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FUNCTIONAL AND ANATOMICAL REORGANIZATION IN DEVELOPING HAMSTERS AFTER EARLY POSTNATAL LESIONS OF THE OLFACTORY SYSTEM

by

ROCHELLE SMALL

A dissertation submitted to the Graduate
Faculty in Psychology in partial fulfillment
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Abstract

FUNCTIONAL AND ANATOMICAL REORGANIZATION IN NEONATAL HAMSTERS AFTER EARLY OLFACTORY LESIONS

Neonatal transection of the lateral olfactory tract (LOT) in hamsters leads to rearrangements of the olfactory bulb projection which result in sparing of adult olfactory-dependent behavior. This study provides evidence that within 10 days after a neonatal transection, LOT fibers have already begun to display most of the features that characterize the rearranged projection in adulthood and that this postlesion growth is already functional in the neonate. The appearance of LOT fibers distal to the tract section is associated with recovery from one of the neonatal effects of olfactory bulbectomy, persistent thermal orientation.

Olfactory lesions made during the first week of life result in a disruption of normal developmental transitions; pups continue to show strong thermal orientation into the second week, a behavior pattern usually seen only in younger pups. Pups with unilateral transections of the LOT initially appear similar to pups with unilateral olfactory bulbectomies, both groups showing levels of heat preferences not seen in normal pups or pups with control lesions. The thermal preference of pups with LOT transections declines to control

levels by day 11, while that of pups with olfactory bulbectomies remains elevated throughout the period of testing (15 days of age).

Analysis of the relationship between individual patterns of thermotaxis and the projection field of the olfactory bulbs in pups receiving early LOT transections, revealed that: 1) Pups showing recovery had LOT fibers extending distal to the lesion and projecting into the olfactory tubercule; 2) Pups not showing recovery had no distal fiber growth and more extensive damage to the cortex underlying the tract; and 3) Pups showing no initial effect of the lesion on thermal behavior had cuts that missed or only partially damaged the LOT.

Alterations in the olfactory bulb projection at levels rostral to the lesion occurred in both recoverers and nonrecoverers. These changes included increases in the density and laminar width of the projection to regions typically receiving olfactory innervation and an extension of the projection to adjacent regions such as the dorsal bank of the rhinal sulcus and the cortex dorsal to the hippocampal rudiment.

These results suggest that among ventral forebrain regions innervated by the olfactory bulb, the olfactory tubercle plays a singularly important role in the transition from thermotaxis to exploratory modes of behavior that occurs at the end of the first week. Previous work has demonstrated that the LOT projection to the tubercle begins to acquire mature degeneration characteristics at about 7 days of age, the time when a strong preference for conspecific odors is first observed in hamster pups.

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ABBREVIATIONS

Anatomical:

AO	Anterior olfactory nucleus
AOB	Accessory olfactory bulb
CNS	Central nervous system
CO	Cortical amygdaloid nucleus
DBh	Horizontal limb of the nucleus of the diagonal band of Broca
EPL	External plexiform layer of OB
ER	Entorhinal cortex
Gl	Glomerular layer of OB
Gr	Granule cell layer of OB
HR	Hippocampal rudiment
IC	Islands of Calleja
ICm	Medial island of Calleja
IPL	Internal plexiform layer of OB
LOT	Lateral olfactory tract
M	Mitral cell layer of OB
ME	Medial amygdaloid nucleus
MOB	Main olfactory bulb
N	Nucleus of the lateral olfactory tract
OB	Olfactory bulb
OB_i	Olfactory bulb ipsilateral to LOT transection
OB_c	Olfactory bulb contralateral to LOT transection
Och	Optic chiasm
OT	Olfactory tubercle
PP	Prepiriform cortex
RS	Rhinal sulcus

Surgical Treatments:

BH	Bilateral aspiration of the dorsal hemispheres
SO	Sham operations
ULOT	Unilateral transection of the lateral olfactory tract
UOB	Unilateral olfactory bulbectomy

Other:

(C)	Contralateral to LOT section
(I)	Ipsilateral to LOT section
NE	No effect: pups in the ULOT group showing no effect of the lesion on the transition from thermal orientation to exploratory behavior

Other: continued

NR	Nonrecovery: pups in the ULOT group showing continued thermal orientation through 15 days of age
R	Recovery: pups in the ULOT group showing a delay and alteration in the transition from thermal orientation to exploratory behavior
SD	Standard deviation
SEM	Standard error of the mean

Introduction

Injury to the central nervous system (CNS) in infancy often results in neurological deficits that differ in degree or kind from those observed after similar damage in adulthood (Kennard, '38, '40; Milner, '74; Stewart & Riesen, '72; Teuber, '71, '74). One principle derived from many studies evaluating the age variable is that early lesions often lead to less severe behavioral impairments in adulthood than lesions sustained at maturity (Kennard, '42).

However, sparing of function is not an inevitable outcome of early injury and some variation has been reported depending upon such factors as the species used, the locus of injury, the postoperative time points at which function is evaluated (Goldman, '74) and the behavioral tasks selected (Nonneman & Isaacson, '73; Schneider & Jhaveri, '74). There are many instances in which neonatal and adult lesions result in comparable deficits (e.g., Bland & Cooper, '69; Goldman, '71; Johnson, '72; Kennard, '38; Lawrence & Hopkins, '70; Murphy & Stewart, '74). For example, olfactory bulbectomy in adult male hamsters results in a complete loss of mating behavior (Murphy & Schneider, '70) and no sparing of this function occurs after neonatal bulbectomy (Winans & Powers, '74).

In other instances, neonatal lesions produce more severe deficits or maladaptive function not seen after adult lesions. Partial transection of the lateral olfactory tract, the output pathway of the olfactory bulbs, has no effect on male mating behavior when done in adult hamsters, but a similar lesion in neonates leads to incomplete and inconsistent mating performance later in life (Devor, '75,'77). Olfactory bulbectomy in neonatal female hamsters leads to marked changes in adult social behaviors such as postcopulatory aggression and scent marking (Leonard, '72) while bulbectomy in adult females does not appear to affect social behaviors (Carter, '73). Similarly, unilateral tectal ablation in adult hamsters results in a loss of turning towards stimuli presented in the visual field contralateral to the lesioned tectum but a neonatal lesion leads to misdirected turning later in life in response to stimuli presented in parts of the affected visual field (Schneider & Jhaveri, '74).

1. Neuroplasticity in the response of the mammalian CNS to injury:

Differences in the nature of altered neuronal connectivity have been suggested as one explanation for the variation observed in the consequences of early and late lesions.

This suggestion is supported by many recent demonstrations showing that the mammalian CNS is capable of axonal reorganization after injury (for recent reviews see Bernstein & Goodman, '73; Eidelberg & Stein, '74; Guth & Clemente, '75), and more specifically by studies showing differences in the neural consequences of lesions in young and adult mammals.

The definitive work on regeneration of nerve fibers in the mammalian CNS done by Ramón y Cajal ('28), led to the conclusion that regeneration, or growth from the proximal stump of transected fibers, is aborted before functional restitution can occur. The few possible exceptions that challenge that conclusion are based on the response of axotomized aminergic fibers originating in the brain stem. These fibers sprout vigorously from the region of the proximal stump, but although the sprouts establish synaptic contacts throughout the brain, they do not restore the axotomized tracts (e.g., Katzman, Björklund, Owman, Stenevi, & West, '71).

Rather than regenerative sprouting, recent work has documented a different form of neuroplasticity in the mammalian CNS, collateral sprouting, which is growth from intact axonal branches in response to the injury of neighboring axons or collateral branches of the same axon (the pruning effect). Collateral sprouting usually results in rearrangement of neural connections, such as an increase in the density of a projection to a terminal field or anomalous projections onto neurons or portions of neurons not normally innervated by a fiber system.

For example, apical dendrites in the plexiform layer of the olfactory cortex receive a complementary laminar projection from the ipsilateral olfactory bulb and from the association pathway arising in olfactory cortical areas. Olfactory bulb fibers terminate in the superficial part of the plexiform layer, in lamina Ia, synapsing on the more distal segments of apical dendrites, while association fibers terminate deeper in the plexiform layer, in lamina Ib, synapsing on the more proximal segments of apical dendrites (Price, '73; Scalia, '66). When the distribution of the association pathway was examined in adult rats who had received olfactory bulbectomies at birth, it was found to project to both the distal and proximal segment of the apical dendrites (Moxley & Price, '74; Westrum, '75a).

II. Age as a factor in the response of the CNS to injury:

Studies which have specifically compared the effects of CNS lesions in young and adult mammals, have reported either quantitative or qualitative differences in the response of neurons and in the resulting reorganization of axonal connections.

Marked differences are observed in both the anterograde and retrograde responses of injured neurons depending upon the age of the animal. As early as the 19th century it was noted that after injury, neurons degenerate more rapidly and in greater numbers in young animals than in adults (Cajal, '28; Gudden, 1870). The cell bodies of axotomized neurons often show more rapid and pronounced chromatolytic reactions in young mammals (for recent reviews see Brodal, '73; Lieberman, '74). While chromatolysis can be followed by rapid recovery (Marinesco, '09), a more common observation is that it leads to the actual degeneration of large numbers of neurons. The vulnerability of neurons to cell death appears to decrease progressively as the neuron matures (e.g., Brodal, '73; Hess, '56; Romanes, '46).

Anterograde reactions to axotomy in young and adult mammals can also be distinguished by temporal parameters. In young animals the distal segment of transected fibers shows degeneration argyrophilia for only a few days after injury, while in adults, argyrophilia persists in the distal segment for weeks after axotomy (Schneider, '70; Leonard, '73, '74b, '75).

The age of the animal also has a marked influence on the rearrangement of axonal connections that results from CNS injury. Since neonatal surgery is often performed at ages before fiber outgrowth is completed, it is frequently not possible to determine if transected or untransected fibers are responsible for altered connections seen later in life; for this reason, nonspecific terms such as postlesion fiber growth will be used in the following discussion rather than regenerative or collateral sprouting.

Injury to some fiber systems results in axonal alterations following both neonatal and adult lesions, but the developmental stage influences specific details of the connections formed. For example, in the hippocampus where many afferent projections converge in a zone of termination, removal of one input induces the remaining afferents to expand their distribution to fill in vacant postsynaptic sites. This process occurs after lesions in neonates and adults, but the specific contributions made by each of the remaining afferents varies according to the age at injury, and leads to different laminar arrays of terminals. After a lesion of the ipsilateral entorhinal cortex in neonates, commissural and association fibers expand their distribution to fill most of the molecular layer while septal fibers intensify only along the superficial border; the same lesion in adults causes the septal projection to expand through the outer half of the molecular layer while commissural and association systems show a much reduced spread (Cotman & Lynch, '76). The developmental stage also affects the rate of postlesion growth. Descending adrenergic fibers reinnervate the spinal cords of young and adult mammals after chemical axotomy using 6-hydroxydopamine, but a far more rapid reappearance of adrenergic fibers is reported after neonatal axotomy (Nygren, Olson & Seiger, '71).

In other fiber systems such as the central olfactory and visual pathways, alterations occur in the pattern of neural connectivity only when the lesions are sustained during an early developmental period (e.g., Devor, '74, '76b; Guillery, '72; Hubel, Wiesel, & Le Vay, '76; Kalil, '72; Lund & Lund, '73, '76; Schneider, '70, '73; Westrum, '75a). For example, tectal ablation in hamsters leads to anomalous retinal projections in the case of neonatal lesions but not adult lesions. After a unilateral tectal lesion at birth, the retinal projection shows an increased distribution and density in those thalamic nuclei which normally receive input from the tectum (i.e., nucleus lateralis posterior and the ventral nucleus of the lateral geniculate) and in addition the retina projects to the ipsilateral tectum through an anomalous tectal decussation (Schneider,'73).

Devor ('76b) has demonstrated that if the postnatal period is subdivided into age intervals, distinct patterns of postlesion growth result from lesions made during particular neonatal age ranges. In some regions, a more extensive pattern of growth is observed after lesions made during a specific neonatal interval than would occur after lesions made at either younger or older age ranges.

III. The choice of the hamster for demonstrating functional and anatomical correlations associated with age at injury:

Despite several anatomical demonstrations that different patterns of neural connections can result from early and late lesions, there are relatively few studies directly relating these differences to the divergent behavioral effects associated with age at injury (e.g., Devor, '75, '77; Hicks & D'Amato, '70; Schneider & Jhaveri, '74). In two of these demonstrations the hamster proved to be a useful subject for contrasting the effects of early and late lesions.

The hamster is particularly well suited for lesion studies evaluating the age variable because the pup is born after a 16 day gestation period (Dieterlen, '59) which is much shorter than that of most other rodents. Consequently the CNS of the hamster is very immature at birth. The major cell migrations in the brain occur after birth (Shimada & Langman, '70) and myelination of the CNS does not begin until the second postnatal week (Clark, '66; Clark & Telford, '64). It is therefore possible to manipulate an immature nervous system without resorting to _in utero_ surgery. Axonal connections have been demonstrated after lesions of hamster neonates that are not present after lesions of other neonatal rodents. Function after neonatal lesions can be contrasted with the effects of adult lesions which do not lead to similar connections.

The difference in visually elicited orientation responses of hamsters receiving neonatal and adult tectal lesions has been explained by the different patterns of retinal projections that result from the lesions. Normally each retina projects primarily to the contralateral tectum and the output pathway of the tectum mediates head turning toward the opposite side. Unilateral tectal ablation in neonates leads to misdirected turning which is related to the anomalous pathway arising from the retina contralateral to the lesioned tectum. This retina now projects into the ipsilateral tectum after the removal of its usual target. The maladaptive consequences of early lesions are due to the anomalous tectal projections formed by the retina. In the adult, unilateral lesions result in a complete loss of turning towards stimuli in the visual field contralateral to the lesion and the retina of that eye has no tectal projection (Schneider & Jhaveri, '74).

IV. The central olfactory pathway and its use in contrasting the effects of early and late lesions:

Devor used the olfactory system of hamsters to contrast the functional and anatomical consequences of early and late lesions. Like the visual system it provides a well described pathway which has been related to a reliable behavioral marker in adults: olfactory bulb removal, whether in neonates or adults, leads to a complete absence of mating behavior in male hamsters (Murphy & Schneider, '70; Winans & Powers, '74).

The pathway from the olfactory bulbs to the olfactory cortex can be described with reference to Fig. 1. Most axons leaving the bulb are contained in the lateral olfactory tract (LOT) which gives rise to a continuous sheet of terminals extending throughout the ipsilateral ventral forebrain. Fibers of the LOT appear to give off many collateral branches, most of which terminate in lamina Ia of the plexiform layer. There is very limited topographic organization between the olfactory bulb and the cortex or between parts of the LOT and the cortex so that discrete regions within the bulb and tract give rise to projections extending throughout the cortex. The two topographic relationships observed are: 1) fibers of the accessory olfactory bulb (a separate structure within the main bulb) travel as a discrete bundle within the LOT to terminate exclusively in the cortical and medial amygdaloid nuclei (Broadwell, '75; Devor, '76; Scalia & Winans, '75); and 2) fibers from the caudomedial part of the bulb project directly into medial portions of the olfactory cortex without entering the LOT (Devor, '76a).

Olfactory bulbectomy and LOT transection both denervate lamina Ia of the olfactory cortex, but LOT section differs from bulbectomy in several important ways: 1) the mitral cells of the olfactory bulb, which give rise to LOT fibers, are not destroyed by the lesion so that it is possible to investigate alterations in their projection which result from cutting their distal branches; 2) only the cortex caudal to the cut is denervated while the rostral projection is spared; 3) fibers from the caudomedial part of the bulb are not interrupted by the cut; 4) the section damages a portion of the cortex underlying the tract; 5) the cut disrupts fibers of the association pathway passing rostrally and caudally through the region of the cut and those arising from the cortex at the site of the lesion. (Fibers of the association system terminate in lamina Ib throughout the projection field of the bulb and also in adjacent regions such as the outer plexiform layer of 'sulcal' neocortex situated dorsal to the rhinal sulcus and in the entire width of the plexiform layer of the dorsal part of the hippocampal rudiment (Devor, '76b; Price, '73)); and 6) the cut severs centrifugal fibers to the bulb which arise in the olfactory cortex and pass forwards in close relation to the LOT (Price & Powell, '70).

Using this system, Devor ('75, '77) was able to relate the different effects of neonatal and adult LOT transections on male mating to the pattern of the olfactory bulb projection remaining after the lesions. Complete section of the adult LOT leads to a permanent and total loss of olfactory bulb efferents caudal to the cut. If the section is made at rostral levels, it duplicates the effect of adult bulbectomy and results in a loss of mating behavior

(Devor, '73). Partial adult transections denervate a small wedge of cortex just distal to the cut, with the remainder of the caudal projection field retaining a sparse but otherwise intact projection from the bulbs. Partial LOT cuts in adults do not affect mating. Only a restricted region of olfactory cortex is denervated because each portion of the LOT contains fibers which project non-topographically to overlapping fields within the cortex.

In the neonate, both partial and complete LOT transections lead to identical deficits in adulthood, which can be described as an impairment, but not a loss of mating behavior. Since no mating occurs after complete adult cuts these results suggest a relative sparing of function following complete neonatal lesions. However, partial lesions in the neonate lead to more severe deficits than in the adult where partial transections do not affect mating.

The similar effects of partial and complete neonatal cuts can be explained by the alterations observed in the olfactory bulb projection of adults which had received neonatal lesions. After complete neonatal LOT section made prior to 7 days of age, fibers from the olfactory bulb had restored the projection to at least the rostral half of the olfactory cortex by adulthood. After partial neonatal cuts, axonal alterations resulted in a pattern similar to that seen after complete neonatal cuts. The projection just distal to the cut was intact, with branches of uncut fibers filling in the denervated wedge, and there was a shortening of the projection to more caudal regions. This shortening resulted either from a failure of intact fibers to continue their growth so as to keep pace with the expanding forebrain during development, or from a secondary retraction of longer branches of intact fibers as a consequence of the lesion.

Proximal to the LOT section, in cases of both complete and partial cuts there was in increase in the width of lamina Ia and in the density of its terminals, as well as an extension of the bulb's projection to the sulcal and cortex dorsal to the hippocampal rudiment. The olfactory bulb had extended its projection proximal to the cut into those sites normally occupied by the association system.

V. An evaluation of neural reorganization in developing hamsters after neonatal lesions:

Both Schneider and Devor evaluated the functional and anatomical consequences of neonatal lesions months later in the adult when the process of neural reorganization was well advanced or completed. Since in both the visual and olfactory systems, neural alterations present in adulthood were shown to have functional implications, it is reasonable to inquire if these rearrangements are already present during the neonatal period and if they have significance for early behavioral development. An evaluation of reorganization in younger animals would allow the process of postlesion fiber growth to be described in terms of its pattern and time course and this information could be related to the onset of specific functions.

Why then have workers traditionally waited until adulthood to evaluate neonatal lesions, rather than looking for early signs of functional reorganization? The data available on the time course of sprouting suggest that axonal alterations observed in adults are probably formed soon after injury. Within three to five days after entorhinal cortex lesions, septal fibers show signs of proliferation in the hippocampus of both neonates and adults (Cotman & Lynch, '76). Sprouting of adrenergic fibers is observed wtihin seven days of axotomy in adults (Björklund & Stenevi, '71; Katzman et al., '71), and in neonates fiber outgrowth appears within a day or two of surgery (Nygren et al., '71).

The major problem hindering an evaluation of functional reorganization in the neonate involves a behavioral constraint: recovery from functional deficits produced by a CNS lesion must be detected against a baseline of behavioral development. The postnatal period could be described as a series of shifting behavioral equilibria as behavior patterns emerge, remain stable for a short time. and then decline as they become replaced by newly emerging patterns. These fluctuations in response strength often tend to be accompanied by increases in response variability. It is within this shifting response repertoire that one must identify a response that can serve as a reliable functional marker of postlesion anatomical growth in the neonate.

The present study sought to evaluate the consequences of early lesions of the olfactory system on development during a 10 day postoperative period. The behaviors of pups with unilateral LOT transections and unilateral olfactory bulbectomies were compared to determine if the two lesions initially produced comparable alterations in behavior and to determine if there were differences in development that would distinguish the groups. The behavior profiles of pups with LOT sections were then examined in relation to the pattern observed in the olfactory bulb projection 10 days after the lesions to see if particular profiles were associated with specific patterns of connectivity.

