Str-S			
2.520	Str	*		
2.740	Str-Hab	*		

Abbreviations: Str-S=stripes-shock; Str=stripes; Str-Hab=stripes-habituation

Table 3.2
Summary Tables For Analysis of Thalamus

Source	SS	df	MS	F	η^2
Group	1.23	2	0.67	4.85*	.618
Error	0.82	6	0.14		
Area	0.03	1	0.03	3.36	
G x A	0.01	2	0.01	<1	
Error	0.06	6	0.01		
Hemi	2.71	1	2.71	176.35**	.967
G x H	0.00	2	0.00	<1	
Error	0.09	6	0.02		
A x H	0.19	1	0.19	51.10**	.894
G x A x H	0.00	2	0.00	<1	
Error	0.02	6	0.00		

**p<.01; *p<.06

of sample size and within group variance. Nevertheless, the general trend observed in experiment 1 was still present.

Cortical Areas

Results from cortical analyses are presented in Figure 3.6. The obtained ratio values from the two hemispheres are given as well as interhemispheric difference scores for area 17 (bottom of Figure 3.6). As with experiment 1, there did not appear to be an overall effect of shock on cortical activity. But when difference scores for area 17 were examined, strong effects of shock and habituation were noted. Difference scores were highest for the group receiving shock, and lowest for the group given prior exposures to the visual display.

No overall group main effect was present from the statistical analysis. An examination of the higher-order interactions yielded a significant group by cortical area effect ($\underline{F}(4,12) = 4.00$, $\underline{p}<.05$) and a group by cortical area by cortical hemisphere interaction ($\underline{F}(4,12) = 5.81$, $\underline{p}<.01$). A oneway analysis on the difference scores for area 17 suggested that the observed pattern between groups was significant ($\underline{F}(2,6) = 66.54$, $\underline{p}<.01$; see Tables 3.3 and 3.4 for analysis summaries).

Post-hoc analyses for visual cortex revealed an interesting pattern involving the two hemispheres. Comparison of the hemisphere fed primarily by the unoccluded eye showed that the stripes and stripes-shock groups had higher ratio values than the habituated group. Conversely, for the hemisphere fed by the occluded eye, the stripes-shock group showed lower ratio values than either of the other two groups. This suggests that the higher difference scores obtained for the stripes-shock group were not solely a function of increased activity in the "exposed" hemisphere, but

