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THE DEVELOPMENT OF THE NEONATAL RAT BRAIN AS MEASURED BY THYMIDINE INCORPORATION

by

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DISSERTATION

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ABSTRACT

THE DEVELOPMENT OF THE NEONATAL RAT BRAIN
AS MEASURED BY THYMIDINE INCORPORATION

KATHLEEN A. DARK

Maturation rates of the cerebral cortex, caudate, thalamus and hypothalamus were examined in the neonatal rat. Male and female Sprague-Dawley rats received two consecutive injections of ^{3}H-thymidine on Days 1-2 postnatally, Days 7-8, Days 14-15 or Days 21-22. Animals were sacrificed on Day 50 and their brains dissected and assayed to determine, by scintillation counting, the amount of thymidine incorporated into the DNA of the developing cells. The brains of these animals were examined for: (1) differential rates of neural development over time, (2) sex differences in the amount of neural maturation taking place at any age, and (3) maturational differences between the left and right sides of these structures.

A significant group effect was found for all four areas of the brain examined. Of the four ages at which thymidine injections were administered, the highest rate of incorporation (and thus the greatest amount of neural development) was observed on postnatal Days 7-8. By postnatal Days 21-22, almost no incorporation was evident.

Although no left-right laterality effects were present for the animals as a group, a strong laterality effect was found in terms of high side of incorporation vs low side

of incorporation regardless of the left-right dominance of the results. This high side vs low side comparison was significant for all four age groups and over all four brain areas.

A trend was evident that suggested that females were undergoing greater neural maturation than males at 12 out of 16 measures (4 structures x 4 ages) however, these results were not statistically significant.

Professor Harman V.S. Peeke, Ph.D.
Chairman, Dissertation Committee

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INTRODUCTION

Within the last fifteen years it has become increasingly apparent that there are many differences between the brains of males and females. These differences are often manifested as sex differences in behaviors or abilities, as well as differences in the susceptibility to injury or differential responsiveness to drug treatments. Pursuing this line of research requires that investigators from broadly different areas of interest combine both their background knowledge and methodologies to lead to cohesive investigations of these issues. At present, investigators from the three seemingly disparate disciplines of early brain development, brain and behavioral laterality and sexual differentiation of the nervous system are attempting to combine forces to address two basic problems: (1) whether or not sex differences exist either in the development of specific brain areas or in the maturation rate of the brain itself and (2) whether or not early development in the brain is lateralized and if sex differences may be evident in this early lateralization. To understand the integration of these areas necessitates a review of the pivotal work from each area.

SEXUAL DIMORPHISM OF THE NERVOUS SYSTEM

Sexual dimorphisms may be reflected in differences in

the synaptic connections of a structure, differences in the cell number or cell size of an area of the brain, changes in neural maturation rate between males and females, or even by differences in how male and female brain areas respond to injury. In addition, the majority of these sex differences appear to be under the control of gonadal steroids. The action of gonadal steroids can be classified as either being "organizational" or "activational" (Goy and McEwen, 1980). The activational hypothesis states that morphological or behavioral dimorphisms are manifested only at the time during which the hormone is present. More and more evidence points to the importance of "critical periods" and the organizational effect of hormones on the development of sexual dimorphisms in the brain. The organizational hypothesis states that it is the presence or absence of hormones during a "critical period" which determines the development of either male or female patterns, respectively, of neural organization. An example of the organizational effects of gonadal hormones on brain organization is given below in the discussion of the preoptic area of the hypothalamus.

THE PREOPTIC AREA OF THE HYPOTHALAMUS: A MODEL FOR THE HYPOTHESIZED EARLY HORMONAL CONTROL OF SEXUAL DIMORPHISM

Perhaps the most well investigated area of the brain with respect to sexual dimorphisms is the sexually dimorphic nucleus of the preoptic area of the hypothalamus (SDN-POA).

Raisman and Field (1973) demonstrated that the number of spine synapses in the POA is about twice as great in female rats as in males. They also have shown that the density of these synapses is reduced by the presence of neonatal testosterone. In their study they found that the density of spine synapses was approximately equal in intact females, Day 1 castrated males, and females given testosterone propionate on Day 16; and significantly greater in these three groups than in intact males, females given testosterone propionate on Day 4, and males castrated as adults. The authors hypothesize that because this area is responsible for the triggering of the ovulatory surge of gonadotropin in females but not in males, this functional dimorphism could account for the greater number of synapses in females.

Sex differences in the SDN-POA of hamsters were also demonstrated (Greenough, Carter, Steerman and DeVoogd, 1977). By mathematical reconstruction of dendritic densities, they showed that male hamsters have a more central distribution of dendrites in the SDN-POA and also show a tendency for slightly longer dendritic length. Female hamsters have shorter dendrites with a more irregular density distribution. The authors suggest that these dendritic differences could reflect differences in afferent connections which could result in functional dimorphisms.

Gorski, Gordon, Shryne and Southam (1978) found gross sexual dimorphisms in the SDN-POA of the rat. In a well controlled and methodologically sound study, they demon-

strated that the SDN-POA has a significantly greater volume in male rats than in females. They also demonstrated that the volume of this nucleus can be modified by hormonal manipulations only in the neonate, not in the adult. They postulate from preliminary evidence that this volume difference may be due to an increase in the number of large diameter cells in the males.

Even though the volume of the SDN-POA in a Day 1 castrated male is significantly smaller than in an intact male, it never reaches the same level as in an intact female. For this reason, Gorski and colleagues (1978) suggested that a genetic component also may be involved. However, further research has shown this is probably not the case (Dohler, Coquelin, Davis, Hines, Shryne and Gorski, 1982). To test this, they injected either an oil medium or testosterone in oil daily from Day 16 prenatally to Day 10 postnatally. With this procedure of extended injections they were able to get a testosterone treated female to the level of an intact male and, therefore, suggested that a genetic component was unlikely. They proposed that testosterone released constantly by the testes of male neonates during the period of differentiation is responsible for the development of the SDN-POA.

In a further study, however, they demonstrated estrogenic effects on the SDN-POA (Dohler, Hines, Coquelin, Davis, Shryne and Gorski, 1982). In this case, they treated female rats with the synthetic estrogen, diethylstilbestrol (DES), in a paradigm of pre- and postnatal injections as above.

They found the volume of the SDN-POA in females to be significantly enlarged to the level of a normal male. Since this procedure was just as effective as giving testosterone propionate, it implicates the role of the conversion of androgens to estrogens (aromatization) in this process. The enzymes necessary for the aromatization of androgens have been identified in this area of the brain.

In contrast to the work of Gorski and Dohler, Young (1982) in his work with mice found no observable sexual dimorphism in the volume of the medial portion of the anterior hypothalamic nucleus (AHm), the region comparable to the SDN-POA of rats. He proposes two possible explanations for this finding: (1) the AHm is not sensitive to testosterone as is the SDN-POA or (2) both male and female mice may be androgenized due to gonadal steroid transfer between littermates in utero. He also states, however, that sexual dimorphisms in the mouse AHm with respect to fine neuronal structure cannot be ruled out.

SEX DIFFERENCES IN SUBCORTICAL BRAIN AREAS: THE HIPPOCAMPUS AND THE AMYGDALA

Milner and Loy (1980) found sex differences in the amount of sympathetic axonal sprouting after damage to the septal afferents to the hippocampus. By determining the amount of axonal sprouting with fluorescence histological procedures, they were able to demonstrate that lesioning of

males as adults produced very different patterns and amounts of new growth into the hippocampus than did lesioning of adult females. In addition, measuring the high affinity uptake of ^{3}H-norepinephrine, showed that males have many fewer sites than females. However, they also found that if the animals received septal lesions on postnatal Day 3 (versus as adults) no male/female differences were found. The authors state two possibilities for the observed sex differences: (1) increased axonal sprouting is due to circulating gonadal hormones in adulthood, or (2) they may be due to the presence of gonadal hormones during development. To test these hypotheses ,they performed both adult and neonatal hormonal manipulations (Milner and Loy, 1982). Male and female adult rats were gonadectomized approximately one week before the septal lesions. For the neonatal groups, males were castrated on postnatal Day 2, while females were implanted with Silastic capsules of testosterone. Some castrate males also were implanted with testosterone capsules. All of these groups were deafferented as adults.

They found that adult gonadectomies had no effect on the axonal sprouting of either males or females. Males who were castrated on postnatal Day 2, however, had sprouting patterns similar to females, while females who were intact but treated with testosterone had patterns similar to normal males. Males who underwent neonatal castrations and received testosterone replacement also were similar to normal males. These results clearly support an hypothesis for the organ-

izational effects of testosterone. The authors do state, however, that the site of action of testosterone (i.e., the neonatal superior cervical ganglion, the septum, or the hippocampus) as well as the mechanism which controls sprouting in response to injury are still unclear (Milner and Loy, 1982).

Utilizing electron microscopy, Nishizuka and Arai (1981a) have found a significantly greater number of postsynaptic elements in the amygdala of male rats compared to female rats. By counting specific synaptic types (shaft, spine, and somatic) they determined that the increase in number between males and females was due almost exclusively to increases in shaft synapses. To determine whether this observed dimorphism was under hormonal control, they performed both neonatal castrations of males and neonatal testosterone injections in females. They found, in fact, that the neonatal castrates had a significant decrease in the number of shaft synapses to a level comparable to normal females, whereas, the females who underwent neonatal androgen injections had a significant increase in the number of shaft synapses to a level comparable to normal males. Animals in this study were sacrificed on Day 11 or Day 100. No sex difference was evident at Day 11 but was clearly evident in those animals sacrificed on Day 100. In a similar study (Nishizuka and Arai, 1981b) they examined normal males, normal females, and neonatally estrogenized females, and found the same effects. Normal males had significantly

greater numbers of shaft synapses than normal females. In addition, the neonatally estrogenized females were significantly different from normal females but very similar to normal males with respect to the number of shaft synapses. The authors imply that this finding is supportive of the aromatization hypothesis of differentiation since the same effect was achieved in both studies; the first using testosterone propionate, an aromatizable androgen, and the second using estrogen.

To examine the time course of the development of this dimorphism, animals were sacrificed on Days 1, 11, 21, 31 and 100. As in the previous study, the sex difference was not evident until after Day 11 (i.e., first evident in the Day 21 group). Since the previous studies indicate that the organizational effects of gonadal hormones are necessary during the critical period of sexual differentiation (approximately Days 1-5 postnatally) the authors postulate that a "latency period" is necessary after the critical period in order for this dimorphism to become evident in the adult.

In addition to synaptic differences, sexual dimorphisms have been found in the nuclear volume of the amygdala (Mizukami, Nishizuka and Arai, 1983). The volume of the medial nucleus (as calculated through computer digitization) in the male was significantly greater than in females. Females who were treated postnatally with estrogen were found to have a significant increase in the volume of the medial nucleus similar to that found in normal males. As in previous studies,

this sexual dimorphism was not apparent until 21 days of age and persisted into adulthood. As a control area, the authors also examined the volume of the lateral nucleus of the amygdala. In this case, there was no significant difference between any of the three groups (normal male, normal female, and estrogenized females). They attribute this finding of sex differences in medial nucleus volume but not lateral nucleus volume to the fact that the medial amygdala contains receptors for steroid hormones while the lateral amygdala does not. Therefore, the medial amygdala may have different levels of receptivity between males and females which may be part of the mechanism of the observed sexual dimorphism in this nucleus.

Nishizuka and Arai (1981b) also postulate a possible reason for the sexual dimorphisms, both in the synaptic elements and nuclear volume, seen in the amygdala. They suggest that since neural connections between the medial amygdala and the preoptic area are sexually dimorphic (see Dyer cited in Nishizuka and Arai,1981b) male/female differences in synaptic connectivity could possibly reflect neuroendocrine functional differences between males and females. These differences are especially important since the medial amygdala modulates the regulation of gonadotropin secretion, and the LH surge seen at ovulation.