In order to accomplish these goals, a behavioral marker was required that: 1) would be appropriate for pups over an extended age range of 10 days, during which time olfactory preferences are known to shift rapidly (Devor & Schneider, '74); 2) would clearly distinguish pups with olfactory bulbectomies from pups with control lesions during this period; and 3) would be relatively free from variability in the pups' day to day performance, so that if recovery from the effects of LOT transection occurred within the testing period, it would not be obscured by variability.

VI. The olfactory system and the development of behavior:

Studies of olfactory orientation in neonatal rodents show that different olfactory functions emerge at different postnatal ages and many functions show only a short life span (see Alberts, '76 for a recent review). Rat pups have received the most attention in studies of neonatal olfaction. Olfactory cues have been implicated in the first nipple attachment of newborn rats (Teicher & Blass, '77) and they also appear to be crucial for attachment at older ages (Hofer, Shair, & Singh, '76). At two days of age rat pups will become quiet in response to maternal odor (Schapiro & Salas, '70) and show bursts of sniffing to perfume odors (Welker, '74).

Rat pups 3 - 4 days of age show a preferential approach to homecage shavings rather than clean shavings and the strength of this attraction increases during the first postnatal week (Cornwell-Jones & Sobrian, '77). A preference for home-cage shavings over strange cage shavings does not appear until 12 days of age in rat pups (Gregory & Pfaff, '71). At 14 days of age, the pups will approach airborne odors derived from the dam rather than a plain airstream or one derived from a non-lactating female (Leon & Moltz, '71, '72).

The few studies that have examined olfactory orientation in hamster pups have also shown a gradual development of olfactory orientation during the postnatal period. Beginning at 3 days of age, hamster pups reared in pine bedding show an aversion to cedar shavings (Devor & Schneider, '74) and pine shavings scented with plant odors such as lemon or garlic (Cornwell, '75) when tested in a two-choice situation relative to clean pine bedding. Exposure to these odors during the postnatal period appears to neutralize their aversiveness. (Cornwell, '75, '76). More complex olfactory discriminations involving animal odors do not appear until the second postnatal week. At 8 days of age hamster pups begin to approach home-cage shavings rather than clean shavings (Devor & Schneider, '74); Gregory & Bishop, '75); this preference then declines after 12 days of age.

Anatomical evidence also indicates that the olfactory system acquires mature characteristics over an extended postnatal period in rodents. While all neural elements of the main and accessory olfactory bulb are present at birth, granule cells continue to be formed into adulthood in both the rat (Altman, '69) and the mouse (Hinds, '68a, '68b) and these late arising neurons receive synaptic innervation (Kaplan & Hinds, '77). Olfactory tract axons have already established some synaptic contacts in the prepiriform cortex at the time of birth in the rat, but it is not until the end of the second postnatal week that the cortex acquires a mature synaptic profile (Westrum, '75b).

Leonard ('75) has described a sequence occurring through the first 13 days of life in which the various regions of the hamster olfactory projection cortex acquire mature degeneration characteristics. The temporal parameters of olfactory tract degeneration change sharply during development, and it may be that the onset of long lasting degeneration coincides with changes in function. For example, in the visual system of newborn hamsters, all signs of degenerating fibers in the optic projection have disappeared with 72 hrs of enucleation. Beginning at the age of eye opening (14 days) and thereafter, degeneration argyrophilia persists for weeks or months after enucleation (Leonard, '73; '74b). In the rat, eye opening marks the beginning of a period of rapid synapse formation (Lund & Lund, '72).

In the olfactory bulb projection of the hamster pup, long lasting degeneration is observed at 2 days of age in restricted portions of the prepiriform cortex and it then gradually appears in the olfactory tubercle and medial nucleus of the amygdala at 5 to 9 days of age, and in the entorhinal cortex and cortical nucleus of the amygdala at 9 to 13 days of age. The onset of specific olfactory functions in the hamster pup may depend upon the maturation of the olfactory bulb projection to localized regions of the olfactory cortex. The projection to the olfactory tubercle and medial nucleus of the amygdala acquires mature characteristics at the age when approach to conspecific odors is first observed.

VII. The use of a nonolfactory task to evaluate the effects of neonatal olfactory lesions:

The aim of this study was to examine the process of functional recovery in individual pups who had received LOT transections. This goal required a marker that would clearly distinguish developmental processes in pups given olfactory bulbectomies and those given control treatment in daily tests. To accomplish this goal using an olfactory task as a marker would have been a formidable challenge since the gradual maturation of olfactory function, as described above, means that lesion effects would have to be evaluated against a baseline of graded, age related fluctuations in response strength. Fortunately, Leonard ('76) has described an effect of olfactory bulbectomy on thermal behavior which makes that response ideally suited to serve as a marker in this study.

Thermal orientation is a behavior that is strongly and reliably expressed by hamster pups during their first week of life (Leonard, '74a). Each pup consistently shows strong responses in daily tests, and little variation is observed in response strength among pups. Their thermal responsiveness has been suggested as one of the factors responsible for the sedentary early life style of the pups (Leonard, '74a). During the first 8 days of life, the pups are not seen out of the nest and they do not stray even on those occasions when the female briefly leaves the nest (Dieterlen, '59; Richards, '66). Like many other mammals, hamster pups are born ectothermic and depend upon the female as a source of heat (Adolph, '57; Hissa & Lagerspetz, '64; Lagerspetz, '66). Leonard has demonstrated that the pups have the capacity to detect and orient to very shallow thermal gradients at birth; when placed on a thermal gradient the pups will move rapidly and reliably to the warm extreme and remain there. Through 8 days of age, the rate and direction of their movement can be tightly controlled by manipulating the thermal properties of their environment.

After 8 days of age, thermal orientation declines sharply and pups appear unresponsive to the thermal qualities of their environment. They now spend their time investigating the substrate rather than orienting along a thermal gradient. The decline of this behavior seems to be tightly controlled by some developmental event with a sharp onset. It is not due to an increase in thermoregulatory abilities since the pups remain poikilothermic through 11 days of age (Hissa, '68) and only then gradually acquire physiological mechanisms to maintain their body temperature.

If the olfactory bulbs are removed in hamster pups during the first week of life, the normal abrupt decline in thermal orientation does not occur (Leonard, '76).

Instead, pups with either unilateral or bilateral olfactory bulbectomies show persistent thermal orientation throughout the second week of life. Since bulbectomized pups continue to show thermal orientation, this suggests that in normal pups the transition is not due to a decrease in the effectiveness of mechanisms mediating thermal behavior, but is instead the result of an increased impact of the olfactory system in influencing the pup's behavior. The sterotyped progression in thermal orientation during normal development makes this response a very sensitive indicator of even subtle alterations resulting from olfactory lesions.

VII. Summary

In the present study, the behavior of hamster pups on a thermal gradient was examined for a ten day post-operative period in pups which had received either unilateral LOT transections, unilateral olfactory bulbectomies or control treatments at 5 days of age. A sequence of behavioral stages was described for pups with control treatments and contrasted to the developmental sequence observed in pups with olfactory lesions. The olfactory lesions were compared to see if both bulbectomy and LOT section produced persistent thermal orientation into the second week and if the effect of the lesions diverged with age.

At the completion of testing, lesions were verified histologically and the olfactory bulb projection was examined in pups given LOT transections, either by removing the olfactory bulbs and staining for degenerating fibers or by injecting the bulbs with a radioactive label and examining the projection with autoradiography.

Behavioral and anatomical profiles were then compared in pups with early LOT sections to see if lesion placement and the pattern of neural connectivity were associated with particular patterns of behavioral development. Fiber rearrangements present 10 days after complete and partial LOT transections were compared with rearrangements that had been observed by Devor in adulthood after neonatal lesions.

METHODS

Subjects

A total of 30 litters of golden hamster pups (*Mesocricetus auratus*) were used in this study. The pups were delivered by random-bred multiparous females ordered from Charles-River Lakeview (Cambridge, Mass.). For the behavioral studies, two groups, each containing 8 pregnant females due to deliver on the same day, were received in the laboratory within one week of parturition. Females were housed in clear plastic cages with solid bottoms and provided with pine bedding and tissues for nesting material. The diet consisted of lab chow and either water or cabbage *ad libitum*, supplemented by feedings of sunflower seeds, apples and carrots. The colony room was kept on a 12-hour reversed light cycle and all behavioral tests were conducted in the colony room during the animals' night. Litters ranged from 8 to 15 pups at birth and were not culled, as it was found that females generally reduced their litters to sizes ranging from 6 to 10 pups by 5 days of age. Pups were given daily handling from birth to accustom the female to having her pups removed from the nest for testing at later ages. Ambient temperature in the colony room was recorded daily and ranged from 21 °C to 24 °C. The day of birth is considered day 0 throughout this paper.

Surgery

Surgery at 5 days of age:

On day 5, the female was removed from the nest and the pups were taken to the operating room. For all lesions, pups were immobilized on crushed ice for 2.5 min and then placed in a specially constructed holder under a dissecting microscope. With care, all operations could be done under visual control in a blood-free field. Litters were given one of the four operations described below:

UOB - unilateral olfactory bulbectomy: The skin was opened at the midline and the translucent bone covering one of the olfactory bulbs was removed with fine forceps. The olfactory bulb was gently aspirated through a 26-gauge blunted and shaped hypodermic needle. Care was taken to scrape the cribiform plate of any remaining olfactory filaments, and the lesion was continued along the ventromedial aspect of the peduncle to ensure removal of the caudal extent of the bulb.

ULOT - unilateral transection of the lateral olfactory tract: A lateral skin incision was made and a small opening placed in the orbital surface of the frontal bone. The LOT is unmyelinated in young hamsters and its position had to be determined in pilot work using Devor's method

('76b) in which reference points are derived from the neonate's vascular pattern. A fine microknife (Circon Corp.) was inserted and gently drawn back and forth so as to cut the LOT fibers at the level of the caudal peduncle. In several cases the blade was drawn through a vertical arc as well as rotated from side-to-side to produce a more extensive transection. The exact locus and completeness of the cut could not be determined before sacrifice and subsequent histological processing.

BH - bilateral removal of the dorsal hemispheres: The skin and bone overlying the frontal pole was opened, and gentle aspiration was used to remove a volume of cortical and subcortical tissue from each hemisphere approximately equal to that of each olfactory bulb.

SO - sham operations: A midline skin incision was made and then closed with sutures.

Pups from any one litter were all given the same lesion, with the exception of one sham operate who was included to maintain lactation during the immediate post-operative period. This procedure has been used by Leonard ('72) and produces excellent survival rates in operated pups. Incisions were closed with sutures and the pups were toe-clipped for identification. After each operated pup had begun moving, it was placed in the home cage with its operated littermates, and when the entire litter was completed, the female was returned to the nest. Survival rates during surgery were excellent and no reassortment of pups among litters was necessary.

Surgery at 15 days of age:

The projection of the olfactory bulb ipsilateral to the ULOT was examined for evidence of fiber rearrangements at 15 days of age. Two neuroanatomical techniques were used to trace the central projections of the olfactory bulb: 1) the degeneration method and 2) autoradiography. Pups at these older ages were anesthetized with ether, or 25 mg/kg Equithesin (Jensen-Salsbury) supplemented with ether and the pup was placed in a sterotaxic apparatus. The skin was opened along the midline and an opening was made in the bone overlying one or both olfactory bulbs. After one of the surgical procedures described below, the incision was closed with wound clips.

1) Degeneration method - Most pups in the ULOT group were given bilateral olfactory bulbectomies on day 15, using the bulbectomy procedure already described. The bulbectomy causes anterograde degeneration of fibers originating in the olfactory bulb, and these fibers can then be visualized

after an appropriate survival period with a silver stain selective for degenerating fibers. The postoperative survival period after bulbectomies was varied between 48 and 120 hrs to confirm previous findings on the duration of degeneration argyrophilia during development (Leonard, '75).

The temporal parameters of anterograde degeneration in the neonatal olfactory system made it possible to use a double lesion technique to evaluate early postlesion growth. Alterations in the olfactory bulb's projection which result from the LOT transection at 5 days of age, are traced 10 days later with a second lesion, a bulbectomy, which causes degeneration in all fibers originating in the olfactory bulb. After lesions of olfactory bulb fibers performed at 5 days of age, the only sign of residual degeneration seen 10 days later is occasional sparse debris found in the middle third of the plexiform layer. Degeneration resulting from an olfactory bulbectomy done at 15 days of age is confined to a dense band in the superficial part of the plexiform layer and is easily distinguished by its position and density from any sparse, lingering debris.

Since the olfactory bulb projects only to ipsilateral forebrain structures, the bilateral bulbectomy permitted a comparison of the projection ipsilateral to the earlier

ULOT and the contralateral projection which had been allowed to develop normally. The use of intra-individual comparisons of the area and intensity of silver impregnated particles between the right and left sides, reduced the possibility that observed differences were the result of variables affecting silver impregnation during tissue processing, rather than actual differences in the fiber populations.

Five pups were given bilateral bulbectomies on day 17 without receiving a prior ULOT, to compare the normal projection at this age with the projection from the olfactory bulb contralateral to the ULOT. Brains of two normal 17 day old pups were examined with silver stains for evidence of degeneration normally present at these ages in the olfactory cortex.

2) Autoradiography - Several pups given early ULOTs received either unilateral or bilateral olfactory bulb injections of tritiated leucine after day 15. The labeled amino acid is incorporated into proteins by cell bodies in the region of the injection, and then transported anterogradely along the axon to its terminals (Cowan, Gottlieb, Hendrickson, Price, & Woolsey, '72; Lasek, Joseph, & Whitlock, '68). The distribution of label seen with autoradiography is free of the fibers of passage problem

that complicates degeneration studies, since fibers and terminals near the site of the injection do not incorporate and transport the label (Heuser & Miledi, '70).

On the morning of the injections, the leucine (L-Leucine-3,4,5-^{3}H, 79.8 Ci/mM, New England Nuclear) was concentrated by evaporating the .01 N HCl solvent under a stream of nitrogen and then adding 0.9% saline to yield concentrations of approximately 50 μCi/μl. In most cases, 7.5 μCi of ^{3}H-Leucine were injected in a volume of 0.15 μl. A Hamilton microsyringe with a 28-gauge needle was lowered into the olfactory bulb by the electrode carrier, to depths that had been predetermined based on the dimensions of the olfactory bulb at this age. A short post-injection interval of 18 hrs was used to limit the transport of label to the fast component of axonal transport which distributes material primarily, although not exclusively, to axon terminals rather than axons (e.g., Hendrickson, '72; Schonbach, Schonbach, & Cuénod, '71).

Difficulties in anesthetizing adolescent hamsters resulted in the loss of three pups during surgery at 15 days of age.

Apparatus

The thermal gradient was produced in a 25 cm^2 Plexiglas box with a metal plate floor. A Cole Palmer heating pad, 1 x 5 in was inserted under one side of the metal plate and a container of crushed ice was attached to the opposite side. The temperature of the heating pad was adjusted through a Thermodyne temperature controller which regulated the percentage of time that power was applied to the heating pad.

Pups were placed on a nylon mesh floor situated 2 cm above the metal plate. The mesh was divided into 16 numbered squares, each 6 cm x 6 cm, so that the pup's position could be recorded. A schematic diagram of the apparatus is shown in Figure 2. Body length of the pup, from the nose to the tip of the tail, increases from 5 cm at 3 days of age to about 8.5 cm at 15 days of age. The mesh was changed after each test to avoid odor trails, and a clean substrate was pulled through from a roll of mesh. After each test session the roll of mesh was washed thoroughly with a detergent solution.

Air temperature at the surface of the mesh was monitored at the two endpoints and midpoint of the gradient by three Yellow Springs Instrument Co. (YSI) air temperature thermistors (No. 405) attached to a scanning telethermometer which switched between thermistors every 20 sec. The output of the telethermometer was recorded on a Grass polygraph with a DC amplifier, to check the stability of the gradient during each test. Slight variations occurred in the gradient during the course of a testing session and the range of air temperature values at the cool end, midpoint, and warm end are shown in the insert of Figure 2. Average values of the range of the gradient were 20 °C to 36 °C; the steepness of the gradient was 0.72 °C/cm over the warmer half and 0.50 °C/cm over the cooler half.

To ensure that variables other than the thermal gradient were not influencing the direction of the pups' movement, the apparatus was rotated through 90° and 180° during pilot tests and on three of the experimental sessions.

An important consideration in selecting the temperature range of the gradient was that very young pups will enter extremes of heat that cause their core temperatures to rise to dangerous levels, but they do not withdraw from the extreme heat (Leonard, '74a). Since the pups were exposed to the gradient daily, a maximum air temperature of 37 °C was selected which approximates the female's body temperature.

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Testing Procedure

Pups were tested daily for 2 min on the thermal gradient from 3 through 15 days of age. Some pups in the first group of litters were not tested on days 3, 4 and 5 to determine if daily testing at early ages affected thermal orientation at older ages. No testing effects were observed.

Pups were placed on the cool end of the gradient with their body axis oriented randomly with respect to the thermal gradient. Latency to reach the warm end, time spent in the warm end, body temperature and activity were recorded on a Grass polygraph using a chart speed of 25 mm/min. Time spent in the heat was defined as the time in which any part of the pup's body was within one of the four squares along the warm end of the gradient (tier 1 in Figure 2). Movements of one hindlimb were recorded to indicate activity levels.

Body temperature was recorded chronically during testing on some days with a YSI No. 511 implantation thermistor inserted rectally and attached to a calibrated thermometer. On other days, the thermistor was attached to the pup's abdomen to record skin temperature. The record of air thermistors monitoring the gradient on each test, appeared along with the behavioral measures on the polygraph record. By day 9, most pups urinated during the test, and this was considered a time-out from the 2 min test until the pup had started moving again. Other behaviors recorded included grooming, climbing, sniffing, digging and biting. Each pup was weighed after testing and placed in a holding cage until all pups in its litter had been tested.

The design of the test required that pups be warm and healthy at the time of testing. Since pups were introduced at the cool end of the gradient, they had to traverse the grid in order to be scored for time in the heat. A pup with a low core temperature at the start of the test, usually as a result of the female being off the nest, tended to circle at the cool end of the gradient, never getting far from its starting position. Therefore, the starting core temperature of the pups was standardized by selecting pups for testing only at a time when the female was sitting on the nest. This meant that pups were being selected for testing in a variable sequence from among several litters being run concurrently.

Histology

Pups in UOB and BH groups were sacrificed at the completion of behavioral testing and their lesions were confirmed histologically. Pups in the ULOT group were sacrificed 48 to 120 hrs after bilateral olfactory bulbectomies or 18 hrs after injections of labeled leucine. Pups were anesthetized with ether and perfused intracardially with 5 cc of isotonic saline followed by 10 to 20 cc of 10% formalin. Brains were dissected out, grossly inspected and then stored in 10% formalin at 4 °C for several weeks. Brains were photographed from the ventral surface during this period. Brains were transferred to a solution of 30% sucrose in 10% formalin for at least one week, and were then embedded in albumin-gelatin for 5 days. Sections were cut on a freezing microtome at 25 μm. Special care was taken to control temperature which is crucial for cutting frozen sections of young tissue. In most cases, serial sections were cut in the frontal plane perpendicular to the dorsal surface, but some brains were cut in sagittal and horizontal planes. Serial sections were collected in 10% formalin and stored at 4 °C for periods of up to one week until staining. (See Leonard, '74b and '75 for notes on processing neonatal tissue for silver stains.)

Series of sections 0.125 mm apart were stained with one or both of the following degeneration stains: 1) Fink-Heimer procedure (Heimer, '70, staining procedure 1, p. 127) which stains degenerating fibers and terminals. This procedure was modified by omitting the permanganate and bleach steps and by diluting the reducing solution with an equal volume of water; and 2) Fink-Schneider procedure (Schneider, '69, footnote 31) which stains normal fibers as well as degenerating fibers and terminals and produces excellent impregnation in young brains.

Series of sections were also stained with cresylecht-violet to determine the effects of LOT section on the mitral cell population in the olfactory bulb and to define the extent of lesions and injection sites.

Three pups who had been given ULOTs on day 5 were sacrificed at 10 days of age and two pups were sacrificed at 15 days of age without a second treatment to determine if any lingering degeneration was present from the earlier tract section and to examine the olfactory bulbs for alterations resulting from the transection.