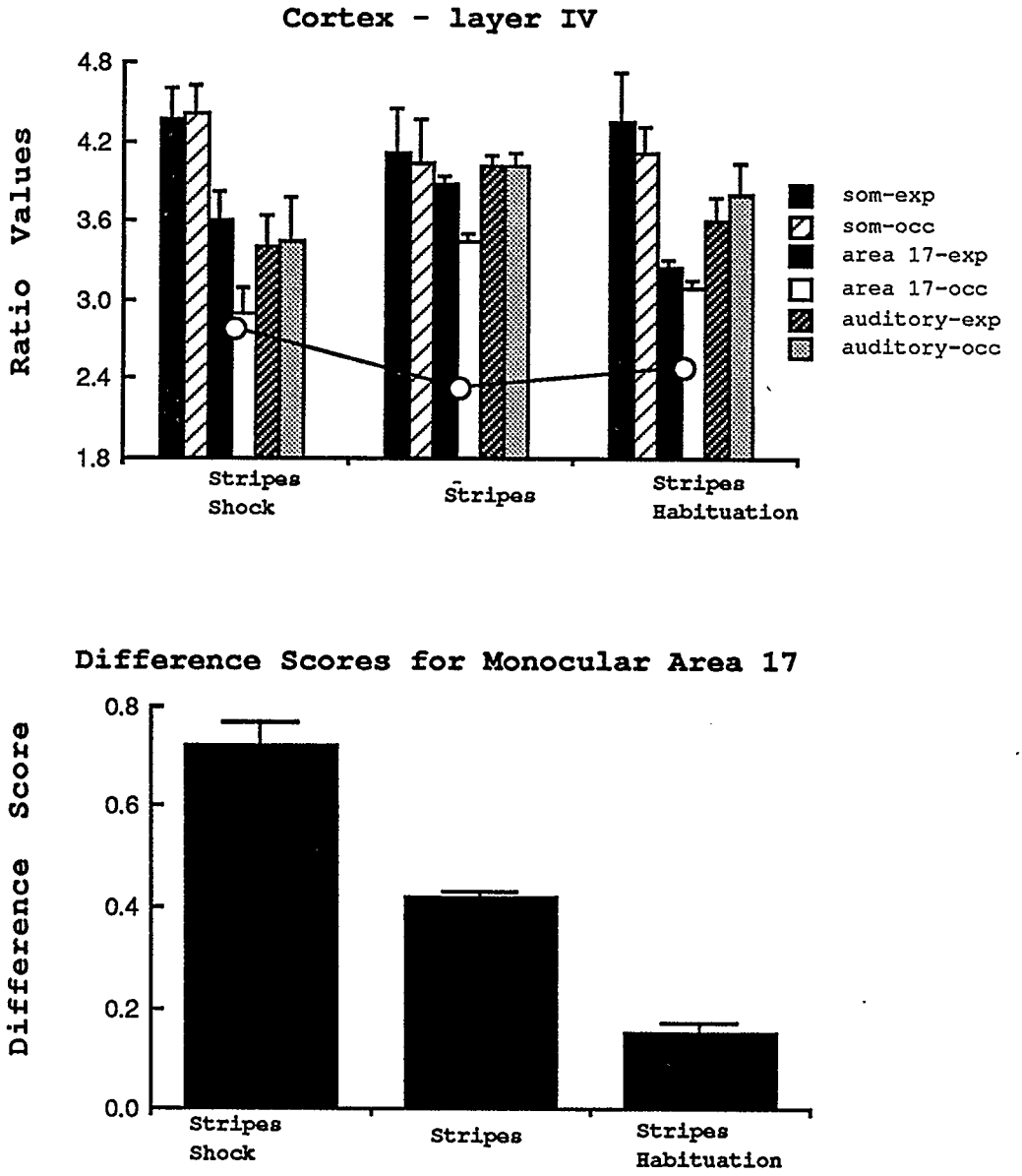

Figure 3.6: Average ratio values for somatosensory cortex (som), area 17, and auditory cortex by group (top of figure). Values for both hemipsheres area shown. Open circles represent average of the VTN, PMN, and SNr. Bottom of figure shows mean interhemispheric difference score from area 17 for each group. Positive scores indicate lower metabolic activity contralateral to the occluded eye

Table 3.3
Summary Tables For Analysis of Cortex

Source	SS	df	MS	F	η^2
Group	0.59	2	0.29	<1	
Error	4.22	6	0.70		
Area	6.94	2	3.47	35.86**	.857
G x A	1.55	4	0.39	4.00*	.571
Error	1.16	12	0.10		
Hemi	0.31	1	0.31	27.63**	.823
G x H	0.06	2	0.03	2.63	
Error	0.07	6	0.01		
A x H	0.59	2	0.29	25.24**	.807
G x A x H	0.27	4	0.07	5.81**	.659
Error	0.14	12	0.01		

*p<.05; **p<.01

Post-Hoc Results (Tukey B, *=p<.01)

Area 17 - Exposed Hemisphere

Mean	Group	Str-S	Str	Str-Hab
3.610	Str-S			
3.870	Str			
3.250	Str-Hab	*	*	

Area 17 - Occluded Hemisphere

Mean	Group	Str-S	Str	Str-Hab
2.890	Str-S			
3.450	Str	*		
3.093	Str-Hab	*		

Table 3.4
Summary Tables For Analysis of Difference Scores from Area 17

Source	SS	df	MS	F	$\eta 2$
Group	0.48	2	0.24	66.54**	.9571
Error	0.022	6	0.0036		

**p<.01

Post-Hoc Results (Tukey B, *=p<.01)

Area 17 - Difference Scores

Mean	Group	Str-S	Str	Str-Hab
0.720	Str-S			
0.423	Str	*		
0.153	Str-Hab	*	*	

also of decreased activity of the "occluded" hemisphere.

Relation of Cortical and Subcortical Metabolic Activity

Figures 3.7 through 3.9 show the within group correlation matrices for cortical and subcortical areas by hemisphere. The reduction in cortical-subcortical correlations in the shock group were quite apparent as in experiment 1 (see Figure 3.9). A reversal in correlations was also present between the stripes and stripes-habituated groups, especially with respect to the LGNd. The stripes group showed high negative correlations between the LGNd receiving projections from the unoccluded eye and all cortical areas, while the LGNd fed by the occluded eye showed high positive correlations (see Figure 3.8). This pattern was completely reversed with the stripes-habituated group (Figure 3.7).

The impressions gained from inspection of Figures 3.7- 3.9 were borne out by statistical comparison of the correlation matrices. A strong group main effect was present as well as group by subcortical area, group by subcortical hemisphere, and a significant three-way interaction of group by subcortical area by subcortical hemisphere. Breakdown of these interactions indicated that the strongest changes were for SGS and LGNd. In both cases the correlations with cortical areas were reduced to zero in the group receiving shock. The differences were larger for the "exposed" hemisphere than the "occluded" hemisphere.

Area 17 and auditory cortex showed the greatest differences in the correlations with subcortical areas between the three groups. The differences were strongest for the "occluded" hemisphere (summaries for this analysis are given in Appendix A).