SEXUAL DIMORPHISMS IN ANATOMY AND NEURAL MATURATION RATES OF RAT CORTEX

Sexual dimorphisms have also been found in cortical thickness. Work by Diamond, Johnson and Ehlert (1979) showed that while there were no significant differences between intact male and female rats, neonatal estrogen is clearly important in determining cortical thickness. Not only was the cortex found to be thicker in Day 1 ovariectomized females versus Day 1 sham-treated females, but they also showed a 12% increase in soma size and a 6% increase in nuclear size as compared with sham females (Pappas, Diamond and Johnson, 1979). Additionally, the authors showed that by ovariectomizing females on Day 1 and giving either estrogen or progesterone on Days 40-90, the cortex became thinner or thicker, respectively, in response to these hormone therapies when compared with sham females (Pappas, et al, 1979).

All of these examples of sexual dimorphisms have dealt with specific neuronal differences between the sexes, i.e., differences in neuronal density or dendritic morphology as well as possible differences in synaptic connectivity. However the sexes have been found to differ along another dimension as well, that of neural maturation rate.

To investigate this, Gregory (1975) compared the development of both nuclear volume and somatic volume of the pyramidal cells of layer 5 in male and female rats. She found that for these two measures development proceeds along similar lines between the sexes from Day 1 to approximately Day 25. Development of the cell was shown to progress

rapidly during the first 16 days and then to slow down somewhat. By Day 25, the somatic volume in females is 10% less than that of males; by Day 35 it is 30% less. As the best explanation of this finding, Gregory states that this could reflect a less mature cell in the male than in the female (i.e., a less mature cell being defined as a cell with fewer synaptic connections). She draws the analogy between a developing neuron and regeneration in a neuron after the severing of an axon. According to a theory postulated by Gregory, during growth there is an accumulation of water by the soma and a concomitant increase in cellular volume, but once synaptic connections are formed, the swelling is reduced as is the cell volume. Therefore, the smaller volume of the female pyramidal cells may represent a more mature cell with more synaptic connections formed.

Evidence supporting a similar conclusion comes from Yanai (1979b) who, through a tritiated thymidine paradigm, also demonstrated that males may have slower rates of maturation of cerebral cortex than do females. He showed that by injecting ^{3}H-thymidine intraperitoneally into male and female rats at 1, 7, 14 and 21 days of age, there was more labelling in the males at every age group. The cortical sections he examined were 6.8% larger in males versus females, but, the packing density of the cortical cells was 5.9% higher in females versus males. Therefore, the actual number of cells was found to be equal between the sexes. Since ^{3}H-thymidine is incorporated only into the DNA of dividing cells,

and males labelled more cells at every developmental stage, he interprets this to mean that more cells in the males are in a state of development when much of the cellular development in females is finished (possibly prenatally, a point I will return to later).

The findings of both of these studies have important implications because, as Yanai (1979b) points out, developing cells may be more at risk to injury than cells which are already formed. It is possible, then, that males could be at risk for a longer period of time during development than females due to delayed maturation, and would be much more vulnerable to early insults.

The discovery of sex differences in many of these brain areas such as the preoptic area and amygdala was surprising but not unexplainable given the fact that most of these areas of the brain are also involved in regulating behaviors which in turn are sexually dimorphic (i.e., ovulatory surges of hormones, aggression, etc.). Diamond, Dowling and Johnson (1981) even explain their cortical differences on the basis that males are more visual-spatial oriented than are females and those are the functions of the cortical areas (areas 17, 18a and 39) where they find the most consistent sex differences. What has been a little harder to explain are sex differences found in laterality effects, both behavioral lateralities and biological.

LATERAL ASYMMETRIES IN THE BRAIN AND BEHAVIOR OF THE RAT

One of the foremost researchers of lateral asymmetries in rodents is Glick who reports findings of numerous asymmetries in rats, mice and hamsters. One of his most consistent findings is that rats have an intrinsic asymmetry in caudate dopamine content. This neurochemical asymmetry is directly correlated with an animal's side preference (Glick, Jerrusi and Zimmerberg, 1977). Normal animals generally turn contralateral to the side of the brain with higher dopamine content. Glick and his colleagues (1977) suggest that an optimal amount of behavioral laterality may be necessary for learning to take place. They found that the greater the side preference exhibited by a normal animal (as measured by %side preference in a T-maze or rotometer) the less hyperactive it was and the better able to learn both passive and active avoidance tasks. Those animals who had almost no side preference were the most spontaneously hyperactive and the poorest learners. In addition, Glick and Greengard (1980) showed that exaggerated laterality, as defined by much greater side preferences than normal control animals exhibit, also results in behavioral deficits. Rats treated with phenylalanine and α-methylphenylalanine were extremely hyperactive, showed significantly greater than normal side preferences, and were impaired in their performance in a T-maze.

Following this line of research, many other investi-

gators found many other asymmetries, and tried to correlate asymmetries found in anatomy and physiology with behaviors which also were lateralized, or with behaviors for which this lateralization might be beneficial. Most studies of lateralization in animals focused on anatomy, biochemistry, histology or electrophysiology. For example: asymmetries of the Sylvian fissure were found in great apes (orangutans, chimpanzees and gorillas) which are remarkable similar to the same anatomical asymmetries found in humans (LeMay and Geschwind, 1975). Biochemical asymmetries of dopamine and norepinephrine were found in the caudate and thalamus of the rat, respectively (Glick, et al, 1977; Oke, Lewis and Adams, 1980; Dark, Ellman, Peeke, Galin and Reus, 1984). Electrophysiological asymmetries, specifically left/right differences in visual evoked potentials were reported in albino rats (Myslobodsky and Shavit, 1978). Pearlson and Robinson (1981) found behavioral and neurochemical changes which differed depending on whether the left or right cortex was damaged in rats. Histological asymmetries also have been reported where the right hemisphere of male rats has been found to be significantly thicker than corresponding parts of the left hemisphere (Diamond, et al, 1981). Denenberg, Garbanati, Sherman, Yutzey and Kaplan (1978) found that by ablating the right or left hemisphere, rats differ on tests of activity and emotionality and that early environmental experience is crucial to the development of this lateralization.

While research on differential maturational rates between the cortices of males and females is ongoing, a differential left-right maturational gradient has also been postulated. A good example of left/right asymmetry in development is a study by Narang (1977) in which he demonstrated that the development of myelination of the optic nerve in the rabbit is asymmetrical where the eye which opens first (usually on Day 10 after birth) showed greater myelination than the other eye which generally opened several hours later. In addition, the author noted it was usually the right eye which opened first (19 out of 24 cases).

None of these early studies, however, clearly addressed the idea of sex differences in laterality. When this point was investigated, researchers discovered a strong gender effect in lateralized behaviors and their neural substrates.

SEX DIFFERENCES IN BRAIN AND BEHAVIORAL ASYMMETRIES

In an experimental paradigm very similar to that of Glick, et al (1977), Robinson, Becker and Ramirez (1980) looked at male/female differences in rotational behavior during amphetamine administration. They found that female rats exhibited greater net rotations than did males. In addition, dopamine was always greater on the side contralateral to the animals rotational preference [which is consistent with the findings of Glick, et al (1977)].

Although there was no relationship between rotational behavior and dopamine asymmetry in males, a surprising finding was that males did, in fact, exhibit a dopamine asymmetry. Dopamine asymmetry (in response to amphetamine) was always higher on the right than the left. The authors state that this finding clearly needs to be replicated.

In a study designed to further determine the effect of gonadal steroids on the activity of the dopamine system, Robinson, Camp and Becker (1981) examined the effect of gonadectomies on electrical brain stimulation-induced rotation. They found that adult female ovariectomies did attenuate rotational behavior while castration of male rats did not. The authors conclude that female gonadal hormones facilitate extra-hypothalamic dopamine activity. Unfortunately the effects of neonatal hormones on the organization of this behavior have not been fully investigated.

Many investigators have hypothesized that in humans, the male brain is more functionally lateralized than the female brain (Trotman and Hammond, 1979; McGlone, 1980). This view was supported in an animal model by Diamond, et al (1981) who showed that there are male/female differences in cortical thickness with respect to laterality. Some regions of the male cerebral cortex were found to be significantly thicker on the right than on the left. This pattern is reversed, though non-significantly, in females. If the female rats are ovariectomized at birth, however, they are found to have the male pattern of cortical asymmetry. This

is a very intriguing finding since females are considered the "neutral sex"; i.e., many female typical patterns of neural development and behavior are seen even in females ovariectomized at birth [for an excellect review of research in this area see Goy and McEwen (1980)]. It is possible that another mechanism of differentiation for the male pattern of development may be operating in this case; i.e., one which requires the absence of estrogen during a critical period as opposed to the presence of estrogen or an aromatizable androgen.

Recent work has shown that the reverse situation (females more lateralized than males) may be true with respect to side preference and rotational behavior in rodents. Glick, Schonfeld and Strumpf (1980) noted that in response to amphetamine, female rats and gerbils consistently rotate more than males. However, as they state in their commentary (1980), this effect was not found in random populations of animals obtained from breeders, but only from animals raised in their laboratory. Generalizations regarding these findings, therefore, should be guarded until they can be replicated in random populations of animals.

Ross, Glick and Meibach (1981) have shown a laterality as defined by the direction of tail curl in the neonatal rat pup, to be present at birth and to be predictive of adult rotational preference. They demonstrated that females had a significant right bias in the position of their tails. Although there was a left bias in the tail position of the

neonatal males, this trend was not significant.

Similarly, Denenberg, Rosen, Hofmann, Gall, Stockler and Yutzey (1982) examined neonates and found that Wistar rat pups show a left bias in tail position as they do in side preference as adults in an open-field paradigm. In addition, they showed that females have a higher left tail bias than males. These data, however, are based on 2223 rats and the statistics include all of the animals which show no preference. It is interesting to note that many more males show no preference than do females (11.4% versus 5.8%) but this group of animals artifactually decreases the percentage of left preference males and contributes to the significant finding of greater left bias in females versus males (57.7% and 51.9% respectively). A more appropriate analysis might be to examine only those animals who show a preference. When this was done, the difference between males and females was much reduced (61.3% females versus 58.6% for males).

THE DEVELOPMENT OF SEX DIFFERENCES AND LATERAL ASYMMETRIES

Recently researchers have begun asking the question, are other sex differences in laterality present at birth, or do they develop over time? And, if they are present in the neonate, are they under the same hormonal, organizational cortrol as are the neuronal sexual dimorphisms discussed previously?

A number of studies have been carried out to investi-

gate these questions. Investigators who are working to consolidate these areas of research (laterality, sex differences and development) have hypothesized five basic points: (1) Sex differences are evident in the neonate (Day 1-21 of age for rodents). There is no dispute over this point. It has been clearly established that sex differences in the brain are laid down early in life [for an extensive review of this work see Goy and McEwen (1980)]. (2) Lateral asymmetries are present in the neonate and can be predictive of asymmetry in the adult. (3) Many neonatal asymmetries are also sexually dimorphic [i.e., tail bias in the neonatal rat as shown by Ross, et al (1981)]. (4) The brain develops asymmetrically, that is, there seems to be a left-right maturational difference. (5) This maturational gradient may also differ between the sexes (i.e., be under neonatal hormonal control).

Diamond (1985) found that, in general, males from Day 6 of life to Day 870-876, have a thicker cortex on the right than the left in 41 of 42 cortical areas. However, these measures reached significance in only 30 of the 42 cases. The results of similar analyses in the female were even more tenuous. The left cerebral cortex was thicker than the right in 33 of 54 measures, but of these 54 measures, only 4 reached significance and 2 measures showed no asymmetry at all. The findings for sex differences and laterality effects in hippocampus paralleled those of the cortex (Diamond, 1985). In the male it was thicker on the right than the left except

at the age of sexual maturity (Day 90) at which time the asymmetry reversed. In females, the left hippocampus was thicker than the right, but again these were non-significant findings except for at one age point. Coincidentally, it was at age 90 days, the point at which the male bias is reversed.