Brains examined for radioactive label were sectioned as above, and then series of sections separated by 0.075 mm through the injection site and 0.125 mm through the olfactory cortex were stained with Luxol Fast Blue for myelinated fibers. Sections were dipped in Kodak NTB-2 emulsion and exposed at 4 °C for periods of up to 60 days. The slides were developed in Kodak D-170 at 18 °C for 6 min and then stained through the emulsion with cresylechtviolet.

Enlarged outline drawings of frontal sections were made with a microprojector at selected levels rostral and caudal to the LOT transection. The distribution of degeneration and radioactive label was then charted on the drawings. The degeneration seen on the side ipsilateral to the LOT transection was described in terms of its location, intensity, particle size and shape, and compared to the appearance of degeneration in the olfactory cortex on the contralateral side of the brain.

The density of mitral cells within the mitral cell layer of the olfactory bulbs was calculated at 5 days after LOT transection from sections containing both of the bulbs. The number of mitral cells per 0.5 mm of mitral cell layer was counted x40 along the medial and lateral walls of 5 coronal sections in each of 4 brains. Sections were selected from regions rostral to and not including the accessory olfactory bulb. The 0.5 mm segment of the mitral cell layer counted was selected with the constraint that glomeruli be present adjacent to the segment and then the same ventrodorsal elevation was maintained for the medial and lateral walls of both bulbs on a given section. Since the mitral cell layer includes only medium sized mitral cells of 15-20 μm and small granule cells of about 4-7 μm, any irregular profile with dimensions larger than those of a granule cell was included in the count.

In order to make a qualitative comparison of the number of fibers emerging from the caudal part of the olfactory bulb on the two sides of the brain, the depth of the fiber bundle was measured x40 at the point where it was maximal, on the first section on which the accessory olfactory bulb was no longer present.

Behavioral Data Analysis

A thermal score was calculated for each pup daily which represented the percentage of the 2 min test time that the pups spent in the warm end of the gradient. Daily group medians and interquartile ranges of the thermal scores for each lesion group were computed. Analyses of behavioral data preceded confirmation of the lesions.

In order to contrast the durability of effects resulting from ULOT and UOB treatments, thermal scores were averaged over two successive three-day intervals representing ages when thermal orientation had already begun to decline in litters with control treatments. This provided an early and late thermal index for each pup, based on scores for days 8, 9 and 10 and days 11, 12 and 13. Differences in the thermal indices of treatment groups at a given age interval and between the two age intervals were evaluated with the t test. The distribution of thermal indices of UOB and control groups at the two age of the ULOT time intervals were used to segregate pups in the ULOT group into 3 subgroups: No effect (NE), Recovery

(R), and Nonrecovery (NR).

Thermal scores were also analyzed as a function of days before and after a transition from high to low levels of thermal responsiveness occurred. The rate of decrease in scores, calculated from the day preceding the transition (day -1) to the transition day (day 0) was compared for treatment groups in a one-way analysis of variance, using the F test (Scheffé method) to make multiple comparisons.

Polygraph records were analyzed in terms of three measures to describe the changing response patterns seen on the gradient. Daily group medians and interquartile ranges were calculated for the following: 1) latency to reach tier 1 - the time taken by the pup to move from his start position in tier 4 and traverse the grid to enter tier 1; 2) maximum bout duration - the pup's longest uninterrupted stay in tier 1; and 3) the number of times the pup entered tier 1 during the 2 min test.

Since all pups within a litter received the same treatment, it was crucial to determine if behavioral effects were the result of selective differences between particular females and their young rather than the result of the experimental treatments. The behavior of litters comprising a lesion group was compared with the Fisher Exact Probability Test and no litter effects were observed.

Much stronger evidence against the operation of litter effects was obtained from the behavioral variability observed within each of the ULOT litters. Lesions of this group could not be visually confirmed at the time of surgery. Subsequent histology showed that each of these litters contained pups with lesions ranging from no damage to the LOT to complete Lot transections, and these differences in lesions were reflected in behavior on the thermal gradient.

Experimental Rationale

Developmental changes in the pattern of thermal responsiveness and exploratory behavior were compared in pups receiving olfactory system lesions and control treatments at 5 days of age. Pups with either unilateral olfactory bulbectomies (UOB) or unilateral sections of the lateral olfactory tract (ULOT) were contrasted to control groups receiving sham operations (SO) or bilateral lesions of the dorsal hemispheres (BH, a control for operative trauma and nonspecific effects of brain damage on thermal responsiveness). Pups were tested daily on a thermal gradient from 3 through 15 days of age, and changes in their preference for the warm extreme and their activity patterns were described as a function of age.

Since the lateral olfactory tract is the main efferent pathway of the olfactory bulbs, LOT transection was used to simulate the effect of bulbectomy in disrupting the bulbar input to the cortex. Moreover, it is known that fiber readjustments after LOT transection lead to a partial restoration of the bulb's projection caudal to the cut and therefore denervation caused by LOT cuts is only temporary in some cortical regions in contrast to the permanent denervation resulting from olfactory bulb removal. The thermal task was used to determine if LOT transection produced effects similar to those observed after bulbectomy and to investigate whether recovery from these effects occurred during the initial post-traumatic period.

After completion of behavioral testing, pups that had received prior section of the lateral olfactory tract were given bilateral olfactory bulbectomies or injections of a labeled amino acid in order to characterize the projection from the bulb ipsilateral to the tract section. Each brain was assigned a number and anatomical analyses were conducted without knowledge of behavioral performance of the pup. The placement of lesions and the projection of the olfactory bulbs were described histologically and then related to the behavioral profiles of individual pups.

A summary of the number of litters and subjects included in each treatment group is presented in Table I.

All behavioral data presented in this paper are based on these subjects. The single sham operate included in each of the experimental litters was treated identically to its littermates and given daily tests, but data from these pups are not included.

Behavioral data came from two groups of litters reared consecutively, with each group containing a sample of UOB, ULOT and either BH or SO litters of pups delivered on the same date. Behavioral results were similar in both groups of litters and they have been combined for presentation.

Evaluating the effects of lesions on the behavior of pups reared sequentially in the laboratory, can confound lesion effects with many uncontrolled variables that can potentially affect developmental sequences. For this reason, a group of litters including both olfactory treatments and a control treatment was run concurrently. Potential variables range from temperamental differences observed in females ordered at different times of the year, to the overall amount and pattern of usage of the colony room. While the rigid sequence of thermal orientation seen in normal pups suggested that this was a robust pattern not readily modified by external factors, it was not known how susceptible lesioned pups might be to such influences.

The procedure of allowing females to cull their own litters did not work well in the case of one large litter containing 14 untreated normal pups. The litter size was not reduced through day 15 and this affected the pattern of weight gain and behavioral data. The data from this litter were not included since they were not consistent with data from intact pups tested previously, and since the data of sham operates resembled previously tested intact pups.

The use of pups with unilateral olfactory system lesions provided several advantages over bilateral lesions, although it required sensitive behavioral measures to detect effects. Pups were vigorous and gained weight steadily. The high mortality rates reported after bilateral olfactory lesions of neonatal rodents (Kling, '64; Singh & Tobach, '75) did not occur in this study, either as a result of the use of unilateral lesions or due to other procedures employed. Mortality rates calculated from the time of surgery on day 5 through 15 were 8.3% for the UOB group, 7.7% for the ULOT group, and 7.7% for the BH group; no deaths occurred after sham treatments. One pup per litter died in 3 of the 4 UOB litters, in 4 of the 6 ULOT litters, and in 1 of the 2 BH litters.

RESULTS

Behavioral Analysis:

Behavior on the thermal gradient clearly distinguished pups with unilateral olfactory lesions from pups with control treatments. Thermal scores representing the percentage of the test spent in the warm extreme of the gradient, tier 1, were calculated daily for all pups. The two main behavioral results are summarized by the median thermal scores of treatment groups shown in Figure 3.

The first finding was that both UOB and ULOT treatments resulted in a tendency for pups to display strong thermal preferences at ages when pups with sham operations and BH lesions had ceased approaching the warm end of the gradient. Prior to day 8, all groups regardless of treatment, spent at least 80% of the test in tier 1. After day 8, thermal scores decreased abruptly for BH and SO groups to less than 30% of the test time, while ULOT and UOB groups continued to show strong thermal responsiveness beyond day 8.

The strong preference for the heat of tier 1 that characterized young pups, was terminated abruptly in both BH and SO groups in a manner identical to that described for normal pups (Leonard, '74a). This sharp decline was altered by unilateral olfactory lesions but was not affected by bilateral lesions of the dorsal hemispheres.

The second and more salient behavioral finding was that while the ULOT treatment initially mimicked the effect of bulbectomy, this effect was transitory. Thermal scores of the UOB group remained high beyond day 9 and showed small gradual decreases with age. Scores of the ULOT group remained high beyond day 8 but then shifted in an abrupt manner at later ages. By day 11, thermal scores of the ULOT group had declined to control levels, while the UOB group maintained elevated thermal scores.

The first result shows that Leonard's ('76) finding that unilateral olfactory bulbectomy extends the period of thermal responsiveness, is an effect that can also be produced by sectioning the lateral olfactory tract. This suggests that maturational changes in either that portion of the olfactory bulb output contained in the tract projecting distal to the cut, and/or that portion of the olfactory cortex underlying the cut, are responsible for the abrupt transition from thermal orientation which normally occurs after day 8.

The second result, that ULOT lesions produced only a transitory effect, while UOB lesions had a more stable effect, suggests that between the time of the operation on day 5 and the time thermal scores reached control levels on day 11, some reorganization of olfactory processing must have occurred which was dependent on the presence of an intact bulb ipsilateral to the tract section. Fibers from the olfactory bulb might have regrown and reinnervated olfactory cortical areas as shown by Devor ('76b) and thus affected the transition from thermotaxis. This possibility will be examined following a more detailed comparison of the behavior of groups.

In order to contrast the persistence of effects resulting from ULOT and UOB treatments, thermal scores were averaged over days 8 to 10 days 11 to 13 to provide a thermal index for each pup at the two time points. The distribution of thermal indices is represented in Figure 4 by the mean and standard error of the mean of each group.

On days 8 to 10, the thermal indices of UOB and ULOT groups were significantly higher than those of control groups ($p < .001$, t test). No differences were observed between the indices of UOB and ULOT groups which spent an average of 65% and 59% of the test in tier 1, and no differences existed between BH and SO groups which spent 36% and 42% of their time respectively in the heat.

On days 11 to 13, the UOB group had significantly higher indices than all other groups ($p < .001$) while the ULOT group overlapped control groups. The BH treatment apparently produced no effect on thermal orientation as BH and SO groups did not differ at either age interval ($p > .05$).

Pups of all groups showed significant decreases in thermal indices with age ($p < .01$). Even though indices of the UOB group decreased with age, the amount of decrease was much smaller than that found in other groups. Their thermal indices remained higher than control and ULOT groups on days 11 to 13 ($p < .001$). The mean percent of time spent in tier 1 on days 11 to 13 was 51% for the UOB group versus 25%, 19% and 20% for ULOT, BH and SO groups respectively. A summary of the significance of t tests comparing group thermal indices is presented in Table II.

Thermal indices were used as a basis for distinguishing subgroups within the ULOT group. As it had not been possible to visualize the LOT or to confirm the accuracy of the lesion on day 5, this uncertainty provided an excellent 'blind' test of the validity of using thermal behavior as a marker for olfactory lesions. It was expected that the actual lesions might form a graded series ranging from total misses of the tract, to partial LOT sections, to complete sections with varying amounts of incidental damage to secondary areas. Thermal behavior of the ULOT group was analyzed with the intention of distinguishing cases forming the two extremes of this continuum.

The distribution of thermal indices of the ULOT group was compared with the distribution of indices of control (BH/SO) and UOB groups on days 8 to 10 as shown in Figure 5a, and two extreme behavioral subgroups were identified within the ULOT group: 1) NE (No Effect) - pups whose thermal indices on days 8 to 10 fell within one standard deviation unit above the mean of the control group were considered to be showing no effect of the lesion on thermal behavior (n = 11). It was expected that these pups had lesions that either totally missed the LOT or caused minimal damage to the tract. 2) Effect - Pups whose thermal indices were larger than a score one standard deviation below the UOB mean were considered to be showing a lesion effect and it was expected that they would have more extensive damage or complete sections of the LOT. These pups were then classified, as shown in Figure 5B, into recoverers, R, (n = 16) and nonrecoverers, NR, (n = 8) using the distribution of thermal indices for control and UOB groups on days 11 to 13 to establish criteria. Cases in the ULOT group which occurred in the region of overlap of UOB and control group distributions on days 8 to 10, or outside of cutoff points, were not included in the analysis.

Of the 6 ULOT litters tested, 5 litters contained pups in all three of the subgroups, NE, R, and NR. The sixth litter which was the first ULOT litter to be operated, contained a higher proportion of NE pups than other litters, presumably due to surgical inexperience, and its pups were only represented in NE and R groups.

Similarities in the developmental sequence of pups in NE and control groups and pups in NR and UOB groups are emphasized in the daily profiles of thermal responsiveness presented below. Pups in the recovery group showed a third pattern of change in thermal responsiveness that was clearly distinguishable from the profiles of pups in NE and NR groups.

The pattern of change in thermal scores shown in the daily group medians of Figure 3, reflected the patterns observed in individual pups. Daily records from 4 typical individuals from BH, SO and NE groups are shown in Figure 6a.

Thermal scores of NE pups show the same pattern of decline as that of pups in control groups. For most pups of these groups, a transition occurred over a one day period in which scores dropped precipitously from very high to very low levels. The fact that relatively few individuals showed an intervening day of moderate scores suggests that pups switched in an all or none fashion between behavior patterns that were probably mutually exclusive. On the first day that pups did not show a strong thermal preference, they frequently had a thermal score of zero; thereafter pups maintained low thermal scores, but the range of fluctuations of scores was broader than that observed during the earlier period of strong thermal responding because behavior at these older ages did not seem to be organized with respect to the thermal properties of the grid.

The similarity in the pattern of change in thermal scores is more easily seen if scores are expressed as a function of days before and after the decline occurred, so that the differences in the day of transition do not obscure changes in the underlying developmental process. The first day on which a pup's thermal score dropped below 30% was considered as day 0. The choice of 30% to define the day of the transition, was a post hoc decision based upon the magnitude of thermal scores seen in control groups after day 8 (see Figure 3).

Data of individual pups are presented as a function of the transitional day in Figure 6b. Note the abrupt shift from high to low levels of thermal responsiveness and the similarity in the pattern of decline observed within control and NE groups.

Median thermal scores of SO, BH and NE groups have been superimposed in Figure 7a as a function of age and in Figure 7b as a function of transitional day. No differences were noted in the pattern of decline in these groups.

Thermal scores from 4 representative individuals in UOB and NR groups are shown as a function of age in Figures 8a and 8b. Thermal scores remained high, although they tended to diminish gradually with age and often showed broad fluctuations. An analysis by transitional day was not possible for pups in these groups since no thermal score could be identified which would separate behavior into two distinct periods of different levels of thermal responsiveness.

Thermal scores of 4 representative pups showing recovery are presented in Figure 8c as a function of age. There is a clear transition from high to low thermal scores, but it is more gradual than that of control and NE groups, with one or more days of moderate scores intervening. In Figure 8d, the scores of these pups have been regrouped on the basis of days before and after a thermal score of less than 30% occurred.

The difference in the pattern of the transition of the recovery group can be seen more easily in Figure 9a which compares the median scores of the R, BH, SO and NE groups as a function of the day of transition. Thermal scores of recoverers begin to decline on days -2 and -1, and then drop on day 0 to a higher level than that of other groups. In comparison, SO, BH, and NE groups maintain high scores through day -1 and then drop sharply to low thermal scores.

The rate of decline in thermal scores of recoverers was contrasted to that of other groups by calculating difference scores on days -1 and 0. The mean difference scores shown in Figure 10 for BH, NE, SO and R groups were 74%, 77%, 62% and 47% respectively. These differences were tested in a one-way analysis of variance and found to be significant, $F(3.40) = 5.76$, $p < .01$. An F test (Scheffé method) showed that recoverers had significantly lower rates than BH ($p < .01$) and NE ($p < .05$) groups, but they did not differ significantly from the SO group. The difference observed between SO and BH groups was also not significant.

When thermal scores of the three ULOT subgroups are compared by age in Figure 9b, the same relationship between groups was observed in the age related change in thermal responsiveness (i.e., compare Figure 9b to UOB, ULOT and control groups shown in Figure 3).

The effect of organizing daily scores as a function of the transitional day can be seen clearly in measures of group variability shown in Figure 11a and b. The magnitude of the interquartile range of daily thermal scores is presented as a function of age in 11a to indicate the variability within each of the groups. A striking difference can be seen when BH, SO and NE groups are compared to R, NR and UOB groups. At early ages, the interquartile range of all groups was small or moderate, indicating homogeneity of thermal preferences within each group. The range expanded sharply beginning on day 8 for BH and SO groups and on day 7 for the NE group, reaching values of 74%, 64% and 58%, respectively. The increase in the range indicates that on these days, pups within each group constituted two separable populations based on their thermal behavior; some pups spent all of their time in tier 1, while other pups never entered tier 1. The range dropped at ages when the group again showed homogeneous behavior, with all pups now spending little time in tier 1. The slight asynchrony among pups in the age at which the transition from high to low thermal scores occurred, accounts for the expansion of the interquartile range.

When thermal scores are regrouped on the basis of the transitional day in Figure 11b, no expansion of the interquartile range is seen.

In comparison, the interquartile range of thermal scores of NR and UOB groups does not show a marked expansion in Figure 11a. These groups showed no sharp transition in their behavior on the gradient and there were no large differences in thermal scores among individuals at a given age. For the recovery group, thermal scores declined to moderate then low levels in a synchronous fashion so that the transition period was not characterized by a rise in group variability.

The percentage of pups reaching the transition at each day of age is shown in Figure 11c for BH, SO, NE and R groups. The modal day on which the transition occurred was day 9 for BH, SO and NE groups and day 11 for the recovery group.

Profiles of Behavior on the Gradient:

A sequence of three stages can be used to describe the changing response patterns observed in pups from control and

NE groups during their tests on the gradient from 3 to 15 days of age. Activity levels as well as the duration and distribution of heat bouts during these 3 stages are illustrated on Figure 12 by representative polygraph records from pups in BH, SO and NE groups. The profiles of pups in R, NR and UOB groups during these stages, are contrasted on the right side of the figure.

Stage I: 3-7 Days of Age

Through day 7, pups in all treatment groups could not be distinguished by their behavior on the gradient. As soon as a pup was placed on the cool end of the gradient, it turned toward tier 1, and moved rapidly across the grid without stopping until it reached tier 1. Once in the heat, the pup settled quietly, usually sprawled with its belly flat on the mesh. Pups occasionally readjusted their position, but they rarely became active, once in the heat, or backed out of tier 1. Pups were usually oriented with their heads deepest into tier 1, frequently with their snouts touching the wall bordering tier 1. The only other behaviors noted in tier 1 were grooming and yawning.

Latency to reach tier 1 and maximum bout duration were complementary measures at these young ages. Many pups traversed the grid in under 10 sec. thus moving at rates that exceeded 2.5 cm/sec. This was remarkably rapid since at these ages pups moved by dragging their bodies forwards with their forelimbs and they often flipped over and reached the heat by squirming on their backs. The group medians presented in Figure 13 show that pups entered tier 1 only once, usually reaching it within 10 sec, and then remained there for the duration of the test so that maximum bout duration was about 106 sec. Interquartile ranges on all measures indicated almost no variability among pups. Polygraph recordings during stage I show rapid activity before arriving in tier 1, followed by a cessation of activity once in the heat (Figure 12).

Rectal temperature recorded chronically during tests on days 3, 5 and 7 showed that pups began the test with core temperatures ranging from 33.5^{c} to 36 ^{c}C. Body temperature decreased by 1 °C or less until after the pup had settled in tier 1 and then increased gradually by about 1 °C during the remainder of the test. In most cases, core temperature was similar at the start and end of the test and the recordings were slightly concave.

Stage II: 8 - 10 Days of Age

The behavior of pups in control in NE groups was very variable on day 8, with some pups continuing the earlier pattern of rapidly entering and settling tier 1, while other pups moved very slowly about in the cooler regions of the gradient and showed no tendency to enter or settle tier 1. Latency to enter tier 1 increased and the interquartile range extended across the scale, while maximum bout length decreased and was also very variable.