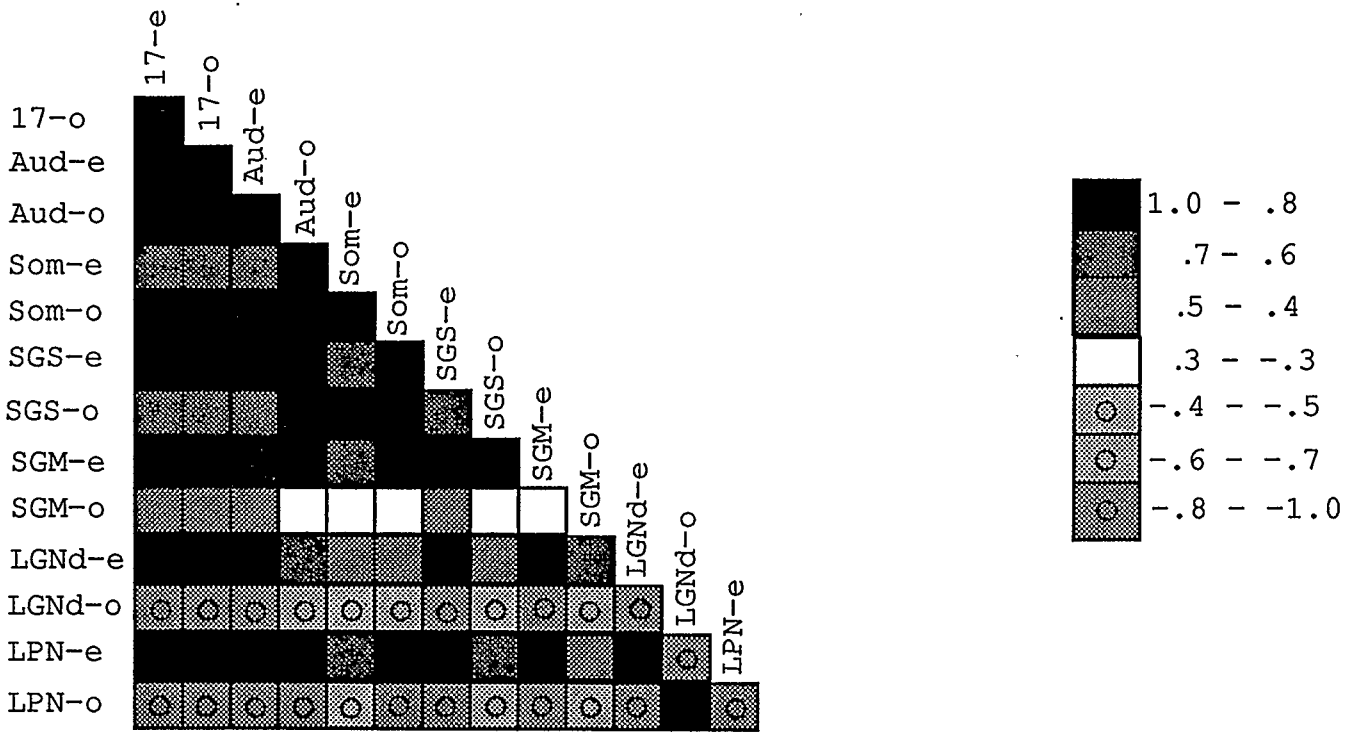

Figure 3.7: Correlation matrix for stripes-habituated group. Legend gives range of values for shading scale. Negative correlations indicated by circle within each shaded square. "e" and "o" indicated "exposed" and "occluded" hemispheres respectively.