Kolb, Sutherland, Nonneman and Whishaw (1982) examined cerebral hemispheric weight and cortical thickness in several animal species. In all cases, even in neonatal rats, they found the right cortex heavier and thicker than the left. No differences were found between the sexes, however. In addition, this right dominance was observed for the four different species they examined: rat, mouse, cat and rabbit. In the rat, they also examined the hippocampus but found no significant left-right asymmetry.

Van Eden, Uylings and Van Pelt (1984) found both laterality and sex differences in subareas of the medial prefrontal cortex and in the orbital prefrontal cortex, but found that these did not persist into adulthood.

Diamond (1985) also found, as early as 6 days of age, and as old as 108 days of age, a right dominant asymmetry in the area of the corpus striatum in the male rat. However, these asymmetries (right greater than left) were significant in only 2 of 10 cases and in one case the bias was reversed although also non-significant. Females were not examined, although this would seem to be a natural next step since many sex differences that are found in the adult asymmetries

are found in the caudate (both in its function as measured by rotation and its dopamine content).

Using ^{3}H-2-deoxy-d-glucose (as a measure of glucose utilization), Ross, et al (1981) attempted to identify metabolic asymmetries in the neonatal rat as well as examine sex differences in these metabolic asymmetries (which they suggest reflect maturational gradients). They found that 2-deoxy-d-glucose incorporation was higher on the right in the hippocampus and diencephalon but higher on the left in the cortex and medulla-pons. These asymmetries were observed only in females. In a later paper, a right-left asymmetry in males, the reverse of that found in females, did reach significance for the medulla (Ross, Glick and Meibach, 1982).

A study examining both the asymmetric function of the hypothalamus as well as a possible left-right maturational gradient was done by Nordeen and Yahr (1982). In a very well designed, well executed study, they implanted estrogen pellets in either the right or left hypothalamus of female rats 1-2 days postnatally. The implant in the right hypothalamus resulted in masculinized development of the female as determined by increased male sexual behavior in these females when hormonally stimulated. The implant in the left hypothalamus resulted in defeminized development (anovulatory sterility and lack of female receptive behavior in response to male stimulation). They suggest three hypotheses resulting from their study: (1) The two sides of the developing

brain may have different sensitivities to steroids. (2) The critical period for masculinization may be earlier than the critical period for defeminization (see also Goy and McEwen, 1980). (3) The two sides of the hypothalamus may pass through critical periods at different times.

CONFLICTING DATA, SOURCES OF VARIABILITY AND LACK OF STATISTICAL SUPPORT

As can be seen from this overview of the literature on sex differences both in laterality and development, a great many inconsistancies exist. One of the major sources of variability in results between laboratories would appear to be differences in the strain of animals used. Glick, when he found his right tail bias in infant females is in direct opposition to Denenberg who found a left tail bias. Glick used Sprague-Dawley rats; Denenberg used Purdue-Wistar. In all of her studies Diamond used Long-Evans rats, a sighted rat that may have a very different brain organization than the relatively non-sighted albinos used in other laboratories. To further illustrate this point, Yanai (1979a) published a paper showing clear differences in brain weight and cellular populations between Long-Evans and Wistar rats, but did not measure laterality differences.

A second issue that needs to be resolved when trying to understand this literature has to do with great differences in the methodologies employed when asking similar ques-

tions. How comparable is measuring cortical thickness to counting actual cell numbers; or measuring cell volume to dimensional measurements and tissue weights? In addition, many of these studies concentrate on measuring very tiny subareas of cortex as opposed to others that take the whole cortex weight measurements. Is it reasonable to compare 2-deoxy-d-glucose incorporation, which is a good indicator of neuronal activity to cell growth and neurogenesis as a measure of neural maturation? All of these techniques have demonstrated asymmetries in some brain areas and lack of asymmetries in others. Likewise, sex differences have been found in some cases but not in others. Many trends are reported but frequently cannot be supported statistically.

TRITIATED THYMIDINE INCORPORATION AS A MEASURE OF THE RATE OF NEURAL MATURATION: A POSSIBLE SOLUTION TO THE PROBLEM

A method which shows promise for answering these questions is the use of ^{3}H-thymidine. It is a technique used in many previous studies in a variety of species (Altman, 1963; Altman and Das, 1865; Camara and Harding, 1984) and to look at many different brain areas (Das and Altman, 1971; Mustari, Lund and Graubard, 1979; Yanai, 1979b; Bayer, 1983; Wyss and Sripanidkulchai, 1985). It has been shown to measure reliably "birthdays" of cells both prenatally if injected *in utero* and postnatally. Tritiated (^{3}H-) thymidine is a DNA precursor that, if injected, is incor-

porated into the DNA of cells undergoing development at the time of the injection. Most investigators use ^{3}H-thymidine in an autoradiographic paradigm, but it also lends itself to scintillation counting which yields reliable, quantitative information on the amount of development taking place over a particular period of time.

This project addressed some basic questions about the sexually dimorphic, left-right gradient of development hypothesis: (1) If animals are injected at varying ages during early development, will there be differential incorporation rates between the age groups? That is, what is the developmental sequence of neural growth? (2) Does the amount of neural growth (as measured by the amount of thymidine incorporation) differ between the left and right sides of the brain at any developmental time? (3) Does the amount of neural growth differ between males and females, and, is this difference found within any particular age group?

METHODS

ANIMALS

Twelve adult Sprague-Dawley rats were bred in the laboratory in groups of two females to one male. When the females were visibly pregnant, they were separated from the males and housed singly. On the day of birth (Day 0), each litter was culled to 8 pups, 4 males and 4 females, to ensure as consistent an amount of maternal nutrition and contact as possible among the different litters. If a litter did not have the required size and sex distribution of pups, it was eliminated from the study. For example, a litter of six males and two females would not be used even though it had eight pups because of the uneven sex distribution.

On the day of birth, each litter was randomly assigned to one of four groups. Group 1 received injections of ^{3}H-thymidine (5μCi/g body weight) on days 1 and 2 of postnatal life (Day 1-2); group 2 received its injections on postnatal days 7 and 8 (Day 7-8), and groups 3 and 4 received injections on days 14 and 15, and 21 and 22, respectively (Day 14-15; Day 21-22). The four groups were further subdivided into subgroups of males and females resulting in 8 experimental groups (Day 1-2 males, Day 1-2 females, Day 7-8 males, Day 7-8 females, etc.). Group size ranged from 8-12 animals per group; a total of 85 animals were studied. All litters were weaned on day 23 and housed with same sex littermates

(4 per cage) until day of sacrifice (postnatal day 50-55). Three animals who had received injections died before the time of sacrifice.

Animals were housed in standard laboratory tub cages in a temperature controlled colony room (37°C) on a 14:10 reverse light/dark cycle (14 hrs on/10 hrs off). All animals had access to food (Purina Rat Chow) and tap water *ad lib*.

INJECTIONS

All experimental animals were injected subcutaneously on 2 consecutive days (as determined by group assignment) with an aqueous solution of (methyl-^{3}H) thymidine (Amersham; specific activity 40 Curies/mmol). The concentration injected was 5μCi/g body weight (Bayer, 1983).

The procedure for injections was first to remove the whole litter from the dam to a separate cage. Each pup was weighed, injected with a calculated amount of ^{3}H-thymidine, and returned to the holding cage. The whole litter was then returned *en masse* to the dam. The entire procedure took approximately 30 minutes per litter. After the injections were completed and the litters weaned, the animals were left undisturbed except for daily routine maintenance for the rest of the experiment.

DISSECTIONS

Brain dissections for all experimental animals took place on postnatal day 50-55, irrespective of group assignment or day of injection. Dissections were performed during the day, while the animals were in their dark phase. Animals were weighed and then sacrificed by ether anesthesia overdose. The brain was immediately removed, weighed and dissected over ice. The left and right halves of the cortex, caudate, thalamus and hypothalamus were removed, labelled and separately wrapped in aluminum foil for processing at a later date. All tissue specimens were then placed in a commercial plastic freezer bag and frozen at -80°C until time of assay.

ASSAY

Tissue samples were processed for analysis in two separate ways, both involving scintillation counting. This is the counting of the emission of β particles released from the compound during radioactive decay. The first step for both procedures involved weighing the tissue sample and homogenizing it in 9 times its weight in grams of ice cold distilled water.

The first procedure involved taking a small aliquot (1/20 th of the total volume) of this homogenate, transferring it into 15 mls of a counting solution (Aquasol; New England Nuclear) and counting the sample for 10 minutes (Packard Scintillation Counter, Model 2009; Counting Ef-

ficiency of 25-28%). The second procedure involved taking an aliquot of one fifth of this homogenate and processing it according to a method used by Shah and Johnson (1983). The difference between these two procedures is that the first method counts all of the ^{3}H-thymidine or metabolites in the tissue sample, which could include non-incorporated thymidine such as that held in amino acid storage pools. The second method, through a series of washings, extracts all of the non-incorporated thymidine from the sample and measures only that which is incorporated into the DNA of the developing cells. The assay procedure to determine thymidine incorporation involved four steps. Two and a half milliliters of ice cold 10% trichloroacetic acid (TCA) was added to the initial 1/5 th aliquot of homogenate. This was centrifuged for 5 minutes at 13,000 rpm, at a temperature of 5° C. The supernatant was discarded and the pellet resuspended with another 2.5 ml cold TCA, and centrifuged. After the supernatant was discarded, the pellet was resuspended in 3.0 ml of 95% ethyl alcohol at room temperature and centrifuged for 5 minutes, also at room temperature. Again the supernatant was discarded, and the pellet was resuspended in 2.0 ml of room temperature 5% TCA. After resuspension, the mixture was heated in a 90° C oven for 20 minutes in order to hydrolyze the DNA and RNA, then centrifuged at room temperature for 10 minutes. An aliquot of 1/4 th of the volume of the supernatant was transferred to a scintillation vial with 15 ml of Aquasol and counted for 10 minutes.

The counts obtained from these samples (minus background counts) were divided by the proportion of the tissue in the sample, and divided by the number of minutes counted to give the total number of counts in the sample of homogenate (total activity). This number was further divided by the weight of the tissue sample to yield a measure of specific activity: the counts per minute per milligram of tissue.

As all animals received the same dosage, the counts/mg can be directly compared rather than having to convert them to quantities of thymidine.

DATA ANALYSIS

Each of the four brain structures were analyzed independently of each other. Each structure was analyzed for (1) Sex differences within each age group for total amount of thymidine incorporation (i.e., left side + right side) for a particular structure; (2) Left vs right differences in thymidine incorporation for each structure within an age group, as well as possible sex differences in left/right incorporation within an age group; (3) Laterality differences within an age group and by sex regardless of side of preference (i.e., high side incorporation vs low side incorporation) and (4) A sex x age interaction for total incorporation (left side + right side) to determine if there is a developmental trend in thymidine incorporation and whether or not this trend differs between the sexes. These analyses were performed for all four structures (Hays, 1973).

RESULTS

The four areas of the brain examined in this study (caudate, thalamus, hypothalamus and cortex) were analyzed separately. The basic measurement obtained from scintillation counting was counts/min/mg tissue (CPM/mg). This was interpreted as the number of counts obtained in a sample, divided by the number of minutes the sample was counted, divided again by the weight of the tissue sample in milligrams. This basic unit of measurement was the standard for all left-right and high-low comparisons. The high versus low comparison was CPM/mg high side vs CPM/mg low side regardless of the left or right dominance of the result. Another measure used in this analysis was Total Incorporated CPM/mg. This was calculated as CPM/mg left side + CPM/mg right side for a particular structure. Percent Incorporation was defined as (Total Incorporated CPM/mg divided by Total Sample CPM) x 100. Total Sample CPM was the CPM measured in the whole tissue sample homogenate. Percent Incorporation gave a measure of what percentage of ^{3}H-thymidine was actually incorporated into the tissue sample versus what percentage of the compound was not incorporated (i.e., extent of tissue incorporation activity). For all analyses, groups were defined as the age at time of injection. Group 1 received injections on postnatal days 1 and 2; group 2 received injections on days 7 and 8; groups 3 and 4 received injections on days 14 and 15 and days 21 and 22 respectively.