By day 9, most pups no longer showed any preference for tier 1; when placed on the grid, they no longer turned toward tier 1 or began to orient along the gradient. Instead, the pups began to slowly investigate the center squares of tiers 2 and 3, moving from one small region to the next with their snouts positioned just above the mesh and often swinging their heads in a manner that suggested sniffing. The slow gaits recorded on the polygraph records of Figure 12 during stage II are the result of this slow exploration of the mesh. When the pups entered tier 1, they continued to be active and they usually left after a short stay causing maximum bout duration to drop to about 24 sec (Figure 13).

By stage II, the activity of pups in control and NE groups no longer seemed influenced by the thermal properties of the grid. It would not have been possible to predict from the pup's movement which tier contained the warm extreme of the gradient.

In contrast, the behavior of pups in UOB, NR and R groups showed no abrupt change from earlier behavior patterns during days 8 to 10. These pups continued to enter tier 1 with short latencies and to remain there for one or more long heat bouts (Figure 13a and b), during which they sprawled quietly on the mesh and often groomed.

The one change noted in the behavior of UOB and NR groups was a small increase in activity in tier 1. The pups readjusted their position sporadically so that at one moment their heads were at the extreme of the gradient and after a short time they would turn so that their rumps were at the extreme. These 'heat circles' became more frequent with age and account for the higher activity levels while in tier 1 seen on the recordings of Figure 12.

It was clear from the profiles of UOB and NR pups that their behavior on days 8 to 10 was a continuation of earlier patterns seen during stage I. There was no evidence for an abrupt change in their preference for the heat or any sign of exploratory behavior.

Recoverers behaved similarly to UOB and NR groups on days 8 and 9, but by day 10 they tended to combine the slow gaited pattern of movement with long quiet bouts in tier 1. Pups divided their time between these two patterns in either order, with some pups going directly to tier 1 for a long bout and then leaving to slowly explore the grid, while others explored first and then entered and remained in tier 1.

Rectal temperature was recorded on days 10 and 11 for 1 to 3 mins at an ambient temperature of 21 °C and showed that pups of these ages do not have mature physiological thermoregulatory ability.

Stage III: 11-15 Days of Age

After day 11, a new pattern was seen in the behavior of pups in control, NE and R groups. Pups now moved rapidly around the grid, covering its limits in only a short time as they followed the walls of the apparatus in a square shaped path. The pups often entered tier 1, but did not slow their gait or show a tendency to settle while there. Thus maximum bout duration was usually less than 10 sec (Figure 13). The pups were in continuous movement except for occasional pauses when they reached a corner and began to climb, or when sniffing or biting at the mesh. Temperature was not recorded during testing beyond day 11 because the high activity levels of the pups resulted in thermistor damage.

During stage III most pups in UOB and NR groups still entered tier 1 with short latencies where they were observed sprawled quietly on the mesh, grooming and making heat circles. Their behavior did change markedly from stage II and they now had multiple brief heat bouts during a test, interspersed with active forays around the grid.

These pups continued to express thermal preferences but had begun to integrate more mature patterns into their behavior. When placed on the mesh, their first tendency was to orient along the gradient so that latency to tier 1 remained short (Figure 13). They settled quietly in tier 1 but only for a brief bout. During a test several of such brief heat bouts were interspersed with periods of active locomotion during which the pups followed the walls in a square shaped pattern and were observed climbing in corners and sniffing. Pups in the UOB group entered tier 1 as frequently as pups in BH and SO groups, but they continued to settle while in tier 1. When they left tier 1, their square shaped path of locomotion brought them again into tier 1, causing them to settle briefly once more. Even on day 15 when these pups entered tier 1, they were observed sprawled on the mesh, grooming and making heat circles. They continued to show organized behavior patterns while in the heat, but they had also begun to respond to attractions outside of tier 1.

Summary of Behavioral Results:

1. Pups receiving sham operations and bilateral aspirations of the dorsal hemispheres responded to the thermal gradient with a developmental sequence identical to that described in normal pups.

Up to 8 days of age, pups showed an exclusive preference for the heat and spent almost the entire test in tier 1. Pups moved rapidly from their start position into tier 1 where they settled quietly for the remainder of the test. Thermal responsiveness did not fade gradually with age, but instead was dramatically suppressed by the strong emergence of exploratory behavior beginning on day 9. These two behaviors were exclusively displayed in a nonoverlapping sequence. Exploratory behavior changed with age from a slow investigation of the center of the grid to a very rapid path of movement around the walls of the apparatus.

2. Unilateral olfactory bulb removal (UOB) delayed the appearance of exploratory behavior and eliminated the abrupt transition from thermal orientation to exploratory behavior. Exploratory behavior appeared gradually with age but it never overcame the pups' tendency to first orient to the thermal gradient or to settle quietly for brief periods while in tier 1.

3. The thermal behavior of pups in the ULOT treatment group was used to define 3 subgroups:

a. No Effect (NE) - pups whose lesions produced no alteration from the normal developmental sequence. These pups displayed an exclusive thermal preference that was abruptly terminated on day 9 by the onset of exploratory behavior.

b. Recovery (R) - pups who showed an effect of the lesion on the onset of exploratory behavior and on the pattern of the transition from thermal orientation to exploratory behavior. These pups continued to display an exclusive thermal preference beyond day 9 but then gradually shifted to exploratory behavior by day 11. The shift occurred over a 1 or 2 day period during which the two behaviors overlapped.

c. Nonrecovery (NR) - pups whose lesions produced a behavior profile similar to the UOB treatment. Exploratory behavior emerged gradually with a delayed onset but it never entirely suppressed thermal responsiveness.

Anatomical Results:

I. ULOT Group:

There was a clear dichotomy between the lesions of those pups which had showed a normal decline in thermal orientation (e.g., NE group), and those pups which had continued to show thermal orientation beyond day 8 (e.g., R and NR groups). Lesions sustained by the NE group either did not involve the LOT or, in three cases, minimally encroached upon the tract, leaving a substantial projection to the olfactory cortex caudal to the cut. In contrast, almost all transections sustained by pups in the R and NR groups involved complete disruption of the LOT fibers with virtually complete denervation of caudal portions of the olfactory cortex. In nonrecoverers, LOT transection was accompanied by substantial damage to deep cortical layers underlying the tract or, in a few cases, damage extended into the contralatereal olfactory cortex.

In cases with LOT damage, indications of all the alterations reported in the adult olfactory bulb projection after neonatal surgery (Devor, '76b) had already appeared 10 days after the transection (see above, pp. 25-26. The extent of axonal alterations proximal to the cut was comparable to those described in adults while alterations caudal to the cut had not assumed their complete adult pattern. By ten days after complete LOT sections, fibers had reinnervated a restricted portion of the cortex behind the cut. After partial LOT sections, stunting was already evident in the caudalmost cortical regions and the wedge of cortex immediately behind the cut had been partially reinnervated.

The olfactory bulb projection of normal adolescent hamsters:

The distribution and organization of the olfactory bulb projection in normal adolescent hamsters at 15 to 18 days of age is briefly described below before discussing the locus of lesions in the ULOT group and alterations in the projection that resulted from LOT cuts made at 5 days of age. In adolescent hamsters, the bulb distributes to the same regions as in adult rodents (e.g., Devor, '76a; Heimer, '68; Powell, Cowan, & Raisman, '65; Price, '73, White, '65) although the cortex has not yet reached its full adult dimensions.

Fibers arising from mitral cells and perhaps tufted cells (Haberly & Price, '75) within the olfactory bulb exit from the caudal bulb and converge into the compact LOT as they pass through the anterior olfactory nucleus (Fig. 1). The terminals of these fibers form a continuous projection sheet in the superficial plexiform layer of cortical regions along the ventral surface of the ipsilateral forebrain. Fibers from the caudomedial part of the olfactory bulb project directly into medial parts of the projection field such as the hippocampal rudiment and medial olfactory tubercle without entering the LOT (Devor, '76a).

Caudal to the olfactory bulb, the projection encircles all parts of the anterior olfactory nucleus and then continues into the prepiriform cortex lateral to the LOT and into the olfactory tubercle and ventral portion of the hippocampal rudiment medial to the tract. The rhinal sulcus marks the dorsolateral boundary of the olfactory cortex throughout its rostrocaudal extent and separates it from the more dorsal neocortex. The medial extent of the projection is bounded by the dorsal portion of the hippocampal rudiment and then by the horizontal limb of the diagonal band of Broca and the anterior amygdaloid area. The projection continues caudally to the nucleus of the lateral olfactory tract at which point the fibers of the LOT splay out and no longer comprise a compact bundle. The bulb projects to the periamygdaloid cortex, the medial and cortical amygdaloid nuclei and to the entire extent of the lateral entorhinal cortex. Portions of the projection are shown in reduced silver stains, after bulbectomy at 15 days of age in Figures 14a-d.

All regions of the olfactory cortex share the basic three layered structure characteristic of paleocortex. Lamina I, the plexiform layer is relatively free of neurons and contains the apical dendrites of pyramidal and stellate neurons comprising lamina II. Lamina III is a deeper polymorphic cell layer. Lamina I has been subdivided into a superficial lamina Iα containing the LOT fibers, lamina Ia, the site of termination of olfactory bulb fibers, and a deeper lamina Ib, the site of termination of the association pathway (Fig. 14e). Fibers of the association system terminate as a dense band in the part of lamina Ib adjacent to lamina Ia (Devor, '76a) and more sparsely in the deeper parts of the ipsilateral olfactory cortex; in addition, this system projects to the sulcal neocortex and cortex dorsal to the hippocampal rudiment. Association fibers, as well as centrifugal fibers destined for the olfactory bulb, travel in close relation to the lateral olfactory tract and are severed along with bulb efferents when the LOT is sectioned. After complete bulbectomy, normal fibers of the association system are seen extending through the plexiform layer with many normal fibers visible in a band intervening between lamina Iα and Ia (arrow in Fig. 14e). The proportion of the plexiform layer receiving input from the bulb varies in different regions, in general decreasing gradually in the more caudal olfactory areas and in the medial and lateral extremes of the projection furthest from the LOT. Lamina Ia attains its greatest width in the plexiform layer subjacent to the tract (i.e., the "tract sulcus").

It is possible, even in normal material, to determine the border between laminae Ia and Ib. As noted by Price ('73) there are many small glial cells within lamina Ia which often form a line at the border between laminae Ia and Ib. Also, the neuropil of the two laminae has different staining characteristics and in a reduced silver stain, such as the Fink-Schneider stain, the neuropil of lamina Ia is sometimes stained distinctly darker than Ib.

Criteria to determine the level and completeness of transections made at 5 days of age:

The site of the day 5 transection was easily identified by a cluster of small cells which extended through the cortical layers perpendicular to the pial surface. The level of transection ranged from the mid anterior olfactory nucleus to the mid olfactory tubercle. In some cases the only evidence of the cut was the path of small cells, with all cortical layers appearing to be otherwise intact. In other cases the cortex showed an irregular contour at the lesion site, and in cases with the most extensive cuts, portions of the cortical layers had actually been removed or notched. Examples of transections from three cases in the recovery group are shown in Figure 15.

Between the time of surgery on day 5 and the time the projection was again investigated 10 days later, fibers of the olfactory bulb had reinnervated regions distal to the cut. Since a projection caudal to the cut present at 15 days of age could have originated from LOT fibers spared in an initial *partial* transection or from postlesion growth following a *complete* transection, it was necessary to establish unambiguous criteria for determination of the completeness of the initial cut.

The two criteria found most useful in defining the extent of the early transection were based on the typical location and appearance of LOT fibers and terminals in degeneration stains after olfactory bulbectomies. By 15 days of age, the LOT contains relatively thick, myelinated fibers which are concentrated in a dense compact bundle subjacent to the pia. The fiber bundle is located in the tract sulcus whose position is marked by a widening of the plexiform layer at the junction of the prepiriform cortex and the olfactory tubercle (arrowhead in Figure 14b). After bulbectomy, the degenerating, silver stained LOT fibers appear as coarse, irregular shaped spherules or elongate particles and form a well defined lamina Iα. In contrast, degenerating terminals of LOT fibers appear as fine homogeneous particles that extend as a dense, continuous band through lamina Ia medial, lateral and deep to the LOT forming a sharp border with lamina Ib (Fig. 16a). A laminar arrangement of coarse and fine silver particles is observed at survival periods ranging from 48 - 120 hrs after bulbectomy in adolescent hamsters.

In cases with previous partial LOT transections that received bulbectomies prior to sacrifice, coarse and fine grained silver particles appeared caudal to the cut in the normal location and typical laminar arrangement. Behind the cut, the width of lamina Iα was reduced and lamina Ia contained a low density of silver particles. Even though the projection was sparse, it could be traced caudally as a continuous band of silver particles and it formed a regular border with lamina Ib (Fig. 16b).

In contrast, after previous complete LOT section, most of the silver-stained particles caudal to the cut were fine grained and even the tract sulcus contained fine particles subjacent to the pia (Fig. 16c). Coarse grained silver particles, when present, were observed in anomalous positions either forming a narrow stratum in the olfactory tubercle or sometimes occurring in a deep part of the plexiform layer in close relation to the glial scar. Silver particles in the olfactory tubercle caudal to the cut were often clustered into discrete pockets separated by gaps free of degeneration (Fig. 16d).

Reduced silver stains have been used to illustrate details of the bulb's projection, rather than autoradiography, because the texture of degenerating silver-stained particles could be used to define different components of the olfactory pathway. The arrangement of different sized silver particles was found to be particularly valuable for distinguishing the caudal projection after complete and partial LOT section. Autoradiography proved a useful technique for defining the distribution of the pathway, but unfortunately it did not distinguish components of the pathway since silver grains overlying labeled parent fibers and terminal zones had an identical appearance. Even selective labeling of one component of the pathway such as axon terminals, is not possible with autoradiography when the post-injection survival period is manipulated.

Site of lesions in pups showing no effect of surgery on day 5 on thermal orientation (NE group):

The brains of 9 pups in the NE group which showed a normal decline in thermotaxis on day 8 were examined. The approximate, reconstructed locus of 8 of these lesions is indicated in Figure 17. In six cases, the LOT was not involved in the lesion. Two transections (273 and 282) were placed in the dorsolateral frontal cortex, with one of these cases overlapping the rhinal sulcus (282). Three cuts (291, 278 and 277) involved the cortex along the medial wall of the hemisphere (i.e., HR, medial portion of AO and medial OT). In case 303 the lesion occurred in the ventral portion of the anterior olfactory nucleus bulb did not overlap the tract.

Three pups had transections that involved the tract but spared a substantial part of the projection to regions caudal to the cut. These lesions resulted in some reorganization of the projection that is described in a later section. In case 289 the lesion damaged the medial extreme of the LOT in the rostral half of the anterior olfactory nucleus, and in case 271 the lateral half of the tract was damaged at the level of the mid tubercle. In the remaining ninth case, the second lesion extended beyond the site of the previous transection. It seemed, however, that the pup had previously received a partial transection since sparse degeneration was found throughout the projection field.

Within the NE group no relationship was found between the locus of injury and thermal scores. Thermal indices on days 8 to 10 are shown below each case number in Figure 17. The distribution of thermal indices of pups with no damage to the LOT ranged from 19% to 43%, overlapping the indices of pups with partial LOT sections which ranged from 18% to 32%.

Since damage to the neocortex or to the medial wall of the hemisphere had no effect on thermal orientation in pups from the NE group, incidental damage to these areas which accompanied some of the larger cuts in the R and NR groups, was probably not the basis for the behavioral effects observed in those groups.

Site of lesions in pups showing persistent thermal orientation after surgery at 5 days of age (R and NR groups):

Histological examination of the brains of 17 pups from the R and NR groups showed that in all but two cases, the lesions sustained by these pups involved complete unilateral destruction of the LOT. In several cases, LOT transections were accompanied by additional damage to the deep cortical layers or to the contralateral cortex.

A sample of three lesions from the recovery group, shown in Figure 15, was selected to illustrate the range of damage seen in the R group. Case 279 (Fig. 15a) had the least amount of damage to the tract and is one of the two cases from R and NR groups with spared LOT fibers. The transection, at the level of the rostral tubercle, removed a large wedge of the plexiform layer through the center of the tract, sparing 25% of LOT fibers at the lateral extreme and 6% of fibers at the medial extreme of the tract, as determined by planimetric measures of cross sectional areas. In case 272, shown in Figure 15b, the LOT was completely severed at the level of the rostral tubercle and the knife tract is marked by a path of small cells. In case 275 (Fig. 15c) the cut removed a substantial part of the lateral portion of the anterior olfactory nucleus lying deep to the LOT, in addition to destroying the tract.

A comparison made of lesions from 12 cases showing recovery and 5 cases showing no recovery on the thermal task revealed that pups in the NR group had more extensive and deeper transections than pups in the R group. Lesions from the NR group are shown schematically in Figure 18, and thermal indices of days 8 to 10, and on days 11 to 13 are shown below the case numbers. In 4 out of 5 NR cases, portions of cortical laminae II and III were destroyed at the lateral margin of the anterior olfactory nucleus, sometimes continuing into the rostral olfactory tubercle, while only 2 out of 12 cases in the recovery group had a similar degree of damage to deep cortical layers. Case 295, the second case with spared LOT fibers, had bilateral partial transections of the LOT which spared 20% of fibers in one tract and 58% of fibers in the contralateral tract. The more extensive damage seen in these cases was probably the result of rotating the knife during surgery. This had been done to avoid the extreme possibility that all ULOT pups might have only partial LOT transections.

By 10 days after LOT transection a remarkable amount of reorganization had occurred in the olfactory bulb projection. These rearrangements are briefly summarized here and described in greater detail in a later section (see pp. 87-99). In cases from both R and NR groups, the projection field rostral to the cut showed an increase in the density of terminals and in the width of lamina Ia as well as an extension of the terminal zone into regions adjoining the olfactory cortex but not typically innervated by the olfactory bulbs (Figs. 19, 20 and 21).

The recovery group showed, in addition, an extension of the projection distal to the cut in all but two cases. Pockets of degeneration were consistently found in the lateral portion of the olfactory tubercle and three cases also had pockets of degeneration in the lateral part of prepiriform cortex adjacent to the tract sulcus. Such distal extensions of the projection were not present in the nonrecovery group. In two nonrecovery cases, fiber growth was present in an anomalous position at the level of the scar. Apparently, the more extensive damage to the cortical laminae in the NR group delayed, but did not prevent the growth process, and perhaps with additional time these cases would also have shown fiber growth distal to the cut. In two recovery cases with deep extensive cuts, fibers had taken the same path through the scar as in NR cases, but a greater number of fibers had penetrated into regions behind the scar by the time of sacrifice than in NR cases.

Two cases included in the recovery group on the basis of thermal scores on days 11 to 13, showed no projection caudal to the cut. This may have been an artifact of histology since degeneration in young brains can fade rapidly prior to staining if sections are not maintained at low temperatures. Another possibility that was not examined in this study is that the optimal survival period after bulbectomy for staining new growth caudal to the transection, differs from the 48-120 hrs range which was found effective for staining untransected fibers in adolescent hamsters. It may be that a shorter postop interval (e.g., 24 hrs) would have resulted in the staining of more postlesion growth. However, it was also noted that these two cases represented the two highest thermal indices included in the recovery group. The shape of the distribution of thermal indices on days 11 to 13 (Figure 5b) actually suggests a continuum rather than two distinct populations.

Within the nonrecovery group, the two cases with the most extreme thermal indices on days 11 to 13 (295 and 298) both had bilateral damage to the lateral olfactory tracts. The thermal indices of these two pups on days 11 to 13 actually exceeded those of most pups in the UOB group. This is of interest because Leonard ('76) has shown that bilateral olfactory bulbectomies lead to even stronger thermal preferences at older ages than unilateral bulbectomies. A third case in the NR group with bilateral LOT damage (286) had considerably more LOT fibers spared in the contralateral tract and its thermal index on days 11 to 13 was consistent with those of the two cases (284 and 281) with unilateral tract damage.

Alterations of the olfactory bulb projection system seen at 10 days after <u>complete</u> transection of the LOT:

Complete LOT transection caused extensive alterations in the distribution and density of the olfactory bulb projection which are shown schematically in Figure 21b. Changes were noted in the ipsilateral olfactory bulb and tract and these are discussed later (see pp. 93-99).