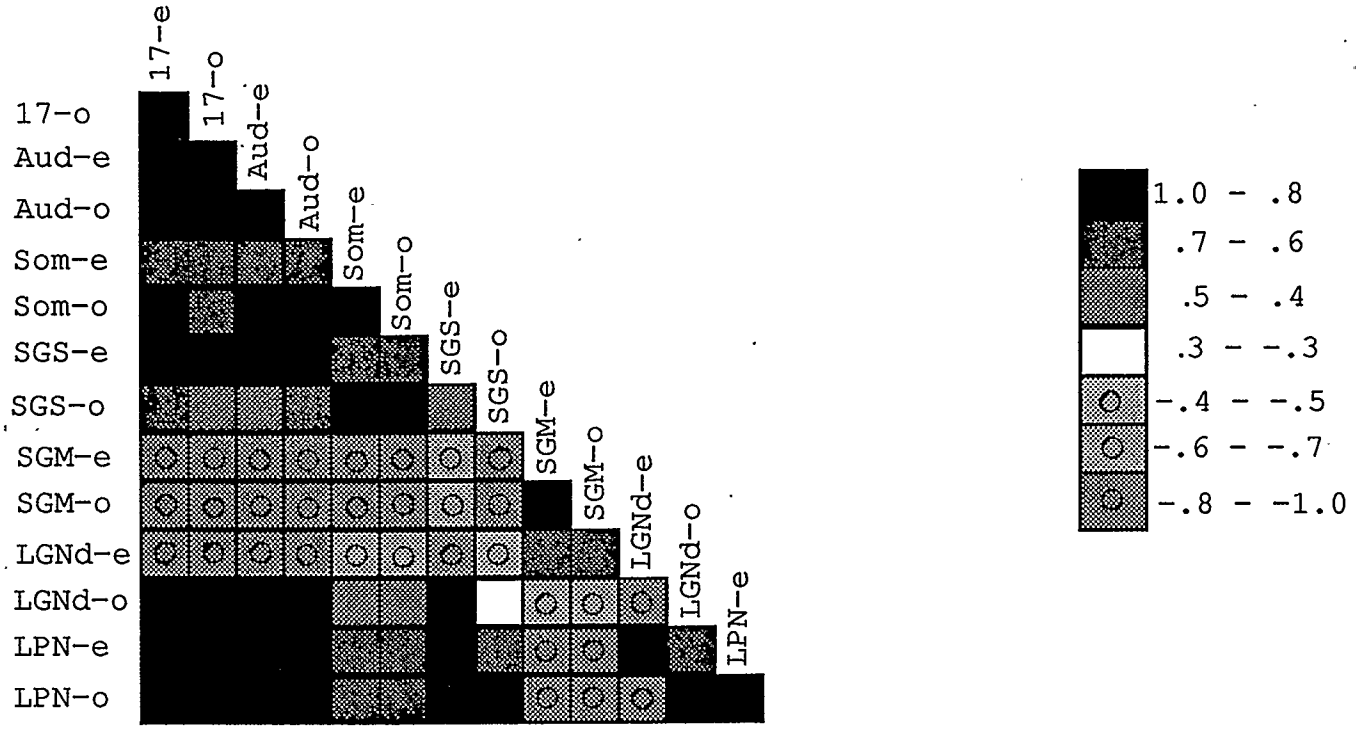

Figure 3.8: Correlation matrix for stripes group. Legend gives range of values for shading scale. Negative correlations indicated by circle within each shaded square.

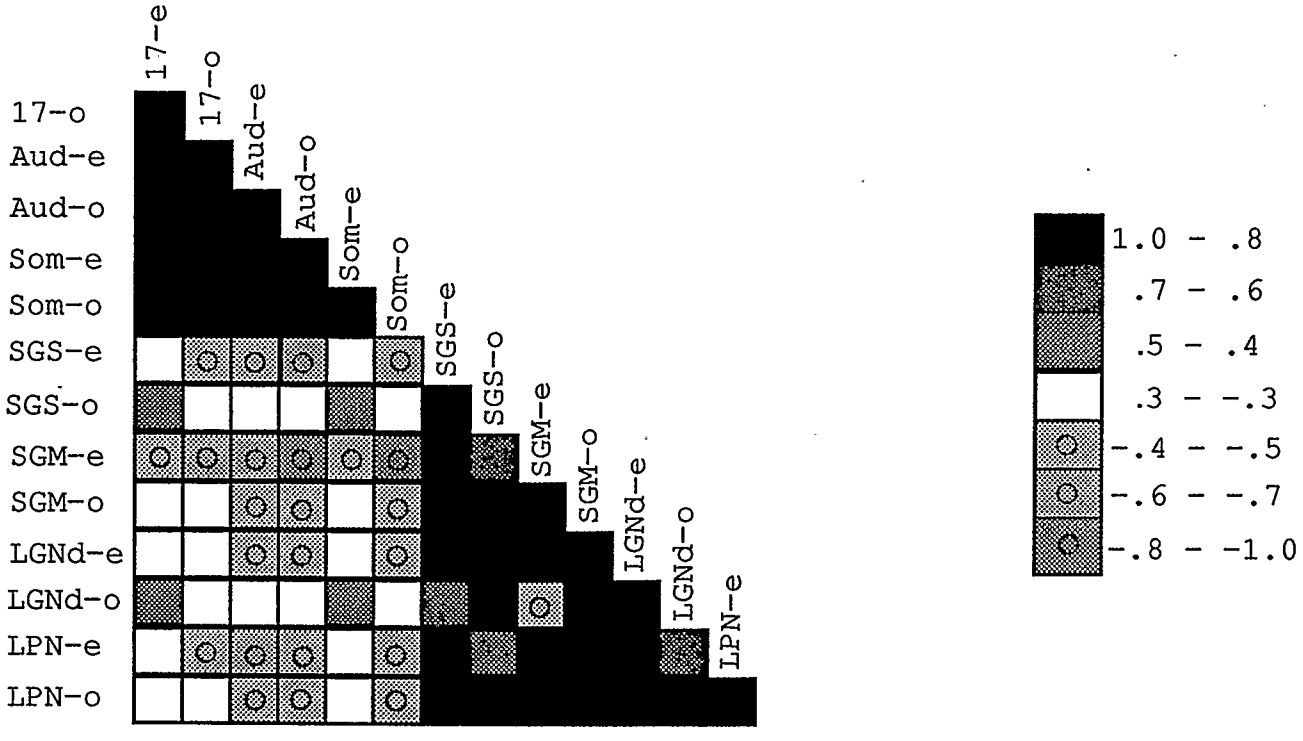

Figure 3.9: Correlation matrix for stripes-shock group. Legend gives range of values for shading scale. Negative correlations indicated by circle within each shaded square.

Summary

The results from experiment 2, in essence, replicate those of experiment 1. Shock-induced arousal appears to generally increase activity in subcortical structures. The effect was additive in subcortex since difference scores were not higher for animals receiving shock.

Experiment 2 also suggests that a true interaction exists between changes in arousal and cortical metabolic activity. Difference scores for area 17 were highest for the shock group, followed by stripes, then by stripes-habituated. The high difference score for the stripes-shock group appears to be the result of lower activity in the "occluded" hemisphere. Greater difference scores for the stripes group compared to the stripes-habituated group replicates the findings of McIntosh and Cooper (1989).