CAUDATE

A group x sex ANOVA for the dependent variables Total Incorporated CPM/mg and Percent Incorporation yielded significant main effects of group [Total Incorporated CPM/mg: $F(3,84)=28.047$, $p<.001$; Percent Incorporation: $F(3,84)=33.135$, $p<.001$; Means and standard deviations for these data are listed in appendices 1 and 2]. Although the females had higher levels of labelling than the males at all four ages, the differences were not significant and the sexes are combined in Figure 1. When CPM/mg left side and CPM/mg right side were used as within subjects factors in a group x sex ANOVA no significant within subjects main effects or interactions were found. However, using CPM/mg high and CPM/mg low as the within subjects factors in the group x sex ANOVA gave a significant within subjects effect [$F(1, 85)=92.154$, $p<.001$] as well as a group x within subjects interaction [$F(3,85)=6.366$, $p<.001$]. The high values were significantly higher than the low values at all four developmental ages (see Figure 2).

CORTEX

Group x sex ANOVAS for dependent variables Total Incorporated CPM/mg and Percent Incorporation again yielded significant main effects of group [Total Incorporated CPM/mg: $F(3,84)=36.515$, $p<.001$; Percent Incorporation: $F(3,84)=52.917$, $p<.001$; see Appendices 3 and 4 for descriptive statistics of these variables]. Again, there was no significant main effect

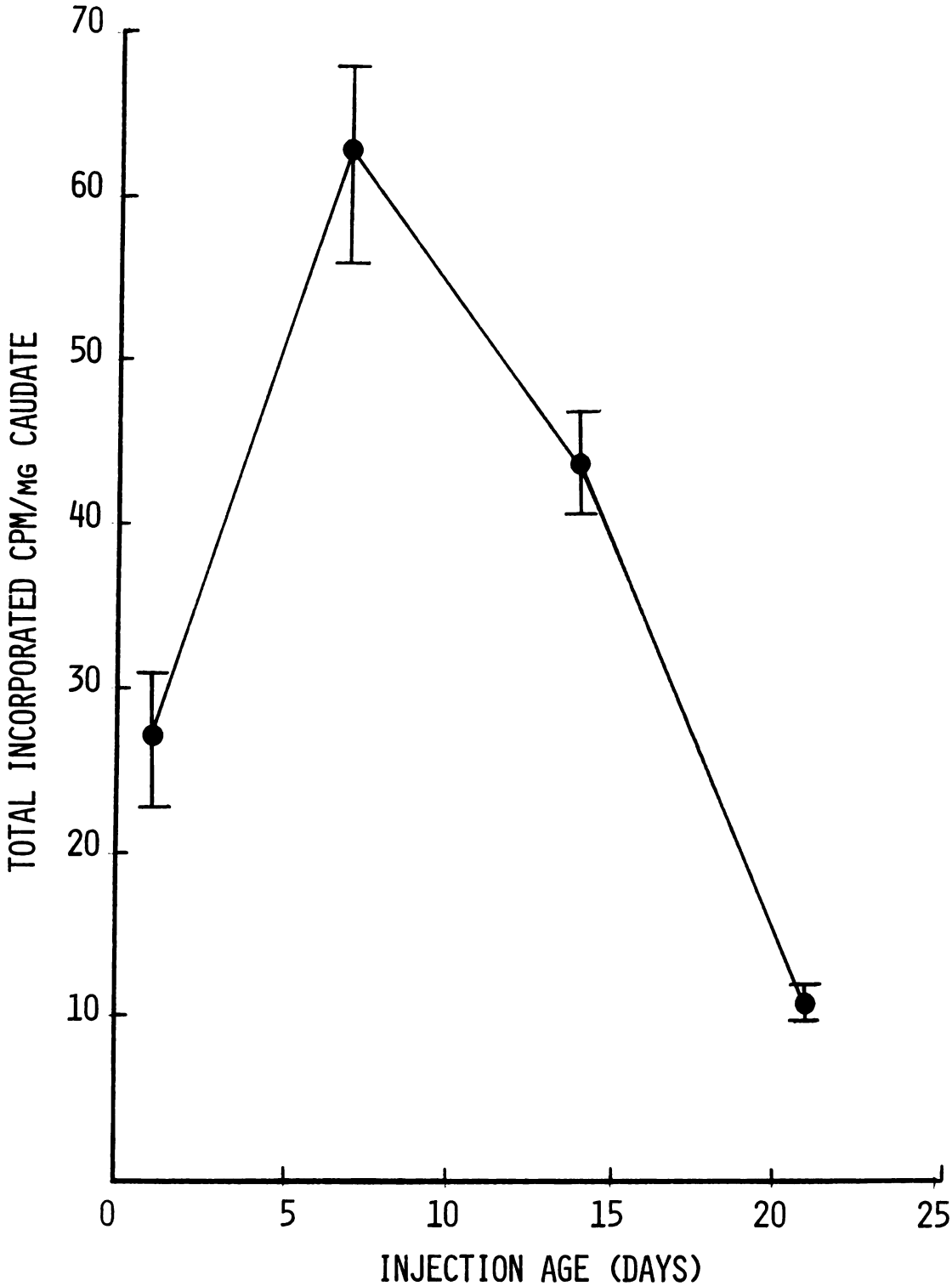

FIGURE 1. CHANGES IN THE AMOUNT OF TOTAL THYMIDINE INCORPORATION IN THE CAUDATE AS A FUNCTION OF AGE AT TIME OF INJECTION.

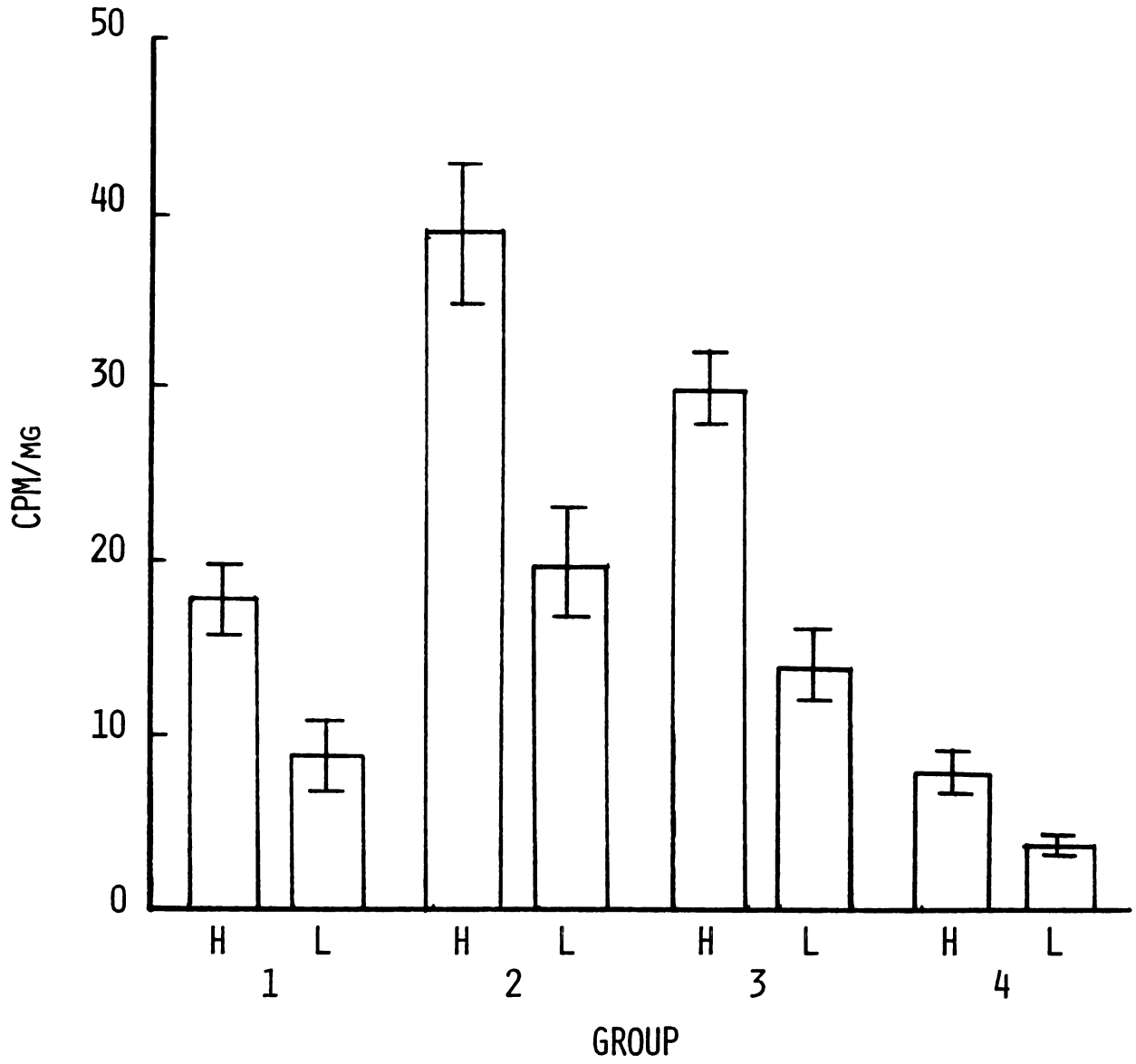

FIGURE 2. COMPARISON OF HIGH SIDE VS LOW SIDE THYMIDINE INCORPORATION IN THE CAUDATE FOR THE FOUR AGE GROUPS EXAMINED. ALL COMPARISONS ARE STATISTICALLY SIGNIFICANT AT ($P < .01$).

of sex or a group x sex interaction, so the sexes were combined for further analysis (see Figure 3). As was found in the caudate, CPM/mg high and CPM/mg low as within subjects factors were significant as a within subjects main effect [$F(1,85)=75.827$, $p<.001$] as well as a significant group x within subjects interaction [$F(3,85)=7.136$, $p<.001$]. Again, all high values were significantly higher than low values (see Figure 4). No left-right differences were found with CPM/mg left and CPM/mg right as within subjects factors.

THALAMUS

The data for the thalamus parallel the findings for the caudate and the cortex. Means and standard deviations for the dependent variables Total Incorporated CPM/mg and Percent Incorporation are listed in Appendices 5 and 6. A group x sex ANOVA for Total Incorporated CPM/mg gave a main effect for group [$F(3,83)=24.634$, $p<.001$] as did the ANOVA for Percent Incorporation [$F(3,83)=30.322$, $p<.001$]. Again, the sexes were combined (see Figure 5). No left-right differences were found, but the CPM/mg high and CPM/mg low main effect and interaction with groups were still evident [$F(1,84)=123.922$, $p<.001$; $F(3,84)=11.757$, $p<.001$ respectively; see Figure 6].