At levels rostral to the site of the lesion, the width of lamina Ia and the density of its terminals was increased in all parts of the anterior olfactory nucleus and hippocampal rudiment (Figs. 19 and 20a - c). At these same levels, the width of the plexiform layer was often reduced by 25% or more. The specific reduction varied in the rostrocaudal dimension and between brains, but in general, the dorsal portion of the anterior olfactory nucleus showed the greatest reduction in width. (Compare Figures 20a1 and a2.) The thickness of the plexiform layer was difficult to determine precisely because in many regions the neurons of lamina II were irregularly arranged after LOT transection. (See Figures 20b1 and b2.) Since the absolute width of lamina Ia had increased in most regions, the decrease noted in overall width of the plexiform layer represented a drastic reduction in lamina Ib. In the plexiform layer adjacent to the tract (e.g., the lateral portion of the anterior olfactory nucleus) the width of lamina Iα was decreased and that of lamina Ia was increased so that the combined width of laminae Iα and Ia was often the same on both sides of the brain.

The projection to the ventral portion of the hippocampal rediment on the medial wall of the hemisphere was increased in density and width. The projection continued into the dorsal part of the hippocampal rudiment, its sulcus and beyond (Fig. 20c), remaining restricted to the superficial part of the plexiform layer. Normally the olfactory bulbs do not project to the dorsal part of the hippocampal rudiment, and the entire width of the plexiform layer is filled by a projection from the association pathway (Price, '73). After LOT transection a maximum extension of 900 μm along the medial wall was seen relative to the dorsal limit of the contralateral projection. At the level where the plexiform layers of the anterior olfactory nucleus (the dorsal portion) and the frontal neocortex (sulcal cortex) lie adjacent with no intervening membrane, olfactory bulb efferents had profusely invaded the superficial part of the neocortical plexiform layer, projecting as a wide band of relatively high density (Figs. 19 and 20a).

The projection caudal to the transection was derived from various routes. In some cases, no coarse degeneration was observed at or behind the level of the scar, and terminal degeneration caudal to the scar appeared continuous with fine degeneration in the tubercle and lateral prepiriform cortex rostral to the cut. In other cases, coarse fibrous degeneration was present in anomalous positions. Coarse particles were observed in several cases as a narrow stratum subjacent to the pia in the lateral olfactory tubercle, extending laterally up to the glial cells of the scar where they took a deep route following the walls of the scar. In cases where the cortex was severly distorted by deep transections that destroyed almost all of the lateral portion of the anterior olfactory nucleus, it was impressive to observe a band of coarse degeneration extending through the lesion site. Regardless of the depth of destruction, the fibers appeared subjacent to the pia, occurring among the cells that now formed the superficial extent of the cortex.

Degeneration caudal to the cut was sparse and showed various distributions. Fine particles were usually located close to the pia but in some regions such as the tract sulcus, they were not densely packed (Fig. 16c). The particles formed a band for short stretches, but more often they occurred as discrete pockets clustered at the

Isles of Calleja in the olfactory tubercle (Figs. 16d and 20d and e). In a few cases fine degeneration was also present in the lateral prepiriform cortex but the projection to the tubercle extended caudally for far greater distances than the projection to the prepiriform cortex. By 10 days after surgery, a projection could be traced for distances ranging from 0.7 mm to 1.65 mm caudal to the scar. At its caudal limit, the projection appeared as an isolated patch of degeneration. The case that showed a projection extending caudally to the cut for the greatest distance (a patch of fine particles could be traced to just beyond the caudal extreme of the olfactory tubercle), showed a relatively small increase in the projection to rostral parts of the cortex when compared to cases with less extensive caudal growth. There was considerable variation noted between brains in the overall amount of postlesion growth as well as in the specific pattern of growth at levels rostral and caudal to the section. These variations were probably related to differences in both the level and severity of the transections.

Alterations in the olfactory bulb projection seen 10 days after partial LOT transections:

The projection field rostral to partial LOT transections showed several indications of the alterations noted after complete cuts but they were expressed in a restricted fashion with extensive variation from brain to brain (Fig. 21c). The most consistent observation was an increase in the density of the projection to lamina Ia in all parts of the anterior olfactory nucleus. Lamina Ia had expanded in width in the lateral part of the nucleus in some cases, but elsewhere there was only slight and sometimes no indication of an expansion in the width of lamina Ia. The fiber layer, lamina 1α, showed a reduction in width while the density of terminals and occasionally the width of lamina Ia was increased. The overall thickness of the plexiform layer did not show shrinkage similar to that seen after complete transections. In one case a slight invasion of the dorsal portion of the hippocampal rudiment was observed and in two cases the projection had just penetrated into the sulcal neocortex.

Immediately caudal to the cut, the density of the projection was reduced throughout the field. When cuts were restricted to the lateral or medial margins of the LOT, the cortex adjacent to the cut showed an even greater reduction in density of terminals than the cortex on the opposite side of the tract, although sparse degeneration argyrophilia suggested some remaining innervation. At the lateral and medial extremes of the projection field. at levels caudal to the cut, the band of degeneration became even more sparse and was separated from the pial surface by a gap. Initially the gap contained a few silver particles, but as the gap widened at more caudal levels, fewer particles were observed between the sparse and displaced band of silver particles that formed lamina Ia and the pial surface. By the level of the nucleus of the lateral olfactory tract a gap had also appeared under the remaining fibers of the LOT as well as throughout lamina Ia.

With increasing distance laterally and caudally from the tract, the width of the sparse band of particles comprising lamina Ia became severly reduced relative to the contralateral lamina Ia. In the prepiriform cortex at the level of the medial amygdaloid nucleus, only occasional silver particles were present in lamina Ia and these were separated by at least 50μm from the pial surface. Signs of a projection disappeared gradually between the caudal prepiriform cortex and the entorhinal formation as the band of particles became more sparse and narrow, and failed to reach the lateral margin of the olfactory cortex marked by the rhinal sulcus. In cases in which the accessory lateral olfactory tract had been spared in the LOT section, a dense projection appeared in and around the nucleus of the lateral olfactory tract. This relatively dense projection was present in the amygdala at levels where almost no projection was evident in the lateral projection field.

Figure 21 contrasts the normal olfactory bulb projection at 15 days of age, with the projection seen in one case with a complete LOT transection and another case with a partial transection. The level of the schematics is indicated on the right of Figure 1.

Alterations within the olfactory bulb and lateral olfactory tract following LOT transection:

Changes were noted in the olfactory bulb ipsilateral to the transected LOT (OB_i) as well as in the lateral olfactory tract at levels rostral to the cut.

By 5 days after surgery, the total volume of OB_i was smaller than the contralateral bulb, OB_c, in four cases observed after either complete or partial LOT section. In Figure 22, sections are presented from a 10 day old hamster sacrificed 5 days after receiving a transection that destroyed most of the LOT. Although OB_i had enlarged considerably during the 5 days following surgery, its rate of growth was less than that of the contralateral bulb as can be seen in Figure 22a. Small reductions ranging from 8% to 15% were noted in the cross sectional area of OB_i as compared to OB_c, determined by planimetric measures of frontal sections, and comparable decreases were observed in the overall length of the mitral cell layer on frontal sections calculated with a map measure. Shortening was also noted in the rostrocaudal dimension of the bulb.

The number of mitral cells contained within the mitral cell layer of OB_i was reduced when compared to OB_c. In Nissl stains, the mitral cell layer of OB_i appeared less densely stained and narrower than OB_c and its cells were arrayed in a less regular manner. The medial margin of the two bulbs has been enlarged in Figures 22b and c, to show the changes within the mitral cell layer. In small regions, particularly along the ventral and lateral margins of OB_i, the mitral cell layer seemed to be represented only by granule cells.

In Figures 22a and b it can also be seen that the external plexiform layer, EPL, of OB_i had become filled with mitral cells throughout the circumference of the bulb. An increased density of relatively large cells was most apparent in the middle third of the plexiform layer, as shown in Figure 22b (see arrows). Cell counts taken from the medial and lateral margins of OB_i showed that the density of mitral cells within the mitral cell layer was reduced approximately 30%, with the lateral margin showing slightly higher reductions than the medial margin. Since the volume of OB_i was smaller than OB_c, the actual reduction in the total number of mitral cells within the mitral cell layer throughout the bulb was probably somewhat greater than 30%.

The decrease in mitral cell density was accompanied by a reduction in the number of fibers seen emerging from the caudal extent of the olfactory bulb. The maximum width of the bundle at the level of the caudal bulb, showed reductions of up to 40% relative to the contralateral tract. This measure did not take into consideration changes noted in the density of fibers contained within the tract (e.g., see Figures 19 and 20b). A decrease in LOT fibers was already evident 5 days after transection, and can be seen in Figures 22e and f which compare the two tracts in a 10 day old hamster at a level rostral to the cut.

Figures 22e and f are enlargements of a section adjacent to that shown in Figure 22d, taken from the regions marked by the arrowheads. The tract has been stained with the Fink-Schneider procedure which impregnates normal as well as degenerating fibers and terminals. The thickness of the fiber band in Figure 22e ipsilateral to the cut (see bracket) is greatly diminished when compared to the undisturbed tract shown in Figure 22f. In addition, the normal fibers present in lamina Ib of the intact side (see arrow), are not observed in lamina Ib on the lesioned side, suggesting a loss of the association projection to levels rostral to the section. Very few degeneration particles were seen in lamina Ib of the lesioned side, suggesting that fibers of the association system which were severed by the transection, had not acquired the characteristic of long lasting degeneration at 5 days of age.

The decrease in the number of fibers emerging from OB_i suggests that many of the mitral cells seen in the external plexiform layer may no longer be contributing fibers to the LOT. There is typically a large population of tufted or displaced mitral cells present in EPL of a normal bulb, but most evidence suggests that few tufted cells contribute an axon to the lateral olfactory tract (e.g., Hinds & McNelly, '77; Price & Sprich, '75; Valverde, '65; also see Haberly & Price, '75 for opposing evidence).

It was noted that the internal plexiform layer, IPL, deep to the mitral cell layer showed a 20% increase in width in OB_i (Figs. 22a, b and c). Since axons of tufted cells as well as centrifugal fibers are thought to distribute to the internal plexiform layer

(Cajal, '11, '55), the expansion of IPL after LOT transection may in part represent an increased projection to IPL arising from the expanded population of displaced mitral cells now present in the external plexiform layer.

An intense glial reaction was noted 5 days after surgery which extended from the external plexiform layer of OB_i (Fig. 22b), through to the plexiform layer of the lateral portion of the anterior olfactory nucleus (Fig. 22d). The glial reaction was probably related to the disruption of rostrally directed fibers of the association pathway which distribute to the plexiform layer of the anterior olfactory nucleus, and to the disruption of centrifugal fibers to the bulb which travel in close relation to the tract to terminate in EPL of the bulb (Price & Powell, '70).

The last change observed at 5 days after surgery in OB_i was a diminished cell density within the inner granule layer. This layer normally contains a dense inner core of cells as shown in Figure 22a (see arrow). After LOT section, the dense core was reduced throughout the rostral half of OB_i. This dense core represents the rostral portion of a continuous stream of cells extending from the anterior wall of the lateral ventricle to the inner layer of the olfactory bulb. Cells of this stream continue to proliferate at the ventricle and migrate into the deep layers of the olfactory bulb throughout the postnatal period in rodents (Altman, '69; Hinds, '68a, '68b). One factor in the reduced granule cell density in OB_i may have been that after LOT section, some of these cells deviated toward the region of the cut, but it is not clear if this observation completely accounts for the reduction in cell density within the granule layer of the bulb.

No effects of the LOT transection were noted in the olfactory nerve layer or glomerular layer of the ipsilateral olfactory bulb.

At older ages OB_i appeared normal on gross inspection, but several alterations observed at early postoperative ages could still be detected in attenuated form. A reduction in the volume of the bulb was most noticeable by a foreshortening in its rostrocaudal dimension, while the internal plexiform layer still showed regions of expanded width. The adult mitral cell layer is not very densely packed with mitral cells and the reduction in the number of mitral cells of OB_i was most easily detected by an indirect measure, a diminished width of the fiber layer emerging from the bulb. The glial reaction noted at 5 days postop, was greatly reduced by 10 days after surgery. Even in adulthood, however, it was still possible to detect a slightly higher density of glial cells in the external plexiform layer of OB_i.

By ten days after complete LOT transections, it was noted that although a considerable number of fibers emerged from OB_i, the size of the fiber bundle dwindled rapidly as the tract progressed through the projection field rostral to the cut. The point of maximum width of rostral portions of both tracts was measured at successive 225μm intervals beginning at corresponding levels just behind the bulbs. The two cases presented in Table III show that the tract ipsilateral to the transection contained fewer fibers at levels rostral to the transection and there was a shortening of its fibers so that very few fibers actually remained in the tract at a level just before the transection. The extent of axonal arborization at levels proximal to the transection, appears to have increased greatly as a result of the lesion , since the reduced fiber population gave rise to a lamina Ia of expended width, density and topography.

II. UOB Group

The brains of 15 pups or 45% of the UOB group were examined at ages ranging from 16 to 24 days to determine the completeness of the bulbectomy. Complete bulbectomy was defined by the absence of mitral cell axons within the LOT. These criteria were applied stringently because there were some surprising alterations seen after bulbectomy.

The gross appearance of these brains was striking. In all cases, the remaining anterior olfactory nucleus had filled in the space vacated by the bulbectomy and it was only by the absence of the fissure normally present on the dorsal surface between the frontal cortex and olfactory bulb and the absence of a myelinated LOT on the ventral surface that the abnormality could be detected.

The lesions had been well standardized and, in most cases, extended along the ventromedial wall of the hemisphere to include a small portion of the rostral anterior olfactory nucleus. In two brains the intact contralateral bulb had shifted to extend slightly beyond the midline; other than a superficial distortion, there was no apparent disturbance to the cell layers or fibers of the bulb. No signs of degeneration from the day 5 bulbectomy remained in lamina 1α and 1a. There was s striking increase in the number of fibers in lamina 1b at the prepiriform cortex and the LOT sulcus (Fig. 23f), suggesting that the sprouting of the association pathway seen in adulthood after neonatal bulbectomy (Moxley & Price, '74; Westrum, '75a) actually occurs soon after surgery. In addition, shrinkage was observed in the width of the plexiform layer of the tract sulcus and adjacent cortex which was most noticeable in the rostral half of the olfactory cortex.

Histology showed that in all these brains there was a thick zone of small 'granule' cells present on the ventral and rostral surfaces of the remaining olfactory stalk. Rostral to the granule cells, there was a plexus of neural tissue resembling the olfactory nerve layer of an intact olfactory bulb. In those brains in which a portion of the nasal epithelium and cribiform plate was left attached to the brain, it was clear that there were indeed olfactory filaments perforating the cribiform plate and occurring in association with the stream of granule cells.

These observations were of interest because of recent demonstrations of continued neurogenesis and synaptogenesis in the primary and secondary olfactory centers of adult rodents (e.g., Graziadei, '77; Kaplan & Hinds, '77; Moulton, '74). An additional study was therefore undertaken to confirm and extend the observations made on the UOB group and these results are presented in the appendix (see p.126).

DISCUSSION

The Process of LOT Reorganization After Early Transections:

The alterations observed in the olfactory bulb projection 10 days after LOT transection can be summarized with respect to the three dimensions of the normal bulb projection: i) Rostral to the transection, the density of terminals to lamina Ia increased and branches proliferated into nonolfactory cortical regions adjacent to the medial and lateral borders of olfactory cortex; ii) Also at rostral levels, terminals branched out from their normal superficial position adjacent to the pial surface, as a continuous band that reached deeper into the plexiform layer. Even at anomalous positions, terminal outgrowth always filled the most superficial position and then continued secondarily for various depths into the plexiform layer; iii) Caudal to the cut, outgrowth was very sparse and occurred as separated clusters of fibers in the olfactory tubercle and, less frequently, in the prepiriform cortex.

The alterations when viewed in conjunction with rearrangements present in the adult after neonatal LOT cuts (Devor, '76b) make it possible to describe the process of axonal reorganization. The proliferation of terminals that occurred rostral to the lesion was very rapid and appeared to be complete 10 days after surgery, since the growth was as extensive, or even greater in some regions, than that seen by adulthood. Devor observed a more extensive projection caudal to the cut in adults, than that present at 10 day survival periods. Growth caudal to the cut either occurred slower and was continued for a more prolonged period, or else it began with a delayed onset. Intermediate survival periods between day 10 and adulthood are needed to decide between these alternatives.

Evidence for a topographic organization in the projection from the olfactory bulb to the cortex has not as yet been demonstrated, although this by no means precludes its existence. In contrast, the projection has been shown to be strictly laminated in the vertical dimension (Price, '73); olfactory axons always fill the most superficial aspect of the plexiform layer before penetrating it deeper. The rearrangement of LOT terminals that occurred after day 5 lesions showed a similar lack of specificity of connections formed in the horizontal plane while the vertical laminar organization was conserved even when local changes in depth occurred or when olfactory terminals invaded nonolfactory regions. This suggests that similar mechanisms may serve to guide and restrict the growth of both developing and regenerating olfactory axons, although the relative influence of these factors may vary with age.

This study, along with many other recent studies, demonstrates that neurons can form new synaptic connections in response to CNS injury and that synapse formation in regenerating systems may result in atypical patterns of connectivity. It is therefore possible that mechanisms regulating the specificity of connections during embryogenesis do not rely upon intrinsic specification of synaptic partners but instead are based upon extrinsic factors such as the staggered time of origin and differentiation of different neuronal populations and the availability of postsynaptic sites at the time of axonal outgrowth.

This suggestion is supported by in vitro studies of synaptogenesis which indicate random rather than directed movement of axonal growth cones (e.g., Pfenninger and Rees, '76; Rees et al., '76). While neurons in cultures are not subject to the complex set of factors that can influence in vivo growth, they do represent regenerating populations whose outgrowth and synapse formation is easily observed. The behavior of outgrowing neurites in culture suggests two general guidance factors: i) There is a tendency for growing neurites to maintain contact with supporting cells (Grainger and James, '70); and ii) axons tend to show mutual contact and grow in bundles (Nakai, '60), With these generalizations in mind, I will review the sequence of events that was observed after early axotomy of the mitral cell axon, and try to determine which aspects of growth can be understood by nonspecific extrinsic factors and which require the postulation of an intrinsic control mechanism.

At the time of surgery, postnatal day 5, mitral cell axons in the hamster seem to be present throughout all but the most caudal regions of the olfactory cortex. Olfactory bulbectomy at this age produces short-lasting degeneration throughout the olfactory cortex except in the entorhinal cortex and cortical amygdaloid nucleus (Leonard, '75). The precocial appearance of axons at their peripheral destinations prior to dendritic or axonal synapse formation is a frequent observation (e.g., Domesick, '74; Stensaas, '67). Leonard inferred that a regional pattern of synapse formation occurred within the olfactory cortex over an extended period from birth through day 13 based on the ages at which long-lasting degeneration appeared in different regions.

An extensive series of studies has been done on the mouse olfactory bulb which covers the time of appearance of neuronal types (Hinds, '68a;b), their differentiation (Hinds, '72a;b) and synaptogenesis within the olfactory bulb (Hinds and Hinds, '76a;b). While developmental timetables in the mouse neocortex (Angevine and Sidman, '61) and hamster neocortex (Shimada and Langman, '70) are quite similar, it is not known if a similar parallel exists for the basal forebrain. Nevertheless, the same overall sequence that is described below for the mouse, probably applies to both species though absolute ages may vary for events.

Mitral cells show the earliest time of origin and differentiation within the bulb and within a day or two of cell birth, their axons course into the LOT (Hinds, '72a). Since the very latest date of birth of mitral cells is E17 in the mouse, this suggests that most mitral cells in the day 5 hamster, probably contribute an axon to the LOT. (The hamster day of birth occurs on E16). All types of synapses present in the mature olfactory bulb, already occur at low frequencies prior to birth in the mouse. The basic circuitry of the bulb would appear to be present at birth, even though synaptogenesis at all levels within the bulb continues well into the postnatal period. Synaptogenesis proceeds in the general direction of superficial to deep layers. The synapses formed the earliest are in the glomerular layer, (present at E14), and primarily involve junctions between olfactory axons from the epithelium and mitral cell dendrites. Synapses are then observed in the external plexiform layer (EPL) and include contacts made by centrifugal fibers onto granule cells and dendrodendritic reciprocal synapses between granule and mitral cells. Synaptogenesis begins in the deeper, internal granular layer last. While there is much conflicting information about the time of synapse formation on axonal and dendritic poles of neurons at different regions, Hinds ('76a) concluded that for mitral cells synapse formation at the axon begins either after or simultaneous with dendritic synapse formation.