As well, relations between cortical and subcortical activity are changed by arousal. Not only did animals receiving shock show large decreases in cortical-subcortical correlations, but also animals receiving prior exposure to the visual display showed a different pattern than animals given 2-DG at first exposure. This lends support to the view that cortical and subcortical areas do not respond the same to changes in arousal level.

Chapter 4

Discussion

It has been established that with transitions from states of sleep to wakefulness, there are specific changes in the pattern of activity in the central nervous system (Steriade, Ropert, Kitsikis, & Oakson, 1980). It now appears that within the waking state as well, shifts in arousal level can produce detectable changes in brain activity. The major findings of the current study may be summarized as follows:

1. An increase in arousal (as a result of shock) leads to an increase in subcortical 2-DG uptake.

2. Cortical and subcortical areas do not respond in the same way to changes in arousal level as indicated both from the modifications in cortical-subcortical correlations and interhemispheric difference scores. Changes in difference scores as a function of arousal were observed in area 17 but not in any of the subcortical structures.

4.1 Subcortical Activity

The elevated 2-DG uptake observed at the subcortical level with increased arousal is consistent with previous research. Stimulation of the MBRF appears to enhance the responsiveness of cells in the LGNd (Doty, Wilson, Bartlett, &

Pecci-Saavedra, 1973; Chi & Flynn, 1967), as does an increase in arousal through peripheral stimuli (Bartlett, Doty, & Pecci-Saavedra, 1973; Swadlow & Weyand, 1985). Livingston and Hubel (1981) have suggested that an increase in arousal leads to an enhancement of receptivity in the visual system. The 2-DG results of Livingston and Hubel suggest that this produces an elevation in glucose utilization. Furthermore, emerging views (Sillito, Salt, & Kemp, 1985) suggest that an increase in arousal leads to an enhancement of both excitatory and inhibitory influences. This would also translate to an increase in 2-DG uptake.

If increased arousal heightens activity at the subcortical level, then it would be expected that a lower arousal level would lead to a decrease in activity, presuming that the relationship is linear. Assuming habituation constitutes a minimal level of arousal, the failure to observe reduced subcortical activity in experiment 2 and the McIntosh and Cooper study (1989) seems contrary to this expectation. However, the effects of arousal seem dependent on the ongoing activity within specific areas (Bartlett & Doty, 1974; Singer & Drager, 1972). It may be that subcortical activity in the visual system is already at its optimal level for the processing of the striped display, regardless of whether the animal had previously been exposed to the display or not. While the arousal level may, in fact, be lower for habituated animals, it may not result in a great enough change in metabolic activity to produce a noticeable difference when compared to animals presented with the display for the first time. As well, the function that describes the relationship between behavioral efficiency and arousal is not linear. It is possible that a similar function exists between changes in arousal and activity in the CNS. Activity levels in low (habituation) to moderate arousal (novelty) may represent either a steady state or plateau in the

relationship. High arousal (shock), however, seems to change metabolic activity enough to produce a difference in 2-DG uptake.

4.2 Cortical Activity

As noted in the introduction, numerous studies have found a difference in the responses of cortical and subcortical areas when arousal level changes. Bartlett and Doty (1974) noted that cells in visual cortex were less responsive to MBRF stimulation than cells in the LGNd (Doty, Wilson, Bartlett, & Pecci-Saavedra, 1973). Livingston and Hubel (1981) observed that single cells in the cat LGNd showed increased responsiveness in the waking state compared to sleep, but found less of an effect in visual cortex. This may suggest that cortex is less affected by changes in arousal level. If changes in functional activity of cortical cells is less evident in the case of sleep versus wakefulness (which may be thought to represent a large shift in arousal), it seems likely that relatively smaller changes in arousal in the waking state would not affect cortical activity to a great extent. This does appear to be the case in terms of general cortical metabolic activity, as suggested by experiment 1.

However, while the overall activity in cortex does not seem to be affected (to the same extent as subcortical areas) by heightened arousal in the waking state, there does appear to be some modification of functional activity within specific cortical areas. For example, interhemispheric difference scores in area 17 were higher for animals given shock, although the *average* level of cortical activity was

not significantly heightened. This effect seems reminiscent of the postulate that an increase in arousal results in an increase in the signal-to-noise ratio in cortex (Livingston & Hubel, 1981). Higher difference scores for the stripes-shock group in experiment 2 were partly a result of a decrease in activity in the "occluded" hemisphere. Since this hemisphere was receiving little visual information, it could be thought to represent "noise" and as such the level of activity within this area would have been attenuated. Responses in the "exposed" hemisphere, conversely, would have been enhanced resulting in increased 2-DG uptake (stripes-shock, and stripes no-shock groups).

That cortical and subcortical correlations were decreased in animals receiving shock may imply independent influences operating at the two levels of the brain. Such findings, as well as those mentioned previously (Livingston & Hubel, 1981; Bartlett & Doty, 1974), have lead to some speculation that there may an intrinsic component in cortex that mediates changes in activity independent of thalamic input (Vanderwolf, 1988), or systems which operate differently at cortical and subcortical levels (Skrebitsky, Chepkova, & Sharonova, 1980).