HYPOTHALAMUS

The hypothalamus results differed from the other struc-

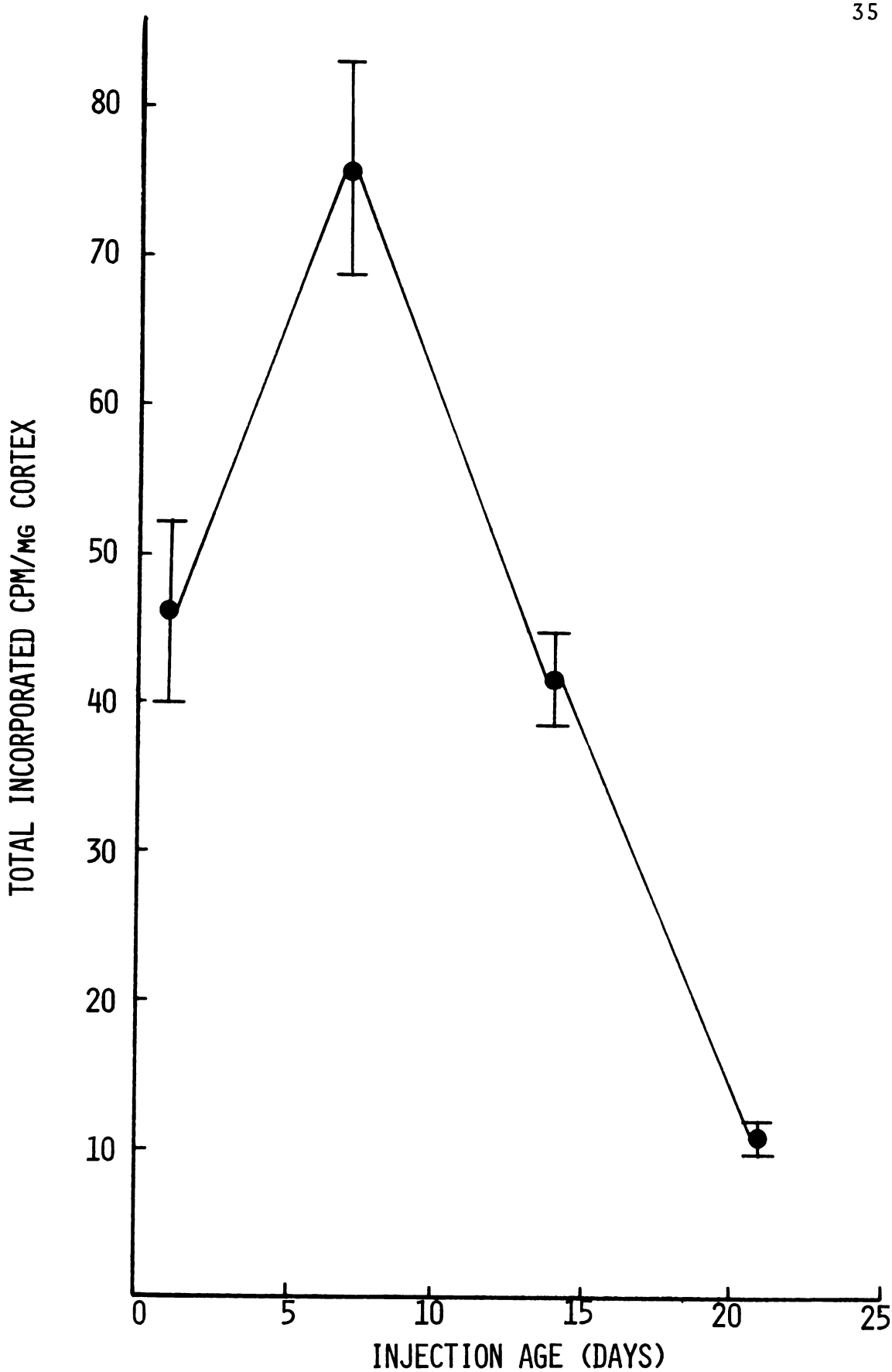

FIGURE 3. CHANGES IN THE AMOUNT OF TOTAL THYMIDINE INCORPORATION IN THE CORTEX AS A FUNCTION OF AGE AT TIME OF INJECTION

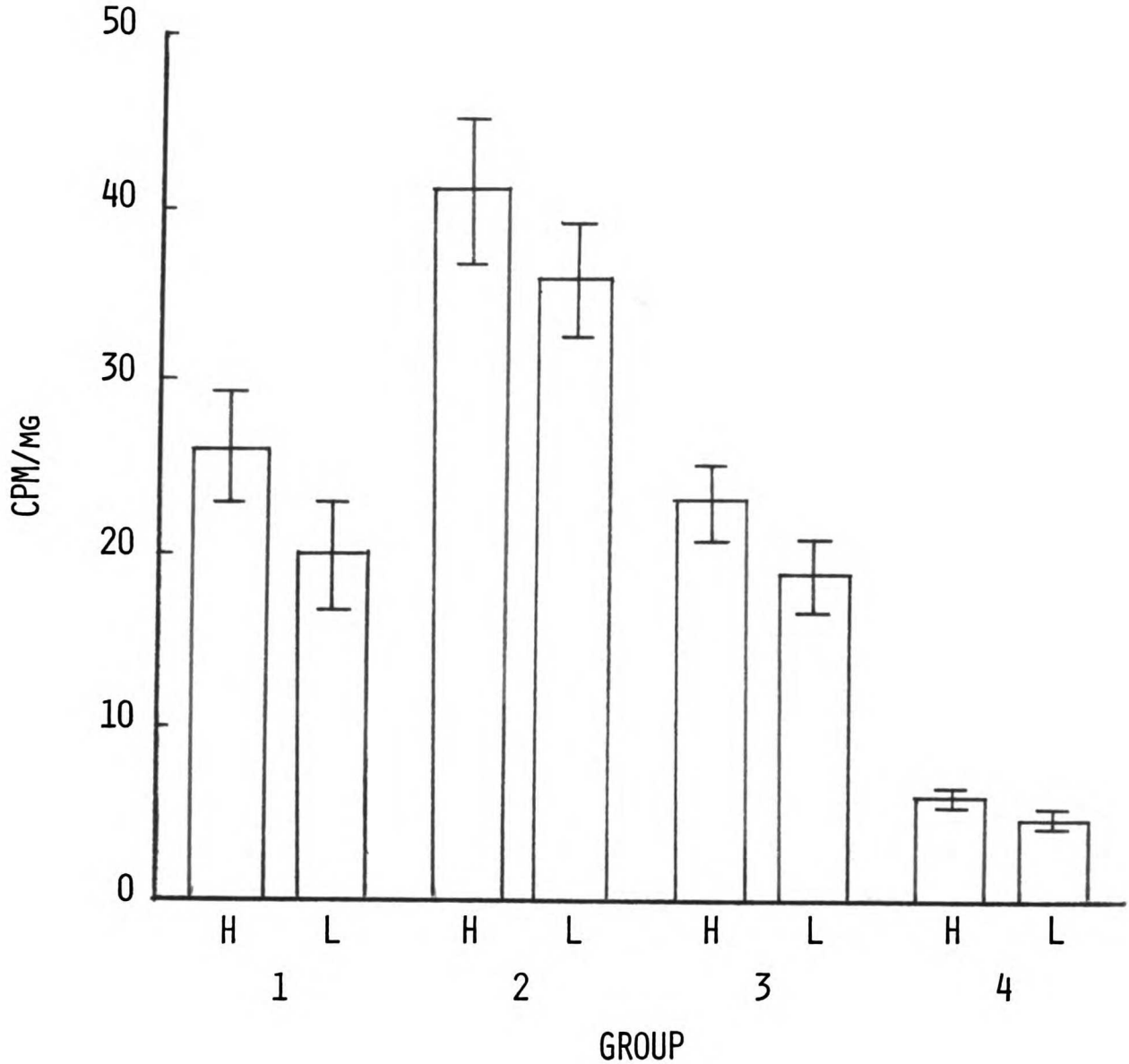

FIGURE 4. COMPARISON OF HIGH SIDE VS LOW SIDE THYMIDINE INCORPORATION IN THE CORTEX FOR THE FOUR AGE GROUPS EXAMINED. ALL COMPARISONS ARE STATISTICALLY SIGNIFICANT AT ($P < .01$).

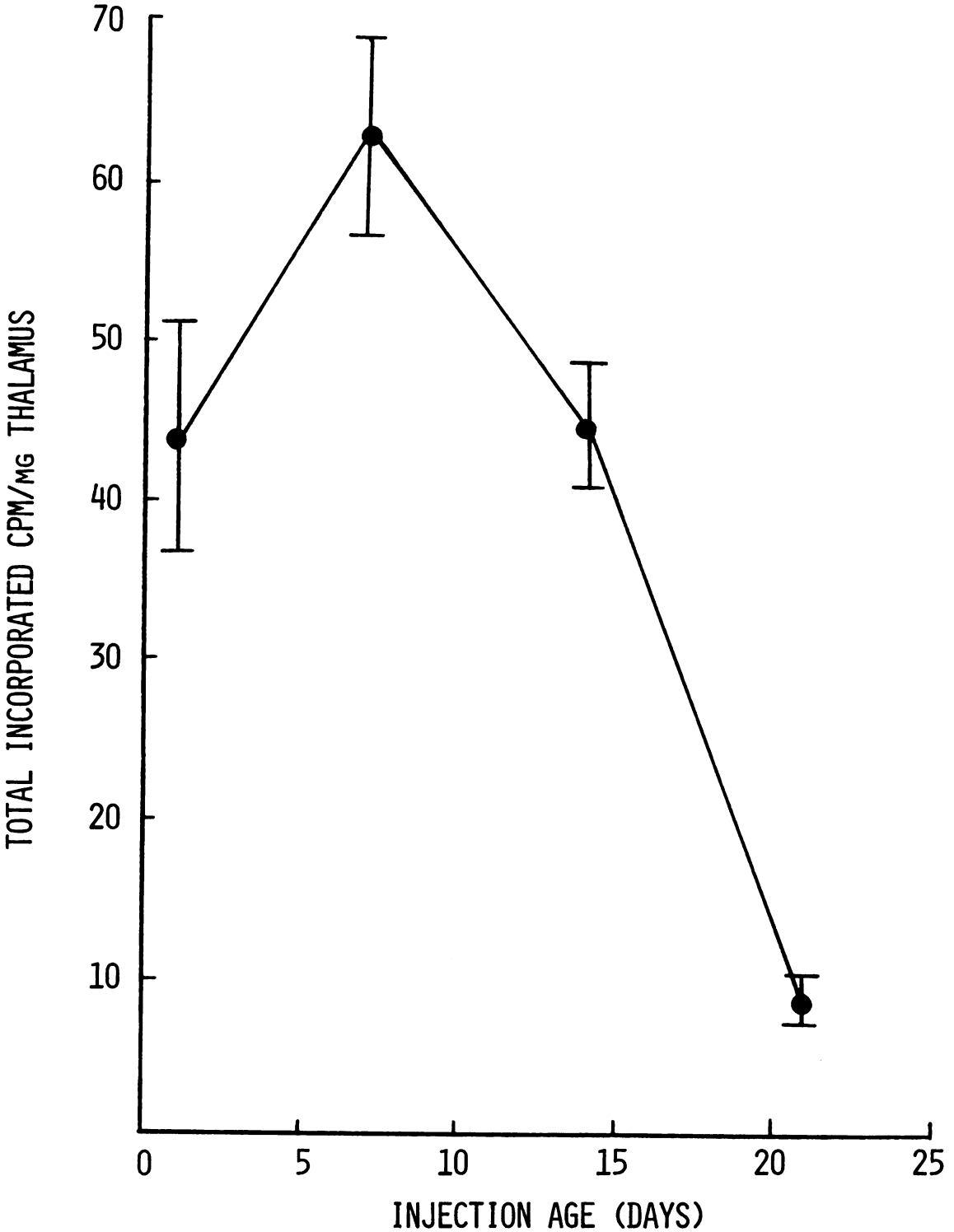

FIGURE 5. CHANGES IN THE AMOUNT OF TOTAL THYMIDINE INCORPORATION IN THE THALAMUS AS A FUNCTION OF AGE AT TIME OF INJECTION.