Extrapolating from these results to the hamster, it seems reasonable to conclude that at the time of surgery on day 5, the axons of most mitral cells have entered the LOT and been transected. Mitral cells have most likely already formed many contacts with primary olfactory axons in the glomeruli, and they have perhaps established some dendrdendritic contacts with granule cells in EPL.

Within 5 days of LOT section almost all signs of Wallerian degeneration is the distal portion of the transected mitral cell axon have disappeared. This result agrees with the rapid onset and time course of anterograde degeneration that is generally observed after lesions at young ages.

Also seen at 5 days survival, was an intense glial reaction in the ipsilateral plexiform layer of the anterior olfactory nucleus and in EPL of the bulb. These regions receive fibers from the association system and centrifugal fibers to the bulb. The glial reaction suggests that axons in both of these systems have been interrupted by the LOT transection. The absence of stainable degeneration in the distal portion of the cut fiber of these systems indicates a rapid clearing of axonal debris.

Another event observed at 5 days postop was a reduction in the number of mitral cells contained with the mitral cell layer and the appearance of many mitral cells in EPL. The decrease in the size of the LOT at caudal levels of the olfactory bulb suggested that many mitral cells no longer contributed an axon to the tract. The reaction of mitral cells probably involves many factors, some of which are indicated below.

The mitral cell reaction might be an anterograde transneuronal effect due to the disruption of centrifugal inputs onto granule cells which then secondarily interrupts the formation of reciprocal dendrodendritic contacts between mitral and granule cells in EPL. The atypical peripheral position of the mitral soma suggests that differentiation of the portion of the dendritic tree contained in EPL has been disturbed. Growth patterns of dendrites are known to be regulated extrinsically by the afferents that dendritic growth cones encounter (e.g., Vaughn et al., '74). The elaboration of the mitral cell dendritic tree occurs simultaneous with afferent synapse formation (Hinds, '76a), and since synapse formation in EPL begins later than in the glomerular layer, at the time of surgery, dendritic differentiation in EPL is probably particularly vulnerable to disruption by deafferentation. Anterograde transneuronal effects are known to be very common after lesions in young animals (for review see Cowan, '70).

As mentioned above, all types of synapses seen in the mature olfactory bulb are present at birth, although they occur in small numbers. Therefore, transection of mitral cell axons on day 5 can produce effects on a system whose basic circuitry is already established. One effect noted within the bulb after the lesion, was an increase in the width of the internal plexiform layer (IPL), a region that normally receives terminals from tufted cells of EPL as well as from centrifugal fibers to the bulb. It is possible that early LOT can produce effects on the circuitry of the bulb similar to the types of reorganization demonstrated in the cortex. The expansion of IPL might be due to an increased projection from the many mitral cells now displaced into EPL; the reduced size of the LOT suggests that if these cells retain an efferent process, its terminal field may be restricted to within the bulb.

An alternative or additional aspect of the mitral cell reaction might involve a direct retrograde response to axotomy. Retrograde effects are particularly rapid and severe at young ages and often lead to cell death. Moreover, the reaction may extend into the dendritic tree causing a separation of afferent terminals (Purves, '75; Rotter et al., '77) and can alter the interaction between glial cells and neuronal soma (Grafstein, '75). Displacement of mitral cell perikarya might be the outcome of several events including a disturbance in the differentiation of the dendrites, a retraction of afferent terminals, an altered association between glia and soma and nuclear eccentricity.

Proximity of the lesion to the cell body is one factor which increases the amount of cell death after axotomy. It is rather surprising to find that the majority of mitral cells survived transection at the level of the anterior olfactory nucleus. Ramón y Cajal ('28) suggested the importance of "sustaining collaterals". The fact that mitral cells survived axotomy near the soma may reflect the presence of terminal branches at levels rostral to the cut as well as recurrent collateral branches that terminate within the bulb.

An interesting question arises with regard to the differences between mitral cells that are displaced and those not showing a reaction to the lesion. Perhaps this is due to differences in the stage of differentiation since there is a spread of a few days in the time of origin of mitral cells. It has been suggested that young cells which are engaged in the synthesis of structural proteins needed for axonal and dendritic outgrowth, are more susceptible to cell death from axotomy than more mature cells which have shifted their metabolism towards the production of enzymes and secretory proteins required for transmitter release (LaVelle and Smoller, '60). Differences in the mitral cell reactions might also indicate that mitral cells differ in their terminal fields; some might contribute terminal branches only to caudal parts of the cortex.

Two extrinsic factors, competition between axon populations and guidance mechanisms, may have contributed to the pattern of axonal outgrowth resulting from the LOT cuts.

LOT transection disrupts fibers of the association system projecting to lamina Ib in AON and to sulcal and infralimbic cortex, the exact regions in the rostral field where olfactory axons proliferated. The lesion has vacated a large number of postsynaptic sites in the immediate area of the proximal axonal stump. The availability of postsynaptic candidates may be the most important factor in determining what is an "appropriate" target for olfactory axons. Normal competition during ontogeny between association and olfactory fibers would result in the usual borders observed in the projections of these two systems. The continued expansion of the forebrain during development, would make additional sites available to olfactory axons with little competition from other fiber systems after LOT transections.

Since the lesion also reduced the number of mitral cells contributing an axon to the LOT, many sites in lamina Ia were also made available and filled in by the remaining LOT fibers. This process can be inferred from the observed high density of terminals in lamina Ia 10 days after surgery.

Caudal to the cut a reciprocal process occurs, and sites in lamina Ia made available by the lesioning of olfactory axons, are filled in by the association system (Moxley and Price, '74; Westrum, '75). This means that the lesion has created a proximal field containing many available postsynaptic sites at which competitive interactions are minimal, and a caudal field also containing available sites at which olfactory axons would encounter heightened competition due to their late arrival.

In normal development the mitral cell precocially extends an axonal process throughout most of the cortex. The axon is elaborated immediately after the mitral cell assumes its position in the olfactory bulb, well before cortical synapse formation occurs. This probably allows the axon to effectively compete for postsynaptic sites as they become available at any point along the course of the axon. The precocial development of the axon is probably necessary for synapse formation to occur independently at different cortical regions as observed by Leonard ('75), rather than along a strict rostrocaudal gradient.

The depth of lamina Ia normally decreases in the cortex with increasing distance medially, laterally and caudally from the tract. Perhaps the greater time required for terminal branches to reach these extreme positions, reduces their ability to compete effectively and thus determines the normal boundaries of the bulb's projection.

The pattern of outgrowth observed after axotomy suggests the influence of contact interactions within the nerve stump in guiding growth. In vitro studies have suggested the importance of interaxonal and glial contacts and in vivo studies of mutant reeler mice suggest that pial contact is very important in guiding olfactory axons (Devor et al., '75b). Terminal branches that arise after axotomy seem to prefer to pile up on existing layers of olfactory axons where contact with other olfactory axons and glial present within the nerve, can be maximized. This leads to an increase in the depth of the existing lamina and the preservation of a tightly packed band of terminals. In contrast, caudal growth along the pial surface offers less opportunity for these contact interactions. Axons that penetrated caudal to the cut occupied positions adjacent to the pial surface and occurred in small, separated fascicles.

The tendency to follow the pial surface can actually prevent outgrowth into the caudal field in some cases since disturbance of the pial layers at the lesion site may deflect axons away from a caudal path.

Observations made after partial LOT transections also suggest the importance of contact interactions in guiding outgrowth. The population of spared LOT fibers maintained a sparse projection throughout most areas of the cortex and partially filled in the denervated wedge behind the cut. However, these fibers failed to elongate at a rate that would keep pace with the expanding forebrain, and consequently, by 10 days after surgery no projection was present in the caudalmost extremes of the olfactory cortex. A possible explanation for the failure of these axons to penetrate caudal levels was suggested by observing the position of terminals at the level at which the projection ended. A very surprising finding was that at the caudal extremes of the projection, these terminals occupied a very sparse and narrow band that was separated by a gap from the pial surface.

This represents the only observation of a gap between the pia and olfactory axons. The gap is probably due to an expansion in the width of the plexiform layer and apparently these fibers were not able to effectively compete for these new sites. Separation from the pia, deprives these terminal branches of a needed guidance mechanism and they can no longer elongate caudally.

One possible factor that might have caused these terminal branches to become displaced from their usual superficial position involves an intrinsic property of the neuron. It was noted that these axons filled in the denervated wedge behind the cut. It is possible that while engaged in outgrowth and synapse formation at that level, the neuron was diverted from or unable to maintain its growth pattern at the more caudal terminals. This interpretation emphasizes the importance of temporal sequencing of synapse formation in determining the pattern of growth. Once the caudal terminals become separated from the pia, they cannot resume their growth.

LOT transection often caused an increase in the density of terminals in lamina Ia. This suggests that in the normal situation there may be an overabundance of postsynaptic sites available to olfactory axons. When the axon is engaged in synapse formation at points along its rostrocaudal extent, it may not be able to fill all available sites perhaps due to intrinsic limits on the rate of synapse formation or the number of contacts that one neuron can maintain.

Proliferations of terminal branches in the rostral field was fairly well completed before the major part of penetration into the caudal field occurred. This could be explained by restrictions on caudal growth due to increased competition and reduced contact relations, but it may also reflect intrinsic restrictions on growth. Axotomy often leads to a retrograde cell reaction that shifts the cell away from the synthesis of molecules required for synaptic transmission, and towards an increased production of structural components needed for axon elongation(e.g., Reis and Ross, '73). Similarly, axons that are undergoing synapse formation show large increases in enzyme levels related to the synthesis of transmitter (Black et al., '74) in addition to the production of synaptic vesicles and junctional densities. Since rostral proliferation and distal elongation occur in a staggered sequence during regeneration, this may indicate that the cell cannot maintain a heavy commitment to both functions simultaneously.

Behavioral Indications of Functional Reorganization in the Neonate After Early LOT Lesions:

Early brain damage has often been shown to lead to behavior effects that differ from similar lesions in adults. In several systems, it has been established that the age at injury can result in different patterns of neuroanatomical reorganization which may be correlated with differences in the behavioral consequences of the lesions.

Most often, neuroanatomical and functional reorganization are evaluated in the adult. Thus it is not known how early the reorganization occurs and when it begins to have functional effects.

Since neonatal LOT transection has been shown to lead to reorganization of olfactory connections that have adaptive consequences for adult behavior, this system was selected to evaluate the time course and impact of reorganization on the development of the hamster.

Thermal orientation of hamster pups proved to be a sensitive marker for detecting disturbances in development resulting from unilateral lesions of the olfactory system and for detecting recovery from such effects. Pups with unilateral bulbectomies continued to express thermal preferences at older ages while pups receiving cortical lesions that spared the lateral olfactory tract showed an abrupt transition from strong thermal orientation to exploratory behavior by 9 days of age.

Pups receiving unilateral LOT transections initially resembled pups in the UOB group and showed persistent thermal orientation beyond day 9. This result suggests that the sharp decline of thermal orientation observed in normal pups is the result of maturational changes in olfactory bulb efferents contained in the LOT which project to regions distal to or underlying the transection.

There was a clear distinction between behavioral profiles of UOB and control pups during the second postnatal week which made it possible to detect the process of recovery which occurred in some of the pups with LOT transections. These pups showed both a delay and an alteration in the pattern of the transition from thermal orientation to exploratory behavior. Unlike control pups who shifted in an all or none fashion between these patterns over a one day period, pups in the ULOT group showing recovery had a gradual transition between behavior patterns with a period of a few days in which both behaviors were observed. By day 11 these pups had recovered to control levels of thermal orientation. Other pups within the ULOT group, nonrecoverers, continued to resemble pups in the UOB group and maintained strong thermal preferences throughout the second week.

Two lines of evidence suggest that maturation of the bulbar projection to the lateral tubercle is involved in the transition from thermal orientation to exploratory behavior: 1) Partial LOT transection of the medial fibers of the tract was sufficient to produce a strong effect on the decline of thermal orientation. Case 279 from the recovery group is of particular interest. This lesion was the most incomplete transection seen in the recovery group, and yet it still produced a strong effect on thermal orientation possibly because the tubercle was drastically denervated. The lesion, which is shown in Figure 16b, destroyed almost all medial fibers of the tract but spared a portion of the lateral margin of the tract. The pup showed a strong lesion effect even though a continuous but sparse projection was retained throughout the prepiriform cortex.

By 10 days after LOT transection, considerable alterations were observed in the olfactory bulb projection. Caudal to the cut, a patchy projection from the bulb was present in the lateral tubercle in all but two cases included in the recovery group, while a projection to the prepiriform cortex was observed only infrequently. In nonrecoverers, the bulb did not project caudal to the transection; only one pocket of sprouts into the tubercle was seen in one animal in the NR group. (The projection in pups showing recovery, as well as those not showing recovery on the thermal task, indicated a proliferation of terminals in cortical regions rostral to the section which suggests that innervation of these regions was not involved in the transition from thermal orientation which distinguished recoverers from nonrecoverers.)

The absence of a projection to the lateral tubercle was most likely responsible for the persistent thermal orientation of nonrecoverers. Since cuts in some NR cases included destruction of deep cortical layers extending into the rostral tubercle, these lesions may have produced effects on thermal orientation by destroying probably target cells rather than just denervating this region. The involvement of the tubercle in the transition from thermal orientation could be tested directly by contrasting the effects of discrete lesions of the tubercle with lesions of the prepiriform cortex, in both cases avoiding damage to the adjacent lateral olfactory tract.

While the appearance of a projection from the bulb into the tubercle was correlated with recovery on the thermal task, a stronger argument for the functional significance of this early postlesion growth would have involved demonstrating that : 1) Destruction of the caudal projection by a second LOT section, at an age after recovery was detected; resulted in increased thermal responsiveness; 2) Removal of the bulb ipsilateral to the transection at an age after recovery, was effective in causing increased thermal responsiveness comparable to that seen in the UOB group. This demonstration would provide evidence that the olfactory bulb projection ipsilateral to the cut was mediating recovery, rather than reorganization of the intact contralateral bulb; and 3) Removal of the bulb contralateral to the transection in recoverers caused thermal responsiveness to increase to a level similar to UOB treated animals but not to as high a level as that seen in bilaterally bulbectomized pups (Leonard, '76).

A second lesion was attempted on days 12 and 13 in five pups who were showing recovery. No conclusion could be drawn from this study because of difficulties encountered in operating on adolescent hamsters. Surgery in adolescent pups is far more traumatic than in neonates. In addition, these pups were returned after surgery to nests containing littermates who had not received a second operation. The pups did not fare well with either their littermates or their mothers, and on the days following surgery, these pups clearly looked unhealthy. An obvious solution would be to operate an entire litter of adolescents even though nonrecoverers and pups showing no effect of the initial surgery would be included.

Unilateral olfactory lesions were sufficient to cause persistent thermal orientation, although by the oldest ages tested, the strength of this response had decreased considerably from the levels observed at younger ages. Two cases in the NR group with extensive bilateral LOT damage, had thermal indices on days 11 to 13 which exceeded the indices of most UOB pups. This agrees with Leonard's (76) demonstration that while unilateral and bilateral both produce persistent thermal orientation, stronger heat preferences are observed at older ages after bilateral lesions.

Two cases included in the recovery group, on the basis of thermal scores on days 11 to 13, showed no projection caudal to their lesions. This may have been an artifact of histology since degeneration in young brains can fade rapidly prior to staining if sections are not maintained at low temperatures. However, it was also noted that these two cases represented the two highest thermal indices included in the recovery group. The shape of the distribution of thermal indices on days 11 to 13 (Figure 5b) actually suggests a continuum rather than two distinct populations.

At present, there are relatively few demonstrations relating innervation of restricted regions of the olfactory cortex to specific behaviors. It is not well understood how the olfactory cortex processes inputs and whether spatiotemporal patterning across the entire cortical sheet or topographic localization within the olfactory cortex is important for olfactory-dependent behaviors (e.g., Devor, '77). Winans and Powers ('77) have provided evidence that the accessory olfactory bulb and its projection to the cortical and medial amygdaloid nuclei are particularly important in the mating behavior of male hamsters.

Leonard's ('75) study of the time course of degeneration argyrophilia suggests regional maturation within the bulb's projection field which may underlie the development of specific behaviors. The projection to the tubercle acquires mature degeneration characteristics at about 7 days of age, just before the transition from thermal orientation to exploratory behavior occurs, and when responsiveness to conspecific odors is first observed in hamster pups.

The age at which these behavioral changes occur corresponds to the age when pups first emerge from the nest and their behavioral repertoire undergoes a rapid expansion. Before 9 days of age, pups are not seen out of the nest. After day 9, pups switch to a mobile life style and can be seen walking about the home cage, feeding at the food pile, stuffing their cheek pouches with seeds, and interacting with littermates outside of the nest (Dieterlen, '59).

Hamster pups will select cool maternal shavings rather than warm, clean shavings beginning on day 9 (Leonard, '74a) which suggests that at this age the olfactory system begins to play a stronger role than thermal inputs in guiding the pup's behavior. The results of the present study would suggest that the olfactory tubercle plays a central role in behavioral transitions occurring at the end of the first week.

The effects of olfactory bulbectomy clearly indicate that developmental changes within the bulb's projection are an important factor in the transition from thermal to exploratory behavior, but it is not obvious how the olfactory system produces an effect on thermal responsiveness. Possible mechanisms could involve a competitive interaction between olfactory and thermal sensory inputs in guiding the pup's locomotion. It was been proposed in one theory of human development that at each stage of development, one sensory system dominates other systems in its effectiveness in controlling behavior, and that systematic alterations in dominance are important factors in behavioral developments (Birch, '62; Birch & Lefford, '67). Another possible mechanism would involve direct inhibitory effects of the maturing olfactory system on CNS circuits mediating thermal orientation.

The fact that the projection to the tubercle was implicated in the transition from thermal orientation to exploratory behavior suggests another interesting possibility. The tubercle is a major site of termination of the mesolimbic dopaminergic (DA) pathway arising in the brain stem. Effects from unilateral lesions of DA systems are frequently reported in the literature. These effects include decreases in spontaneous exploratory behavior, unilateral sensory neglect including reduced responsiveness to odorants, deficits in self-stimulation (e.g., Ungerstedt, '74), and deficits in food and water intake (e.g., Baez, Ahlskog, & Randall, '77). Gilad and Reis ('77) have reported that DA innervation of the tubercle matures at about 7 days of age in the rat pup.

If the time course of maturation of DA fibers is similar in the rat and hamster, it may be that behavioral transitions involving the olfactory tubercle are the result of an interaction of both the bulb and DA projection to the tubercle since both systems appear to mature at similar ages. Since bulbectomy prevents transitions, the DA projection to the tubercle by itself is apparently not sufficient to account for these developmental changes.

Bulbectomies performed in rats at 10 days of age or older result in a proliferation of DA terminals in the tubercle, while bulbectomies performed soon after birth, do not result in DA sprouting in the tubercle (Gilad & Reis, '77). Devor ('76b) observed that LOT transections made at 10 days of age or older, did not result in a projection from the bulb caudal to the lesion. It may be that sprouting of DA terminals is one factor which limits growth caudal to LOT transections to lesions made prior to 10 days of age.

The results of this study suggest that postlesion alterations in the olfactory bulb projection occur soon after neonatal surgery, and that this early postlesion growth has adaptive functional consequences for the neonate. The early postoperative interval would appear to be a valuable period in which to focus attention for those wishing to study mechanisms controlling postlesion growth, or to correlate altered patterns of neural connectivity with functional consequences of early CNS lesions.

APPENDIX

Histological results from the UOB group suggested that olfactory axons were present in the cranium after neonatal bulb removal. An additional study (Small, '77) sought information of the following questions: 1) Were olfactory axons continuously present in the cranium from the time of surgery or did they reappear gradually after some interval? 2) Did axons present after bulbectomies show evidence of degeneration? 3) If the olfactory axons had indeed regrown, was there any indication that they were capable of interacting with neural tissue other than the olfactory bulbs?