It seems counterintuitive to expect that as arousal increases all brain areas should similarly increase. The seeming "dissocation" of cortical and subcortical areas in states of high arousal may represent the exertion of cortical control over both intrinsic activity and ascending influences. Cortical and subcortical activity seems to vary together in moderate states of arousal, but when arousal increases still further the relationship changes. This is most likely a reflection of both an increase in excitation/inhibition and modifications in functional activity within specific cortical areas. If such a mechanism did not exist it is likely that cortical

processes would be degraded in high states of arousal given the apparent non-specific increase in subcortical activity.

4.3 Neural Mechanisms

The fact that shock-induced arousal produced a general increase in metabolic activity of subcortical structures is consistent with the actions of the MBRF. As stated in the introduction, stimulation of the MBRF may lead to activation of cholinergic fibers from the pedunculopontine tegmental nucleus (PPTg) which, in turn, could act to increase the functional activity of tectal and thalamic targets. It has been shown that direct application of ACh to relay cells in the LGNd of the cat increases the responses of these cells to their optimal stimuli (Sillito, Kemp, & Berardi, 1983). As well it seems that ACh inhibits GABAergic interneurons in the LGNd (McCormick & Pape, 1988), which may also act to increase thalamic activity and facilitate thalamocortical transmission.

Cholinergic activity may also have contributed more directly to the observed cortical effects. The major afferent source of ACh to cortex originates in the basal forebrain (Fibiger, 1982). The actions of ACh and cholinergic agonists on cortical activity results in general cortical desynchrony (Vanderwolf, 1988) similar to that produced by MBRF stimulation. Iontophoretic application of ACh to visual cortex neurons results in an increase in the response of the cell to their optimal stimuli, without affecting the level of spontaneous activity. However, some cells in visual cortex show a definite inhibitory response to applications of ACh (Sillito, Salt, &

Kemp, 1985). This suggests that an increase in cortical ACh turnover would not result in the same "summary" effect as in subcortical areas.

Cholinergic influences alone may not fully account for the effects of shock and habituation. It may be stated that, in general, the actions of ACh are excitatory/facilitory and may represent the mechanism that maintains the tonic level of activation (as suggested by the single cell studies of Steriade et al., 1980). While this may be the source of the metabolic increases observed in subcortical structures, it does not seem to hold as well for cortical structures. Moreover, Gonzalez-Lima (1989) has suggested that there may be an inhibitory relationship between the MBRF and the basal forebrain. Both MBRF stimulation and increases in arousal through peripheral stimuli show that as activity in the MBRF increases, basal forebrain nuclei show lower activity. Given that the basal forebrain is the major source of cortical ACh, it seems unlikely that the cortical results can be accounted for by the same cholinergic influences that act subcortically. However, the fact that the interhemispheric difference scores were affected in cortical and not subcortical areas, and that the higher difference scores were partly a function of decreased activity in the "occluded" hemisphere, may imply the involvement of non-cholinergic system(s) that *modulate* the general level of excitation originating from the ascending projections of subcortical nuclei (eg. medial thalamus and sensory nuclei).

One potential source of this modulation could be noradrenergic (NA) inputs from the locus coeruleus (LC). This area projects widely to both thalamic, tectal, and cortical areas and appears to have reciprocal connections with portions of the MBRF (Loughlin & Fallon, 1985). Moreover, connections from the LC to the basal forebrain have also been noted (Moore, 1980). Unlike the cortical desynchrony

produced by MBRF stimulation, stimulation of the LC produces a reduction of spontaneous cortical activity (Foote & Morrison, 1987). As well, LC cells seem to be more responsive to phasic shifts in arousal (Foote, Bloom, & Aston-Jones, 1983). Specifically, LC cells show increases in mean discharge rates when stimuli are novel, both noxious or non-noxious, or have some significance for the organism (eg. food).

Noradrenaline itself has been shown to exert differential effects consistent with its hypothesized role as a neuromodulator. It has been shown that NA applied either intravenously or iontophoretically results in a decrease in the spontaneous activity of cells in visual cortex, but does not affect the response to optimal stimuli (Kasamatsu & Heggelund, 1982). Such results have also been found in auditory (Foote, Freedman, & Oliver, 1975) and somatosensory cortex (Waterhouse, Moises, & Woodward, 1980). NA has also been shown to enhance cellular responses to iontophoretically applied ACh and GABA (Waterhouse et al., 1980); NA potentiated ACh-induced facilitatory responses and augmented GABA-induced cortical inhibition.

An increase in arousal could activate cells in the LC. This would lead to a release of NA onto target neurons in thalamus and especially cortex. NA would then act to enhance the selectivity and vigor of responses to subsequent sensory input or other synaptic inputs (eg. inhibitory influences). Such speculation is consistent with Livingston and Hubel's (1981) postulate that arousal is partly mediated through the LC, hence the increase in the signal-to-noise ratio in an alerted organism.