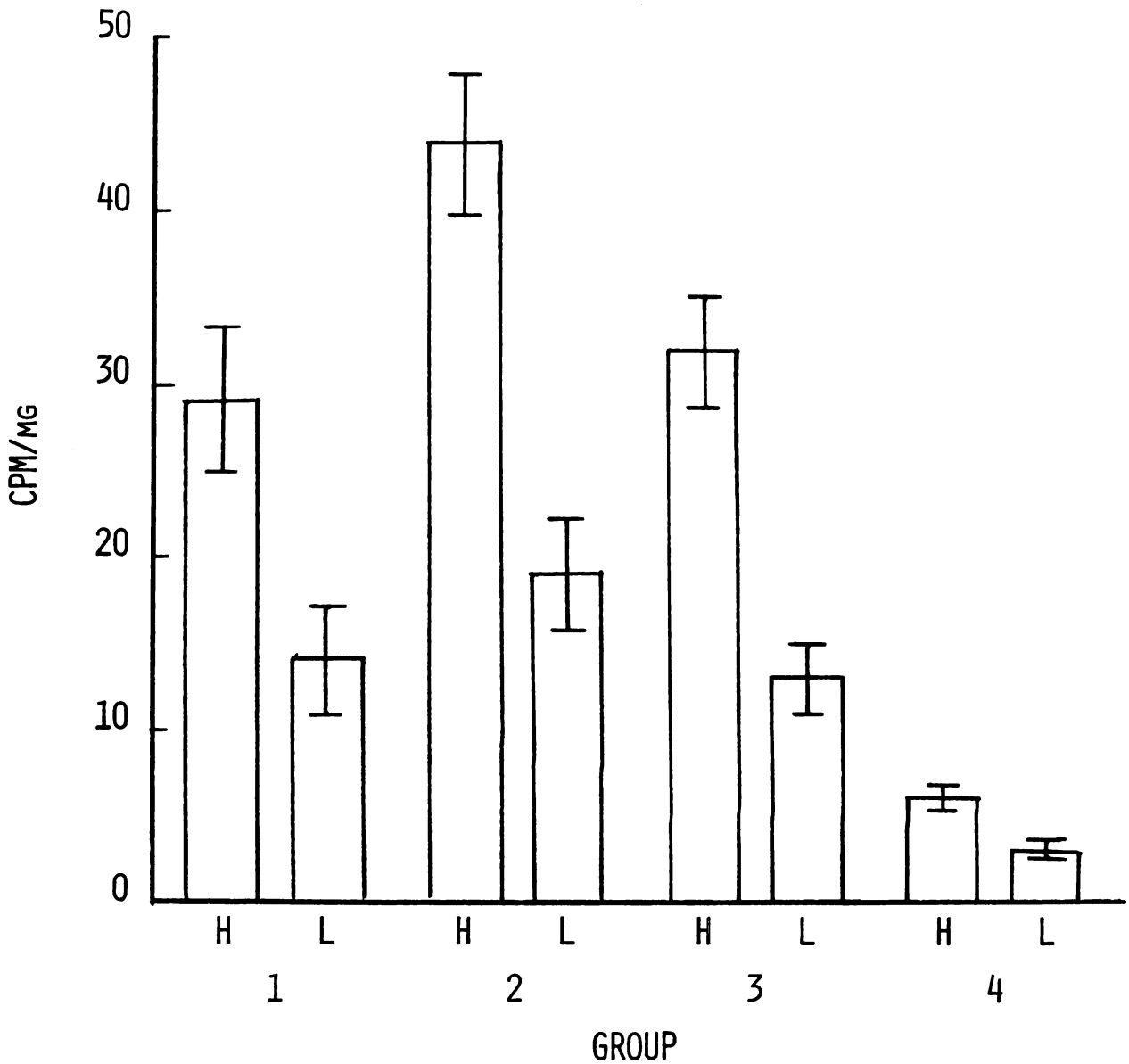

FIGURE 6. COMPARISON OF HIGH SIDE VS LOW SIDE THYMIDINE INCORPORATION IN THE THALAMUS FOR THE FOUR AGE GROUPS EXAMINED. ALL COMPARISONS ARE STATISTICALLY SIGNIFICANT AT ($P < .01$).

tures. The group x sex ANOVA for the dependent variable Total Incorporated CPM/mg showed a significant main effect for group [$F(3,83)=9.186$, $p<.001$], in addition to a significant group x sex interaction [$F(3,83)=2.816$, $p<.04$]. These data are shown in Figure 7. The group x sex ANOVA for Percent Incorporation did not show either a main effect for sex or a group x sex interaction, but did maintain the group main effect [$F(3,83)=8.331$, $p<.001$]. No sex differences were found with regards to CPM/mg high and CPM/mg low but as with the other structures, a significant within subjects main effect [$F(1,84)=62.763$, $p<.001$] as well as a significant within subjects x group interaction was found [$F(3,84)=4.676$, $p<.001$]. Again, all CPM/mg high vs CPM/mg low comparisons were significant (see Figure 8). Descriptive statistics for Total Incorporated CPM/mg and Percent Incorporation are listed in Appendices 7 and 8 respectively.

PERCENT INCORPORATION

No significant sex effects or group x sex interactions were found for any structure for the dependent variable Percent Incorporation, so the sexes were combined in each group for further analysis. As can be seen in Figure 9, the shapes of the curves for all four structures were similar, showing highest incorporation at the earliest injection age.

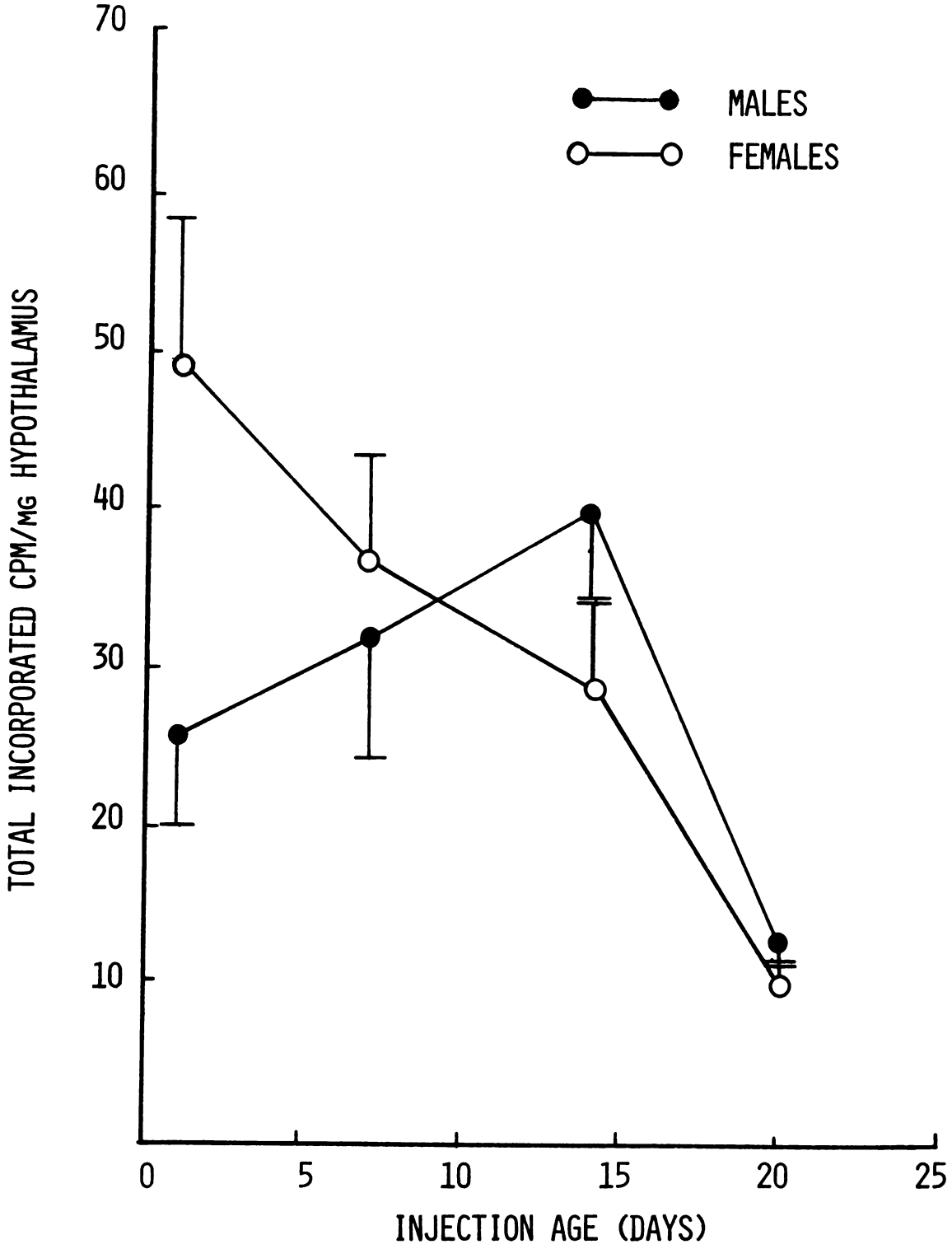

FIGURE 7. CHANGES IN THE AMOUNT OF TOTAL THYMIDINE INCORPORATION IN THE HYPOTHALAMUS AS A FUNCTION OF AGE AT TIME OF INJECTION.

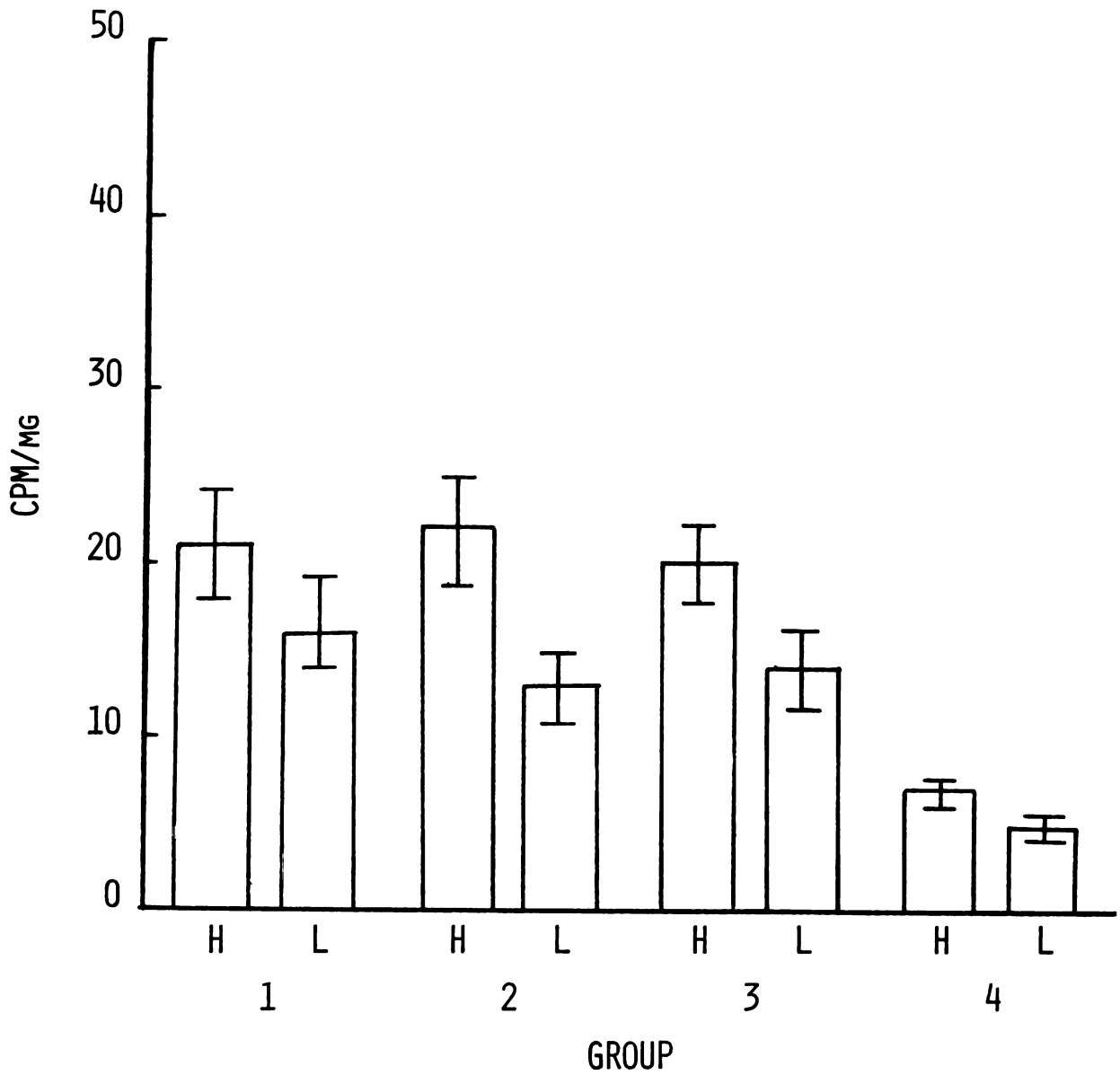

FIGURE 8. COMPARISON OF HIGH SIDE VS LOW SIDE THYMIDINE INCORPORATION IN THE HYPOTHALAMUS FOR THE FOUR AGE GROUPS EXAMINED. ALL COMPARISONS ARE STATISTICALLY SIGNIFICANT AT ($P < .01$).