A group of 45 hamster pups were given bilateral olfactory bulbectomies at 5 days of age. All operations were performed on the same day, and the pups were then randomly assigned to postoperative survival groups of 8 hrs, 6 days, 12 days and 20 days. The brains were treated as in the previous experiment except that they were not dissected out. The skulls were decalcified in 10% EDTA, embedded in egg-yolk gelatin and then frozen sections were cut at 25 μm. Most brains were cut in the horizontal plane so sections included the nasal cavity, cribiform plate and brain. Normal skulls of pups of ages comparable to the sacrifice age of the bulbectomized pups were also examined.

The following sequence was observed with increasing postoperative intervals after neonatal bulbectomy:

In the 12 pups sacrificed in the 8 hr survival group, it was clear that most cases of bulbectomy were complete and included the most caudal and medial extent of the bulb, as well as a small portion of the anterior olfactory nucleus. In 3 cases, a region of easily recognized olfactory bulb remained at what would have been the caudomedial extent of the bulb. Since fibers arising from this part of the bulb do not project into the LOT (Devor, '76a) and would therefore not have been detected, it was decided to exclude the medial wall from consideration in studying regrowth of the olfactory nerve.

In the 8 hr survival group, fascicles of olfactory axons were seen within the cribiform plate, but they had been sheared off at or just above the inside of the cribiform plate. Since these fascicles did not terminate in glomeruli, it was probable that their terminal endings had been destroyed by the bulbectomy. Fascicles at the cribiform plate were separated by a zone of necrotic tissue from the remaining olfactory peduncle. In some cases, the peduncle had already begun to fill some of the space vacated by the bulbectomy, but in most cases there was considerable empty space in the cranium above the cribiform plate

(Fig. 23b).

Within 6 days of bulbectomy, the rostral and ventral poles of the peduncle were capped by a thick zone of small granule cells. These cells were continuous with the stream of cells arising from the anterior wall of the lateral ventricle. The anterior olfactory nucleus had filled in the space previously occupied by the olfactory bulb so the granule cells were sitting flush against the cribiform plate (Fig. 23c).

Within 12 days of the bulbectomy, olfactory axons could be traced through the cribiform plate into the cranium where they formed an irregular plexus rostral to the band of granule cells. This plexus occurred at all positions along the medial to lateral extent of the bank of granule cells.

By 20 days and for longer survival periods, the deepest portion of the olfactory nerve plexus appeared in some brains as spherical regions of neuropil surrounded by granule cells. All brains showed signs of an olfactory nerve plexus at these survival periods, but the degree to which it was organized into discrete glomeruli varied between brains. Three cases showing different amounts of glomerular formation are shown in Figures 23g - i. Most glomeruli contained very few silver particles, comparable to the levels of degeneration seen in reduced silver stains of the glomeruli of normal bulbs, but an occasional glomerulus showed heavy degeneration.

In several cases, thick dendritic shafts were observed extending into the glomeruli, that arose from large neurons that were present immediately adjacent to the glomeruli (Fig. 23j). The origin of these large neurons is not known, but they differ in several respects from normal mitral cells. The relatively large mitral cells of the normal bulb send a thick apical dendrite through the external plexiform layer to terminate in the glomeruli. The mitral cell bodies are usually separated by some expanse of the plexiform layer from the glomeruli. Dendritic shafts within the glomeruli of normal bulbs are not often impregnated in reduced silver stains, although apical dendrites of mitral cells are well stained as they pass through the external plexiform layer of the bulb. Neurons typically observed adjacent to the glomeruli of normal bulbs are periglomerular cells which are considerably smaller than the neurons seen in Figure 23j.

A few animals with day 5 bulbectomies were observed at 60 days postop. Histological examination showed no increase in the size of the nerve plexus or in the number of glomeruli it contained, from that seen at 20 day survivals. Three males were given an extensive series of mating tests in the week prior to sacrifice. They showed no signs of mating behavior in agreement with previous findings (Winans & Powers, '74) that neonatal bulbectomy leads to a loss of male mating in adulthood.

The histological observations after neonatal bulbectomy are of interest because it has been demonstrated that the olfactory receptors, which are primary sensory neurons, are continuously replaced throughout life or as a result of axotomy. Ultrastructural studies show that after olfactory nerve section in adult mice, all mature receptors degenerate and new receptors are differentiated from basal cells in the epithelium (Graziadei, '73). Axons of new receptors penetrate the cribiform plate to establish synaptic contacts within the glomeruli sometime after day 15, and by day 40 synapse formation seems to be fairly complete (Graziadei & Graziadei, '76). After axotomy, biochemical markers specific for the olfactory pathway (Margolis, '72), decrease to a minimum at the end of one week and then begin to increase, reaching 80% of their initial values by the end of one month (Harding, Graziadei, Monti Graziadei & Margolis, '77). Ultrastructural and biochemical results suggest that after axotomy, newly formed olfactory axons are able to establish synaptic contacts in the bulb.

Since bulbectomies were performed only in neonates in the present study, it is possible that olfactory fascicles that appeared in the cranium after surgery are the consequence of a normal postnatal growth of the primary olfactory pathway. The observation that the size of the nerve plexus and number of glomeruli it contained, did not increase between 20 days postop and adulthood could be taken to indicate that this growth is ended or nearly ended by the age of 20 days. This possibility would be easy to test, since, if it is the result of continued postnatal maturation, no plexus would appear after bulbectomies performed in adults. The observations made after neonatal bulbectomy would still suggest that olfactory axons pursue a normal course into the cranium in the absence of the olfactory bulb, and that they are capable of forming glomeruli. The additional observation that large neurons were present adjacent to the glomeruli with dendrites extending into the glomeruli presents the interesting possibility that in the absence of their normal target cells, these olfactory axons are capable of interacting with other neurons that they encounter, and perhaps even able to establish functional interactions with these neurons.

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TABLE I

SUMMARY OF THE NUMBER OF LITTERS AND PUPS INCLUDED IN EACH TREATMENT GROUP

Treatment on Day 5	No. Litters	Total No. Pups
UOB* (Unilateral olfactory bulbectomy)	4	33
ULOT* (Unilateral section of the lateral olfactory tract)	6	48
BH* (Bilateral aspiration of the dorsal hemispheres)	2	12
SO (Sham operations)	1	6

*Each of the litters in these treatment groups contained one sham operated pup (not included in the total number of pups).

TABLE II

SIGNIFICANCE OF THE DIFFERENCES OBSERVED IN THE STRENGTH OF THERMAL ORIENTATION WITH TREATMENTS AND WITH AGE[a]

A. Significance of the differences in thermal indices[b] of treatment groups at two age intervals:

	Days 8-10	Days 11-13
BH vs SO[c]	ns	ns
ULOT vs BH/SO	**	ns
UOB vs BH/SO	**	**
ULOT vs UOB	ns	**

B. Significance of differences in the thermal indices of each treatment group with age:

	Days 8-10 compared to days 11-13
BH/SO	**
ULOT	**
UOB	*

*p .01; **p .001; ns = not significant

[a] All comparisons were made with t tests, pooling the variance for tests in A, and using 2-tailed significance levels.

[b] Thermal indices were obtained by averaging thermal scores over three-day intervals on days 8-10 and days 11-13.

[c] Since BH and SO groups did not differ at either age interval, these groups were combined for other comparisons.

TABLE III

A COMPARISON OF THE SIZE AND RATE OF DECREASE OF THE ROSTRAL LATERAL OLFACTORY TRACTS 10 DAYS AFTER A UNILATERAL LOT TRANSECTION AT 5 DAYS OF AGE[a]

		LOT ipsilateral to transection		LOT contralateral to transection	
	Level	Maximum width (µm)	Cumulative percent decrease	Maximum width (µm)	Cumulative percent decrease
Case: 272	1[b]	112.5		137.5	
	225 µm				
	2	100.0	.11	131.3	.05
	3	87.5	.22	125.0	.09
	4	68.8	.39	125.0	.09
	5	50.0	.56	112.5	.18
	6	Scar			
Case: 281	1	62.5		137.5	
	2	50.0	.20	143.8	.00
	3	37.5	.40	137.5	.04
	4	31.3	.50	125.0	.13
	5	25.0	.60	125.0	.13
	6	Scar			

[a] The point of maximum width of the LOT was measured to provide a relative estimate by which to compare the number of fibers and rate of decrease in both tracts.

[b] Levels 1 through 6 begin at a position proximal to the olfactory bulbs and move caudally towards the cortex.

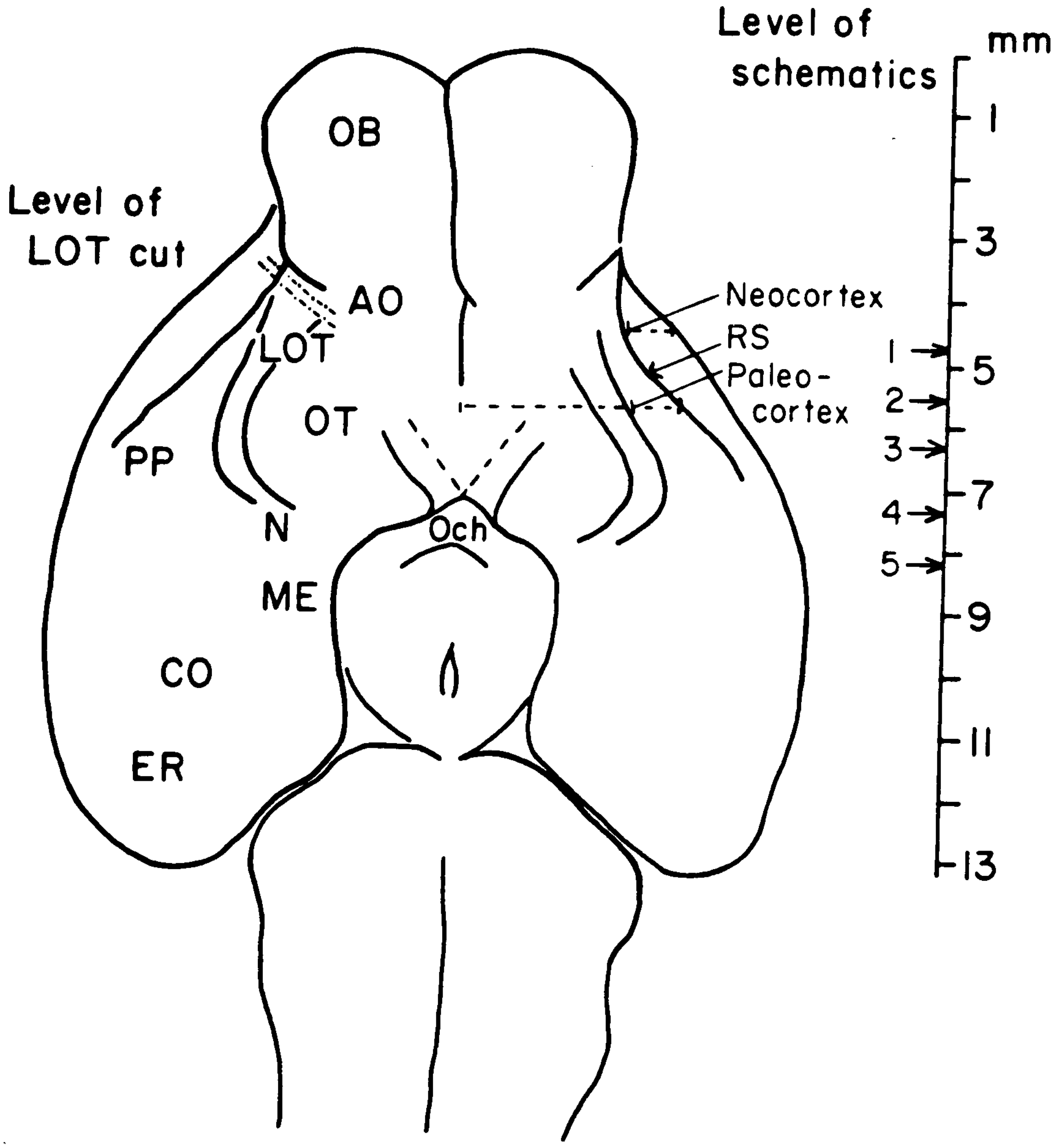

Level of schematics
mm
1
3
5
7
9
11
13
1
2
3
4
5
Level of LOT cut
OB
AO
LOT
OT
PP
N
Och
ME
CO
ER
Neocortex
RS
Paleo-cortex

FIGURE 1

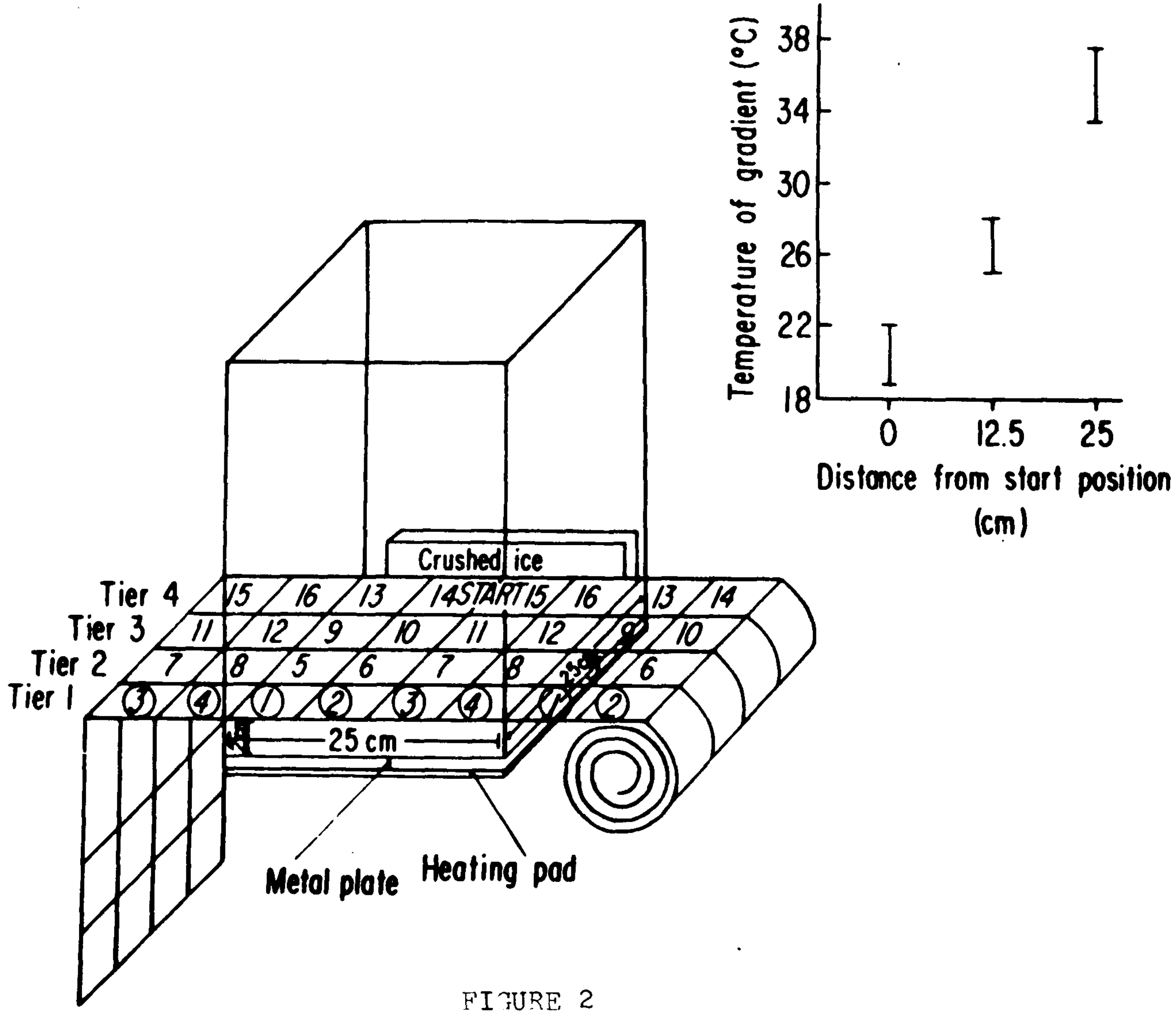

Temperature of gradient (°C)
38
34
30
26
22
18
0
12.5
25
Distance from start position (cm)
Crushed ice
Tier 4
Tier 3
Tier 2
Tier 1
15
16
13
14
START
15
16
13
14
11
12
9
10
11
12
9
10
7
8
5
6
7
8
5
6
③
④
①
②
③
④
①
②
25 cm
Metal plate
Heating pad

FIGURE 2

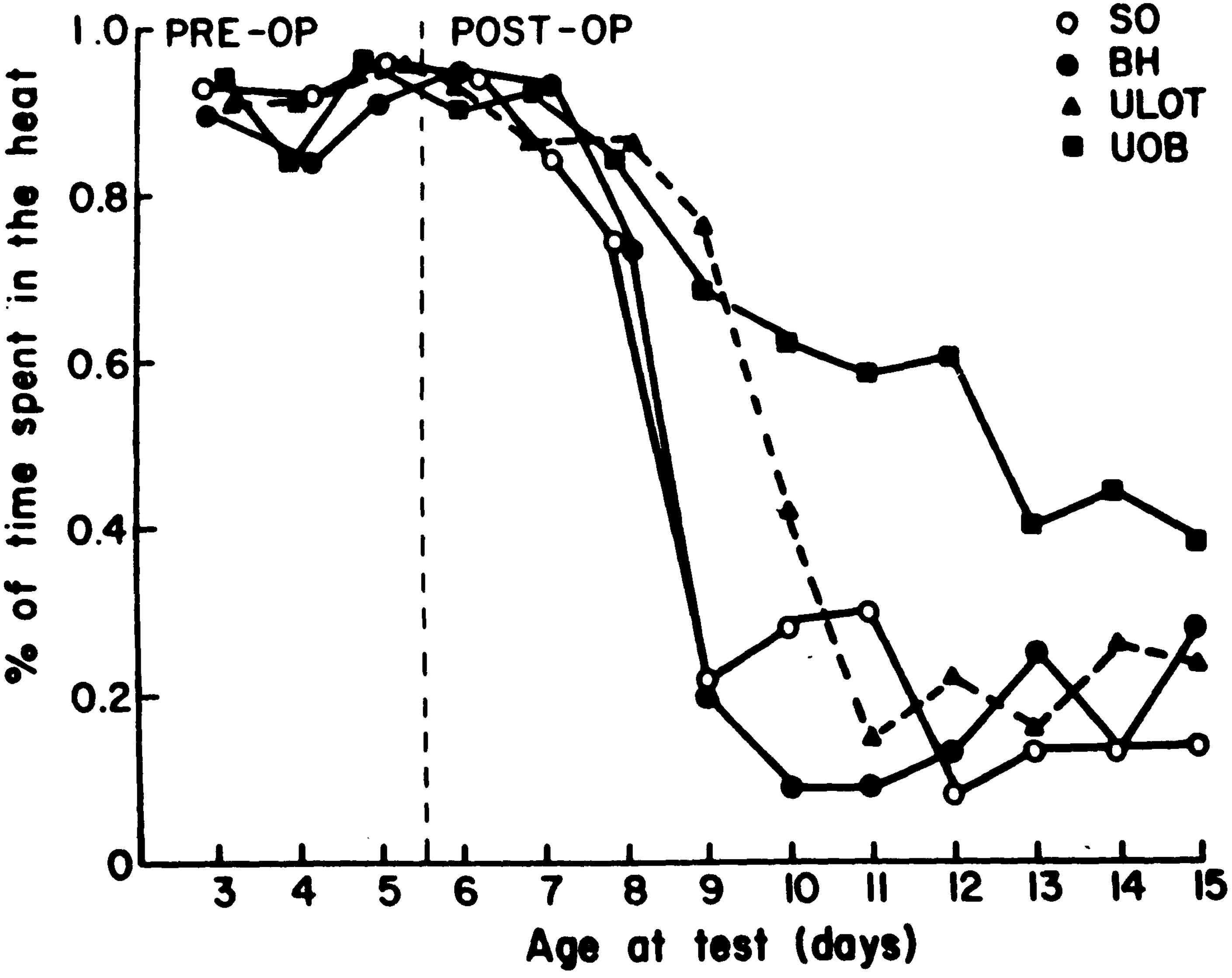

1.0
0.8
0.6
0.4
0.2
0
% of time spent in the heat
PRE-OP
POST-OP
○ SO
● BH
▲ ULOT
■ UOB
3
4
5
6
7
8
9
10
11
12
13
14
15
Age at test (days)