4.4 Future Considerations

The results of this study lead to several potential lines of research which may help to further define specfic mechanisms that operate through different stages of arousal.

1. Since there appears to be specific changes in CNS activity when the organism is aroused by aversive stimuli, it would be useful to determine if different types of stimuli that are arousing produce the same types of effects. Stimuli related to food or sex may produce different patterns of activity since the behavioral responses to these types of stimuli are different than responses to aversive stimuli. Studies such as Hand (1987) suggest that there may in fact be changes in cerebral metabolic activity with different degrees of stimulus significance. As well, it may be of interest to determine if increasing arousal level can overcome the cortical effects of habituation.

2. Pharmacological manipulations of ACh and NA could yield further insight into the relation of the neurotransmitters involved in arousal. For instance, it would be of interest to observe the influence of specific transmitter agonists and antagonists on the effects of shock. Specifically, if the lower metabolic activity observed in the portion of area 17 served primarily by the occluded eye following shock (experiment 2) was a function of NA, then cortical difference scores should be reduced by a NA antagonist.

3. Influences of neurotransmitter systems generally take place in very short

time intervals. Such changes cannot be quantified with the 2-DG technique with its limited temporal resolving capacities. It may therefore be useful to implement electrophysiological techniques to test the time course of the influences of these systems. For example, if the influence of the LC is phasic then there should be changes in the EEG profiles of sensory structures with different stimuli or the same stimulus presented in different contexts. Along with this, pharmacological manipulations may also further elucidate the roles of specific transmitter systems.

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Appendix A

Comparison of Within Groups Correlations

Typically, testing differences between group correlation patterns involves procedures such as comparison of variable loadings obtained through factor analysis. However, these procedures are extremely sensitive to sample size and linear dependency. Calculations of the determinants for each correlation matrix revealed that three of the four matrices from experiment 1 and all from experiment 2 had zero or near-zero determinants suggesting high degrees of linear dependency. Because of this, another method had to be derived to compare the matrices.

It was decided that a multi-way univariate analysis of variance was possible, using the correlations as the dependent variable. Since the main interest was in the relations between cortical and subcortical areas, only the correlations between these areas were used. For experiment 1, four factors were used: condition (shock vs. no shock); visual stimulation (stripes vs. dark box); cortical area (area 17, auditory, and somatosensory cortex); and subcortical area (SGS, SGM, LGNd, and LPN). For experiment 2 the factors were: (a) group (stripes and shock, stripes, stripes and habituation), (b) cortical area, (c) cortical hemisphere (exposed vs. occluded), (d) subcortical area, and (e) subcortical hemisphere.

Sampling distributions for correlations can differ dramatically depending on the expected value for the population correlation (Howell, 1987). If the expected value is zero, then the sampling distribution is normal. However, as the expected value

deviates from zero, the sampling distribution becomes increasingly skewed (the direction of the skew depending on the direction of the correlation). This introduces problems with any test involving correlations as the values for the standard error become more difficult to compute. This problem also exists when comparing correlations obtained from independent groups.

A method to compensate was developed by Fisher (1929; cited in Johnson & Kotz, 1970). It involves a logarithmic transformation of the correlations to Fisher's Z_r which normalizes the sampling distribution of the correlations giving it a mean of zero and a standard deviation of one. Since the population values for cortical-subcortical correlations cannot justifiably be expected to be zero and the comparisons of interest were between groups, each correlation was converted to its equivalent Z_r. As such, the converted correlations could be thought to represent standardized or Z scores.

As the transformed scores are derived from a distribution with a known population mean and standard deviation, they can be related to other types of distribution. A χ^2 distribution, for instance, can be thought of as a sampling distribution of the sums of squares for Z scores (Lewis, 1960). For example, a distribution of the sums of squares based on single samples of Z scores yields a χ^2 distribution with 1 degree of freedom. Sums of squares distributions based on Z scores of samples sized 5 gives a χ^2 distribution with 5 degrees of freedom.

Given this relationship it is possible to evaluate an analysis of variance, where the dependent variable is standardized, by comparing the obtained sums of squares for an effect with the critical value from a χ^2 distribution with the number of degrees of freedom equal to the degrees of freedom for the effect in question. This obviates

the need to compute F-ratios to test each effect.

Such a procedure may be somewhat conservative since the estimate of the standard error used to test each effect will typically be larger than the actual standard error. This possibility, however, would keep the probability of a Type-I error low, which is necessary given the large number of significance tests that would be employed with the designs in question.

A.1 Computation Involving Pairwise Comparisons

As stated previously, the tranformation of correlations to Z_r yields a normal sampling distribution with a known standard error. The standard error of the sampling distribution for any pairwise comparison can then be estimated using the formula:

$$\frac{1}{\sqrt{N-3}}$$

which would subsequently be used in the formula:

$$\frac{Z_{r1} - Z_{r2}}{\sqrt{\frac{1}{N_1-3} + \frac{1}{N_2-3}}}$$

where Z_{r1} and Z_{r2} are the two transformed correlations of interest and N would be the sample size of each group.