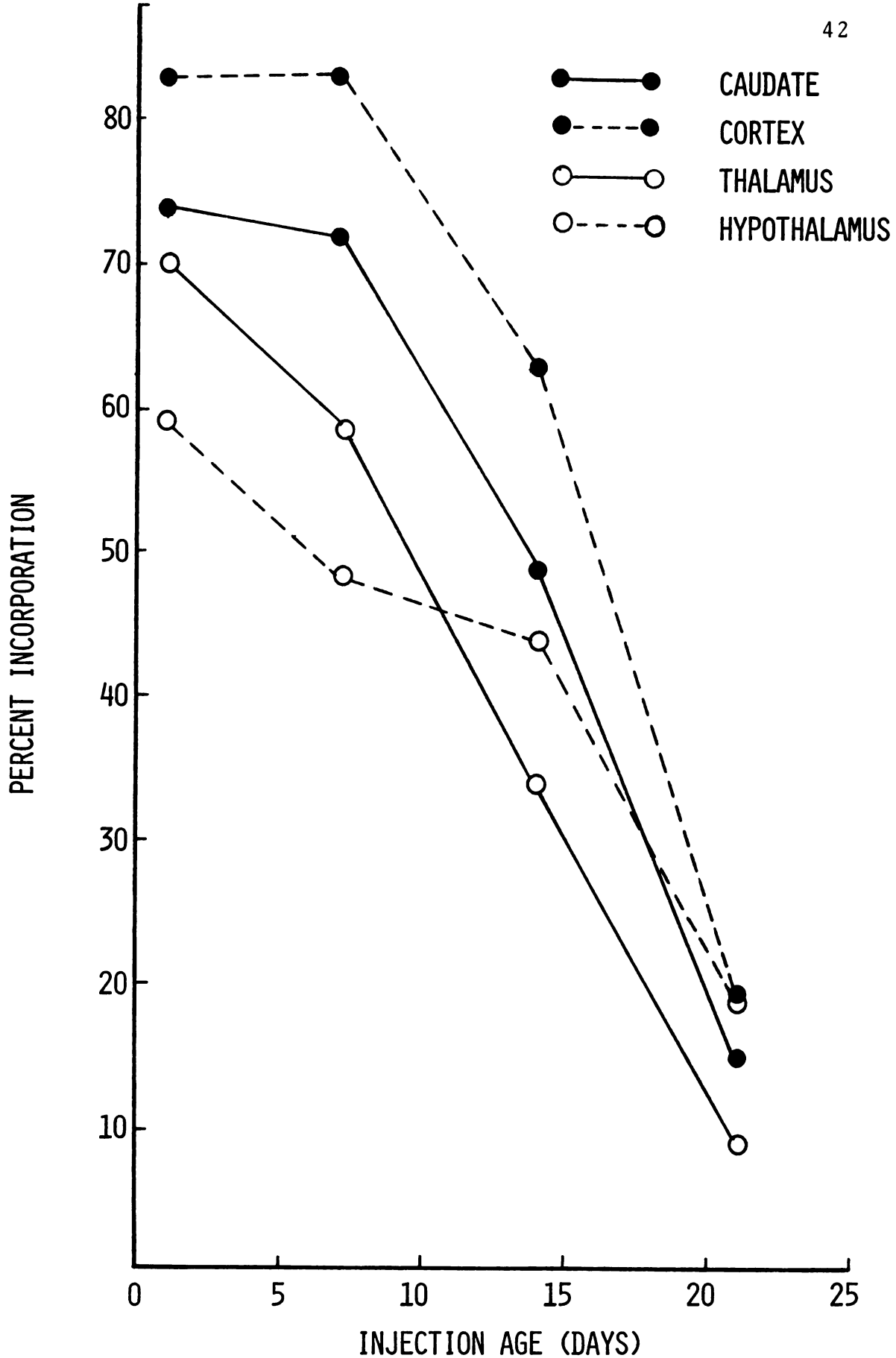

FIGURE 9. PERCENT INCORPORATION AS A FUNCTION OF AGE AT TIME OF INJECTION FOR THE FOUR STRUCTURES EXAMINED.

LEFT VERSES RIGHT INCORPORATION

The actual distribution of CPM/mg left vs CPM/mg right did not differ from chance as measured with a Chi-square for the groups as a whole, or by age or sex. When the data from all structures were collapsed over sex and group, the distribution of left greater CPM/mg vs right greater CPM/mg were as follows: In the caudate the left side is greater 42.4% of the time while the right side is greater 57.6% of the time. The left-right distribution in the cortex is 49.4% left greater vs 50.6% right greater. The thalamus is distributed 50.6% left vs 49.4% right, while the hypothalamus is distributed 53.6% left greater vs 46.4% greater. None of these differences were statistically significant.

WHOLE BRAIN ANALYSIS

When the Total Incorporated CPM/mg for all four structures were combined by group, a significant main effect of group was found [$F(3,337)=76.723$, $p<.001$]. The same group effect was found for Percent Incorporation [$F(3,337)=89.711$, $p<.001$]. No significant group x sex interactions or main effects of sex were found.

DISCUSSION

SUMMARY OF RESULTS

Group effects: The results of this study indicate that the time course of neural maturation of the brain does not follow a linear progression in the neonatal rat. Of the time points examined in this study, the extent of incorporation was greatest, as indicated by the increased amount of thymidine incorporation, at 7-8 days postnatally. By postnatal days 21-22, almost no neural development was observed. In addition, this course of development was consistent throughout the four structures examines.

Laterality effects: Laterality, as a population effect, has traditionally been defined as a "left-right" effect. In this study, no clear left-right patterns in development were found. However, when the side of the brain with the greater amount of thymidine incorporation was compared to the side with lower incorporation, the difference was always significant (see Figures 2,4,6 and 8). It appears that a laterality effect does, in fact, exist but it was not evident at a population level; that is, there is no consistent left-right relationship for all the animals as a group. Since no left-right patterns were evident within the groups, it is possible that the laterality in neural development, at least in the rat, may be a phenomenon peculiar to each individual.

The finding of no clear left vs right differences within groups, is in contrast to the human literature in which there is a clear left-right difference evident during development (Wada, Clarke and Hamm, 1975; Chi, Dooling and Gilles, 1977). It is of interest that the left-right asymmetries found in development in the human are in brain areas which control highly lateralized functions (i.e., language and handedness). It is likely that a consistent left-right population asymmetry in the brain may only be evident when it is involved with behaviors which also show this asymmetry.

The question could be raised as to why the caudate did not show a left-right asymmetry in development when in fact, it is primarily responsible for lateralized behavior in the adult rat (Glick, et al, 1977). One possibility is that the left-right differences appear later in life. Since the behavioral asymmetry seen in the adult rat is under gonadal hormonal control (Robinson et al, 1981), it is conceivable that a left-right asymmetry might appear around the time of sexual maturity in the animal when the activational effects of hormones are evident. Furthermore, the evidence for left-right asymmetries in the rat as a population effect is not unequivocal. It is a very small effect based on an exceptionally large sample size and may actually be an individual effect as the percent of animals with a left preference is nearly identical to the percent of animals with a right preference (Glick and Ross, 1981).

Sex effects: Although a trend was evident for females to have greater amounts of incorporation than males (12 out of 16 cases), there were no significant sex differences at any age level or for any structure. Additionally, a comparison of male vs female total thymidine incorporation for the whole brain (i.e., male vs female collapsed over structure) also showed no sex difference at any age.

RELATION TO PREVIOUS WORK

The group effect for all four brain areas was consistent with Yanai's (1979b) findings for group differences in total thymidine incorporation for specific cortical areas. Both studies show the greatest amount of incorporation on Day 7. In terms of a sex difference in maturation, a similar conclusion was reached in each case, but by different means. In this study, the trend of females having more labelling than males at each time suggests that females may be undergoing more development than males; that is, males show a maturational delay.

Yanai's study suggested that males had greater amounts of thymidine incorporation than females (though results were non-significant). But, since females in his study showed the same total number of labelled cells (by autoradiography) he concluded that female development possibly took place prenatally while the males were still in the process of development postnatally, again, suggesting a male maturational delay.

A possible reason for these contradictory conclusions is a difference in the strain of rat used (Wistar vs Sprague-Dawley), a point discussed in the introduction. To further investigate this discrepancy between Yanai's findings and mine, the next step would be to inject animals prenatally. An injection given to a pregnant mother has been shown to reliably label pup development (Bayer, 1983). This would answer the question as to what sex differences in development are occurring in utero.

Any failure to find left-right differences in rates of neural growth does not preclude laterality effects in development. As Ross, et al (1982) conclude in their study, lateral effects in 2-deoxy-d-glucose utilization change over time. These results indicate asymmetries are present in the brains of individuals without respect to side while clear left-right differences of groups are not always evident.

In this study, clear laterality effects have been found in terms of high side vs low side amounts of thymidine incorporation. The finding of a high-low dichotomy as opposed to a consistent left-right dichotomy in brain development does not rule out the likelihood of there being a functional left-right difference without an underlying left-right difference in neural structure. Most of the sex differences in laterality have been investigated in the adult animal, and failure to find these effects in neural growth in the neonate does not mean that these findings in the adult are invalid.

CLINICAL IMPLICATIONS OF SEX DIFFERENCES IN LATERALITY

Dyslexia: Dyslexia is a disorder which affects significantly more males than females (Witelson, 1977) and some investigators have postulated that it is due to the effects of testosterone on the brain, both pre- and postnatally. Some researchers propose that testosterone slows the maturation of the left cerebral cortex in males, which may predispose them to developmental language disorders (Geschwind and Behan, 1982). This would appear to fit with some of the current animal models of male CNS developmental delays, but other investigators do not support this theory (Galaburda and Kemper, 1977; Witelson, 1977). They propose, instead, that developmental dyslexia is the result of neural abnormalities in the left hemisphere of the male, specifically, cytoarchitectural abnormalities of the left temporal speech areas.

The main proponents of the male developmental delay theory are Taylor (1969) and Ounsted and Taylor (1972) who studied patients with temporal lobe epilepsy. Their work proposes two basic points: (1) injuries which can result in a seizure will affect the hemisphere which is less functionally active than the other and (2) males are more likely to experience seizures associated with left hemisphere damage than females. This indicates that the left hemisphere of males is less functionally active and therefore at risk for a potential seizure producing injury for a longer period of

time than females.

Geschwind and Behan (1982) also propose that this testosterone modulated, left hemisphere delay in maturation is responsible for a greater degree of left handedness in males, and additionally, they have shown higher rates of left handedness among dyslexics.

Autoimmunity: Another aspect of the left hemisphere delay theory in males involves the effects of testosterone on the developing immune system. Geschwind and Behan (1982) state:

> "During fetal life the immune system is also maturing. Testosterone has important suppressive effects on the thymus both in utero and after birth (Dougherty, 1952; Frey-Wettstein and Craddock, 1970). Thus, during periods of increased testosterone effects on left brain development, maturation of the immune system is also likely to be affected. There are studies that support the hypothesis that the fetal thymus controls development of lymphocytes which are responsible for recognition of self-antigens and thus for prevention of autoimmunity (Littman, 1980; Rocklin, Kitzmiller and Kay, 1979). Suppression of thymic growth during fetal life might therefore favor the development of autoimmunity in later life." (p.5099)

Their investigations have shown that left handers (individuals with right hemisphere dominance due to left hemisphere maturation delay) have a higher incidence of immune disorders than right handers.

In addition to these findings linking cortical developmental delays and altered immune functions, other work has demonstrated the role of the neocortex in regulating a com-

ponent of the immune response (Renoux, Biziere, Renoux, Guillaumin and Degenne, 1983). Partial (1/3) ablations of the left cortex in rats resulted in decreased numbers as well as decreased effectiveness of spleen T-cells, a major component of the immune system. An identical lesion of the right hemisphere resulted in the opposite response; that is, increased numbers and increased effectiveness of T-cells. Unfortunately, sex differences were not investigated in this study.

Mast cells are another component of the immune response. These cells contain histamine and when they are located peripherally (i.e., not in the central nervous system) they contribute to allergic reactions and anaphylaxis (Goldschmidt, Hough, Glick and Padawer, 1984). Centrally, the role of mast cells is not clear but histamine has been implicated as a neurotransmitter in the brain. Goldschmidt et al (1984) have found that not only are mast cells asymmetrically distributed in the brain, but this asymmetry is sexually dimorphic. Mast cells were found to be in greater numbers in females than in males, and greater in the left thalamus than the right. The clinical implications of this study are not yet clear.

Cerebral Infarction: Left vs right cortical ablations similar to those of Renoux et al (1983) have also been shown to have profound behavioral and biochemical effects. Right hemisphere lesions in rats produced by middle cerebral artery

ligation (Robinson, 1979) or by suction ablation (Pearlson and Robinson, 1981) result in an increase in spontaneous hyperactivity as well as a bilateral decrease of both norepinephrine in the cortex and locus coeruleus and dopamine in the substantia nigra. Identical lesions of the left hemisphere produced none of these effects. In a third study involving more circumscribed lesioning (Kubos and Robinson, 1984) the effect of right hemisphere lesions remained the same, but a decrease in activity with left hemisphere lesions was found. Catecholamine levels with left hemisphere lesions remained unaltered. This paradigm is now being used as an animal model for cerebral infarctions and stroke. Unfortunately these studies used only males. It is unclear whether or not females react similarly to males in response to cerebral injury.

CONCLUSION

The study of laterality and sex differences often results in creating more questions than it answers. This is due in part to conflicting methodologies, differences in species and strains used and the great amount of variability inherent in the animal models used. To approach these two research areas from a developmental point of view can lead to important discoveries which could shed light on the neural substrates of adult brain and behavior relationships as well as answer questions concerning the incidence of disorders

which seem to be influenced by gonadal hormone effects on brain laterality.

By examining brain development in the normal animal we can investigate two important concepts: (1) can injuries early in life to one hemisphere or the other result in deficits in the adult, and can the effect of the gonadal hormone mileau in the neonate prevent or accentuate the disorder and (2) are predispositions to disease in later life (such as autoimmune disease or susceptibility to stroke) set down in the neural structure of the prenatal or pubescent animal, and can these in turn be prevented or even treated with gonadal steroids.

The present study would seem to eliminate the period from birth to Day 22 as crucial in the establishment of sex differences in group left-right asymmetries. The next step would be to study the same effect (i.e., thymidine incorporation in developing cells) in both the prenatal animal and in the animal as it reaches sexual maturity. In both cases the animal is in a stage in which the hormonal status of the animal has a direct and profound effect on the brain.

If these fail to show any sex differences or left-right effects on neural growth, it would appear that the development of an animal model for left-right differences in the brain will be a long and arduous process.

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APPENDIX 1

Means and standard deviations for the dependent variable: Total Incorporation CPM/mg for the caudate

DAYS OF INJECTION	N	SEX	CPM/mg MEAN	CPM/mg S.D.
1-2	8	M	26.289	13.200
	8	F	27.088	18.884
7-8	11	M	55.000	26.734
	12	F	69.657	34.543
14-15	11	M	43.552	20.075
	12	F	43.711	12.165
21-22	11	M	10.955	5.269
	12	F	11.735	4.642

APPENDIX 2

Means and standard deviations for the dependent variable: Percent Incorporation for the caudate

DAYS OF INJECTION	N	SEX	MEAN	S.D.
1-2	8	M	75.056	29.278
	8	F	72.891	26.056
7-8	11	M	66.945	28.809
	12	F	77.398	32.246
14-15	11	M	45.211	19.627
	12	F	50.343	13.951
21-22	11	M	15.127	7.002
	12	F	15.819	5.705

APPENDIX 3

Means and standard deviations for the dependent variable: Total Incorporation CPM/mg for the thalamus

DAYS OF INJECTION	N	SEX	CPM/mg MEAN	S.D.
1-2	8	M	52.803	27.800
	8	F	34.231	23.050
7-8	11	M	58.688	22.203
	11	F	66.489	35.095
14-15	11	M	43.387	19.037
	12	F	46.603	21.401
21-22	11	M	7.868	4.489
	12	F	9.343	5.623

APPENDIX 4

Means and standard deviations for the dependent variable Percent Incorporation for the thalamus

DAYS OF INJECTION	N	SEX	MEAN	S.D.
1-2	8	M	82.359	33.733
	8	F	57.701	34.254
7-8	11	M	57.460	23.606
	11	F	60.922	33.819
14-15	11	M	32.533	13.865
	12	F	35.281	14.121
21-22	11	M	8.486	5.205
	12	F	9.486	5.783

APPENDIX 5

Means and standard deviations for the dependent variable: Total Incorporation CPM/mg for the hypothalamus

DAYS OF INJECTION	N	SEX	CPM/mg MEAN	S.D.
1-2	8	M	25.515	16.146
	8	F	49.041	24.870
7-8	11	M	32.250	26.811
	12	F	37.150	21.840
14-15	11	M	39.583	20.674
	11	F	28.535	18.075
21-22	11	M	12.683	6.116
	12	F	9.911	4.198

APPENDIX 6

Means and standard deviations for the dependent variable: Percent Incorporation for the hypothalamus

DAYS OF INJECTION	N	SEX	MEAN	S.D.
1-2	8	M	48.083	30.653
	8	F	69.600	34.157
7-8	11	M	44.985	36.270
	12	F	50.840	32.063
14-15	11	M	52.173	29.675
	11	F	35.881	21.551
21-22	11	M	21.863	10.954
	12	F	15.751	7.190

APPENDIX 7

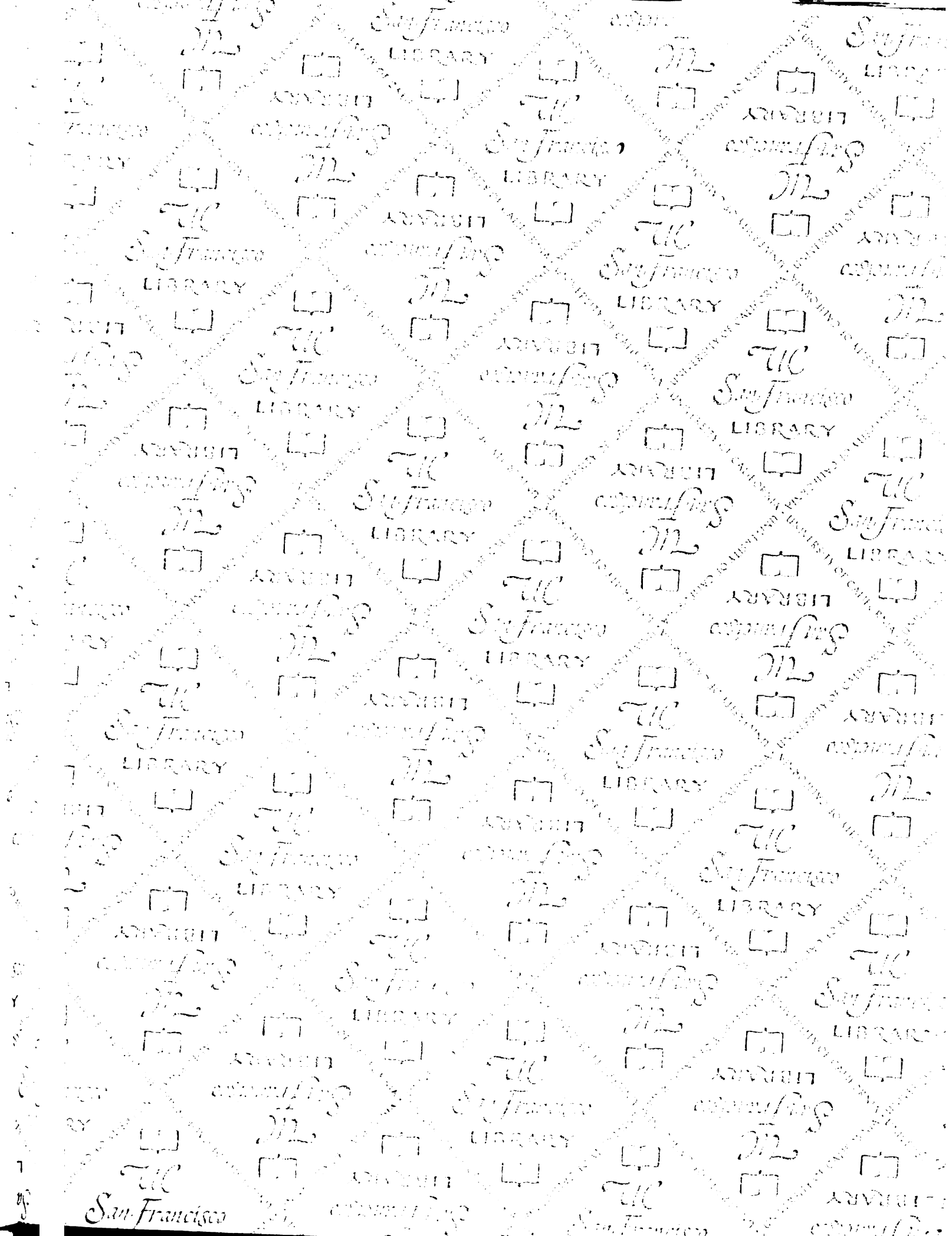
Means and standard deviations for the dependent variable: Total Incorporation CPM/mg for the cortex

DAYS OF INJECTION	N	SEX	CPM/mg MEAN	S.D.
1-2	8	M	43.310	26.280
	8	F	49.139	24.895
7-8	11	M	66.203	26.654
	12	F	85.781	34.723
14-15	11	M	44.818	15.982
	12	F	39.796	15.832
21-22	11	M	8.376	3.167
	12	F	12.514	4.717

APPENDIX 8

Means and standard deviations for the dependent variable: Percent Incorporation for the cortex

DAYS OF INJECTION	N	SEX	MEAN	S.D.
1-2	8	M	81.603	26.078
	8	F	84.125	21.342
7-8	11	M	79.144	27.851
	12	F	87.136	22.586
14-15	11	M	64.365	18.253
	12	F	62.478	18.545
21-22	11	M	16.051	5.685
	12	F	20.788	7.875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