This estimate poses no problem as long as N is greater than 3. However, when N is less than or equal to 3, the standard error based on the above formulae would equal zero (or less) and hence the equation would be undetermined. It is possible though to compute the standard error if the population correlation can be

estimated. If zero is given for the expected population correlation, the computed standard error will most likely be higher than is actually the case. While this again would lead to an overly conservative test, it would further protect against Type-I error.

Given the estimate of the population correlation, the standard error can then be derived using the formula:

$$(n-1)^{-1}[1+\frac{1}{2}(4-\rho^2)(n-1)^{-1}+\frac{1}{6}(22-6\rho^2-3\rho^4)(n-1)^{-2}]$$

(where ρ is the estimated population correlation) and taking the square root of the result (Johnson & Kotz, 1970).

A.2 Results of Analyses

The results stemming from the analyses in experiments 1 and 2 are presented in the tables which follow. For clarity only the sums of squares for significant effects are presented. Each source was evaluated against the critical values from a χ^2 distribution with alpha set at .01. Pairwise comparisons were evaluated against the values from a unit normal distribution again with alpha set at .01.

Table A.1
Summary Tables from Analysis of Within Groups Correlations
Experiment 1

Source	df	Sums of Squares
Shock	1	27.703
Visual	1	23.781
Cortical Area	2	65.14
Subcortical Area	5	65.79
C x V	1	11.27
C x SA	5	45.02
V x CA	2	11.60
V x SA	5	73.94
C x V x CA	2	37.02
C x V x SA	5	38.19

Critical values based on Chi Square distributions (alpha=.01):
1 df = 6.63
2 df = 9.21
5 df = 15.09

Pairwise comparsions of Z_r

Shock versus No Shock

Area 17 and SGS:
Shock: groups differ at p<.01
No-shock=2.096
Shock =0.762

Auditory Cortex and SGS:
Shock: groups differ at p<.01
No-shock=1.494
Shock =0.33

Area 17 and LPN:
Shock: groups differ at p<.01
No-shock=2.78
Shock =1.44

Auditory Cortex and LPN:
Shock: groups differ at p<.01
No-shock=2.39
Shock =0.30

Breakdown by Group

Area 17

Group: groups differ at p<.01

Stripes - No shock =2.43

Stripes - Shock =0.57

Black box - No shock=0.78

Black box - Shock =1.50

Auditory Cortex

Group: groups differ at p<.01

Stripes - No shock =1.386

Stripes - Shock =0.211

Black box - No shock=0.80

Black box - Shock =0.35

Somatosensory Cortex

Group:

Stripes - No shock =0.738

(two group do not differ)

Stripes - Shock =0.772

Black box - No shock=0.06

(groups differ at p<.01)

Black box - Shock =-0.88

SGS

Group: groups differ at p<.01

Stripes - No shock =1.47

Stripes - Shock =0.31

Black box - No shock=1.71

Black box - Shock =0.31

SGM

Group: groups differ at p<.01

Stripes - No shock =1.47

Stripes - Shock =0.70

Black box - No shock=-1.74

Black box - Shock =-0.19

LGNd

Group: groups differ at p<.01
Stripes - No shock =1.61
Stripes - Shock =0.48

Black box - No shock=-0.26
Black box - Shock =0.59

LPN

Group: groups differ at p<.01
Stripes - No shock =1.54
Stripes - Shock =0.68

Black box - No shock=2.49
Black box - Shock =0.51

Table A.2
Summary Tables from Analysis of Within Groups Correlations
Experiment 2

Source	df	Sums of Squares
Group	2	28.14
Subcortical Area	5	98.52
Subcortical Hemisphere	1	11.52
G x SA	10	171.66
G x SH	2	91.16
G x SA x SH	10	62.51

Critical values based on Chi Square distributions (alpha=.01):
1 df = 6.63
2 df = 9.21
5 df = 15.09
10 df= 23.21

Pairwise comparsions of Z_r

SGS(exposed hemisphere): groups differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
-0.499	Str-S			
2.27	Str	*		
2.34	Str-Hab	*		

SGS(occluded hemisphere): groups differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
0.154	Str-S			
1.144	Str	*		
1.57	Str-Hab	*		

LGNd(exposed hemisphere): groups differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
-0.29	Str-S			
-2.03	Str	*		
1.136	Str-Hab	*	*	

LGNd(occluded hemisphere): groups differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
0.215	Str-S			
1.503	Str	*		
-1.47	Str-Hab	*	*	

Area 17(exposed hemisphere): groups differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
0.06	Str-S			
0.828	Str	*		
0.657	Str-Hab			

Area 17(occluded hemisphere): groups differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
-0.232	Str-S			
0.733	Str	*		
0.677	Str-Hab	*		

Auditory Cortex(exposed hemisphere): groups **do not** differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
0.083	Str-S			
0.098	Str			
0.381	Str-Hab			

Auditory Cortex(occluded hemisphere): groups differ at p<.01

Mean	Group	Str-S	Str	Str-Hab
-0.484	Str-S			
0.828	Str	*		
0.310	Str-Hab	*		

Abbreviations: Str-S=stripes-shock; Str=stripes; Str-Hab=stripes-habituation.