FIGURE 3

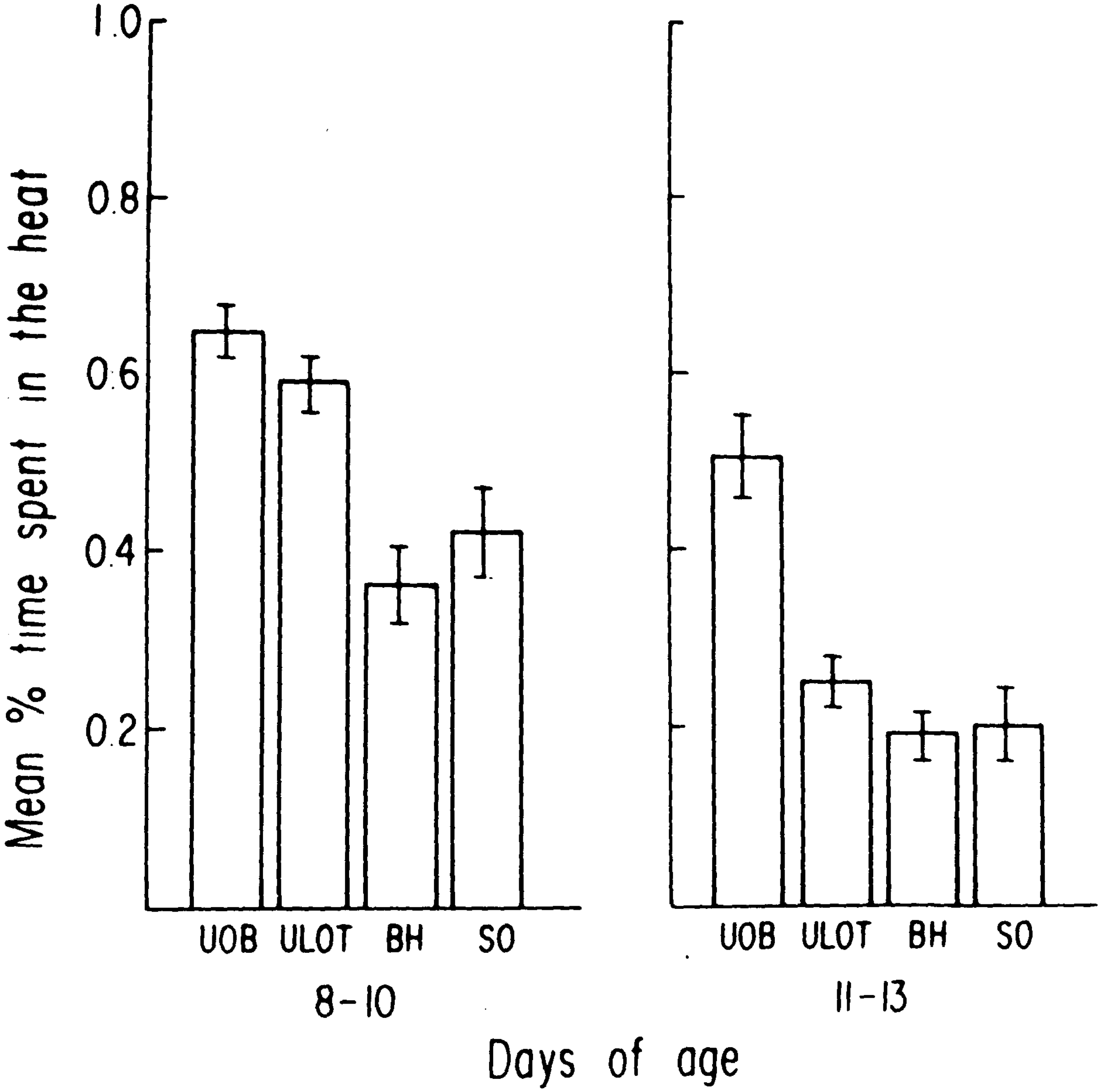

Mean % time spent in the heat
1.0
0.8
0.6
0.4
0.2
UOB
ULOT
BH
SO
8-10
UOB
ULOT
BH
SO
11-13
Days of age

FIGURE 4

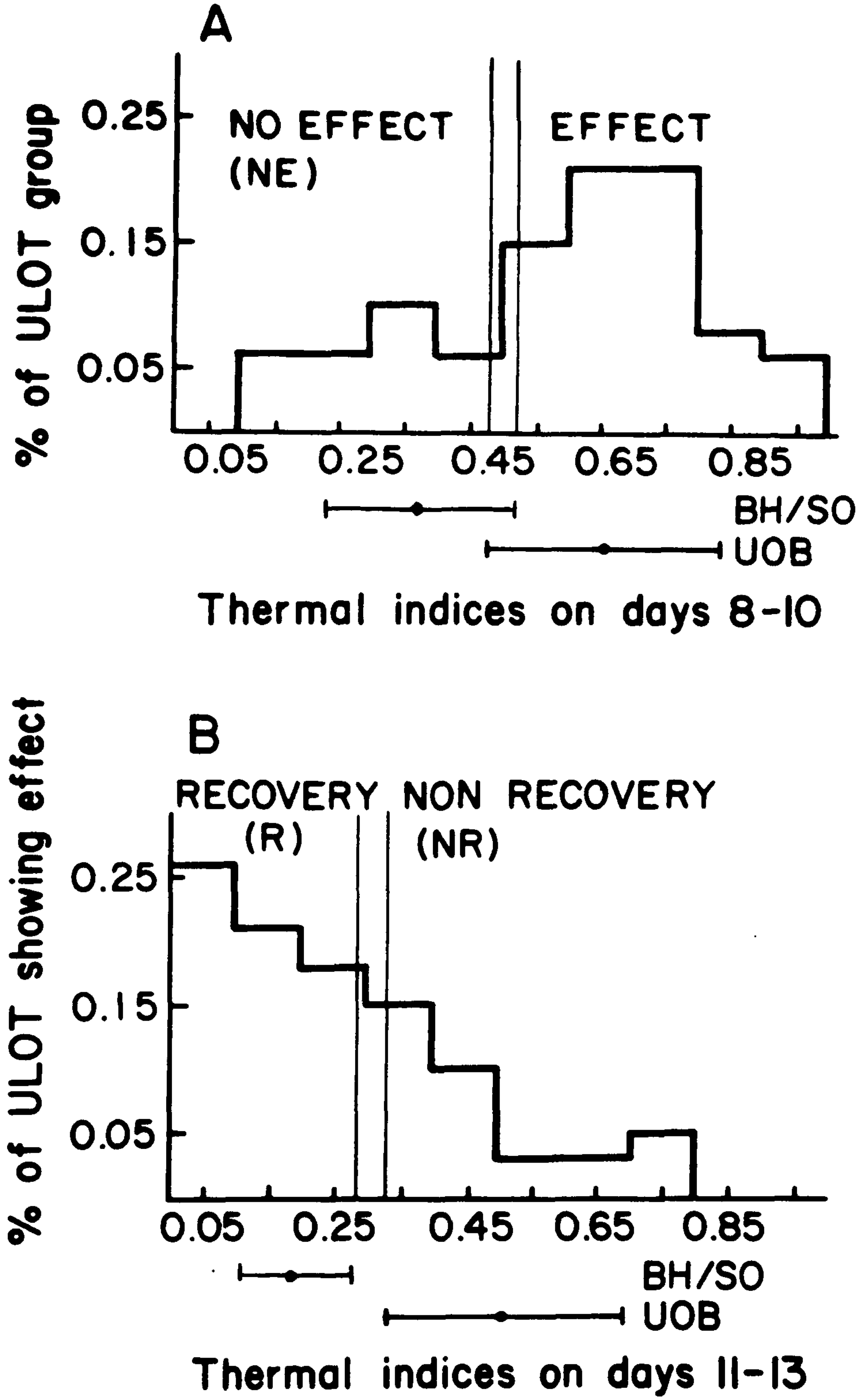

A
% of ULOT group
0.25
0.15
0.05
NO EFFECT
(NE)
EFFECT
0.05
0.25
0.45
0.65
0.85
BH/SO
UOB
Thermal indices on days 8-10
B
% of ULOT showing effect
RECOVERY
(R)
NON RECOVERY
(NR)
0.25
0.15
0.05
0.05
0.25
0.45
0.65
0.85
BH/SO
UOB
Thermal indices on days 11-13

FIGURE 5

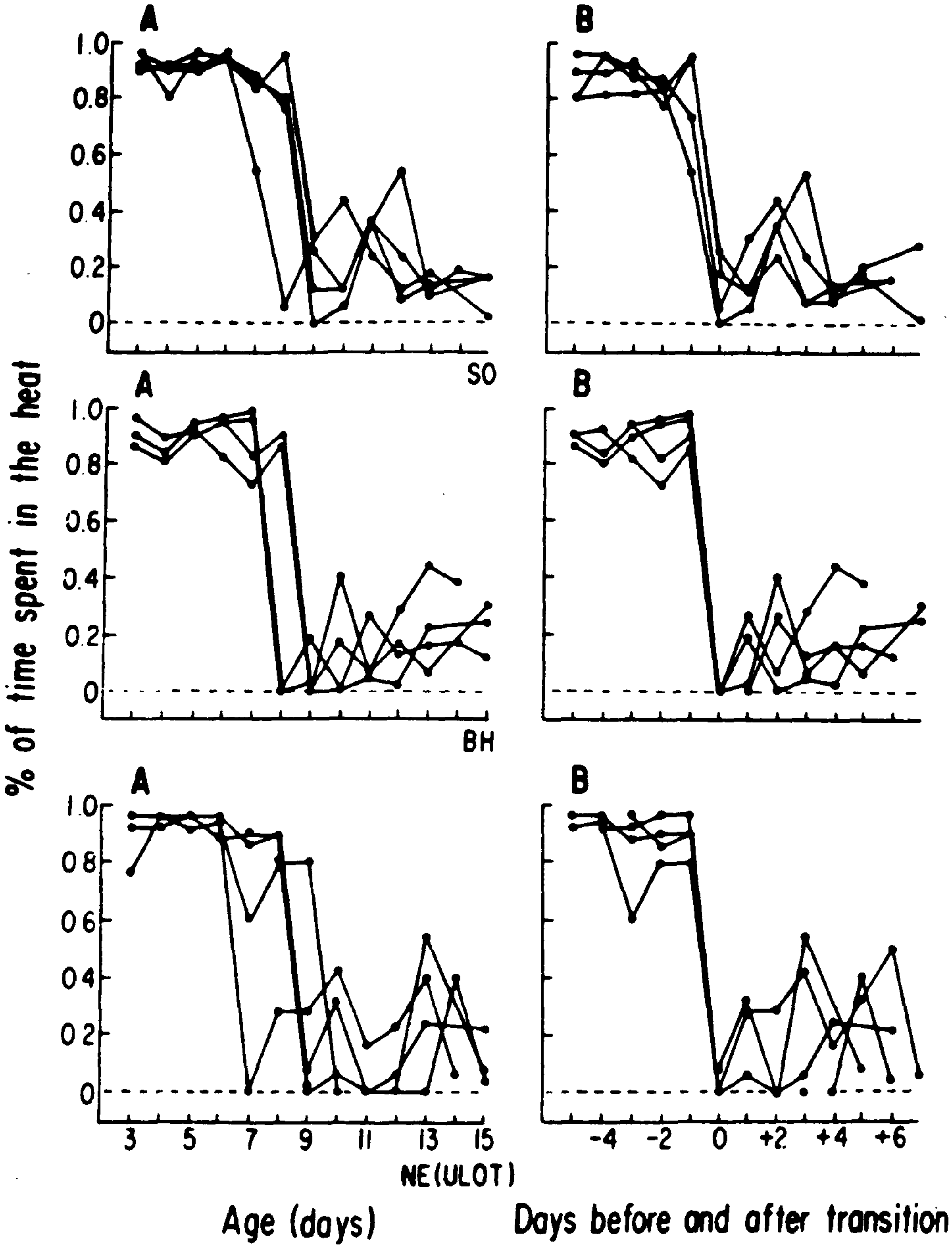

A
B
1.0
0.8
0.6
0.4
0.2
0
SO
BH
NE(ULOT)
% of time spent in the heat
3
5
7
9
11
13
15
-4
-2
0
+2
+4
+6
Age (days)
Days before and after transition

FIGURE 6

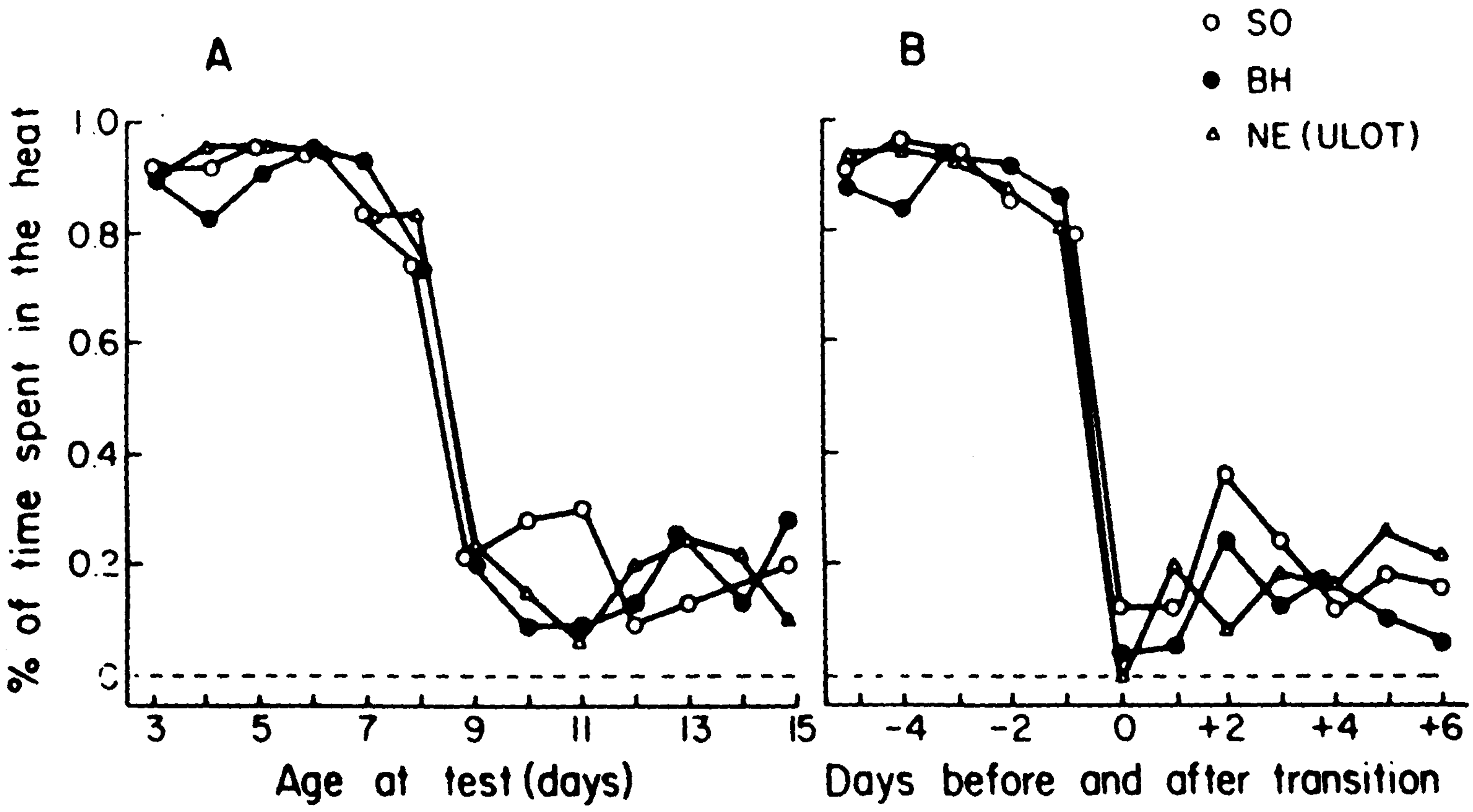

A
B
○ SO
● BH
▵ NE (ULOT)
% of time spent in the heat
1.0
0.8
0.6
0.4
0.2
0
3
5
7
9
11
13
15
Age at test (days)
-4
-2
0
+2
+4
+6
Days before and after transition

FIGURE 7

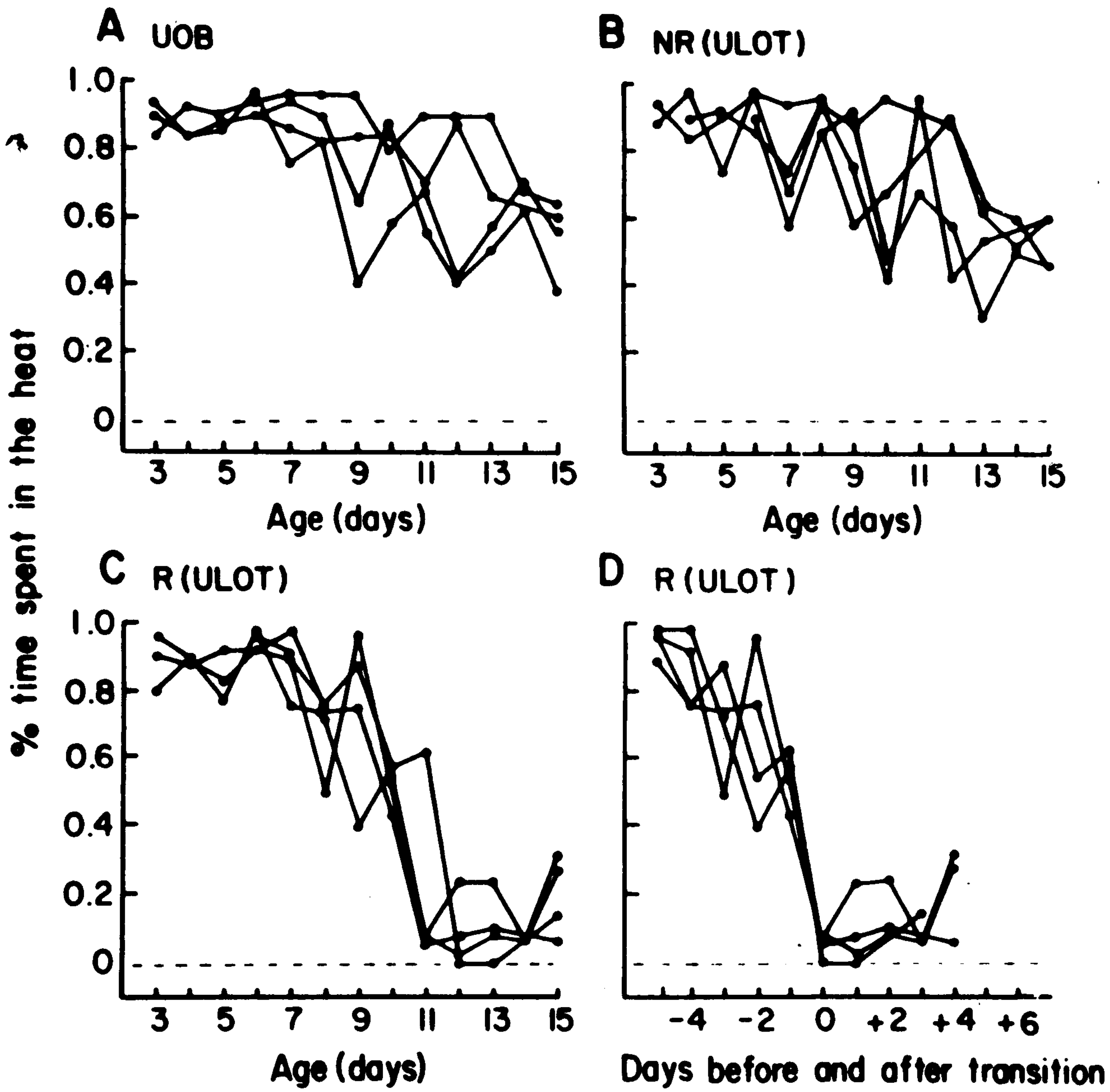

A UOB
B NR (ULOT)
C R (ULOT)
D R (ULOT)
% time spent in the heat
1.0
0.8
0.6
0.4
0.2
0
3
5
7
9
11
13
15
Age (days)
-4
-2
0
+2
+4
+6
Days before and after transition

FIGURE 8

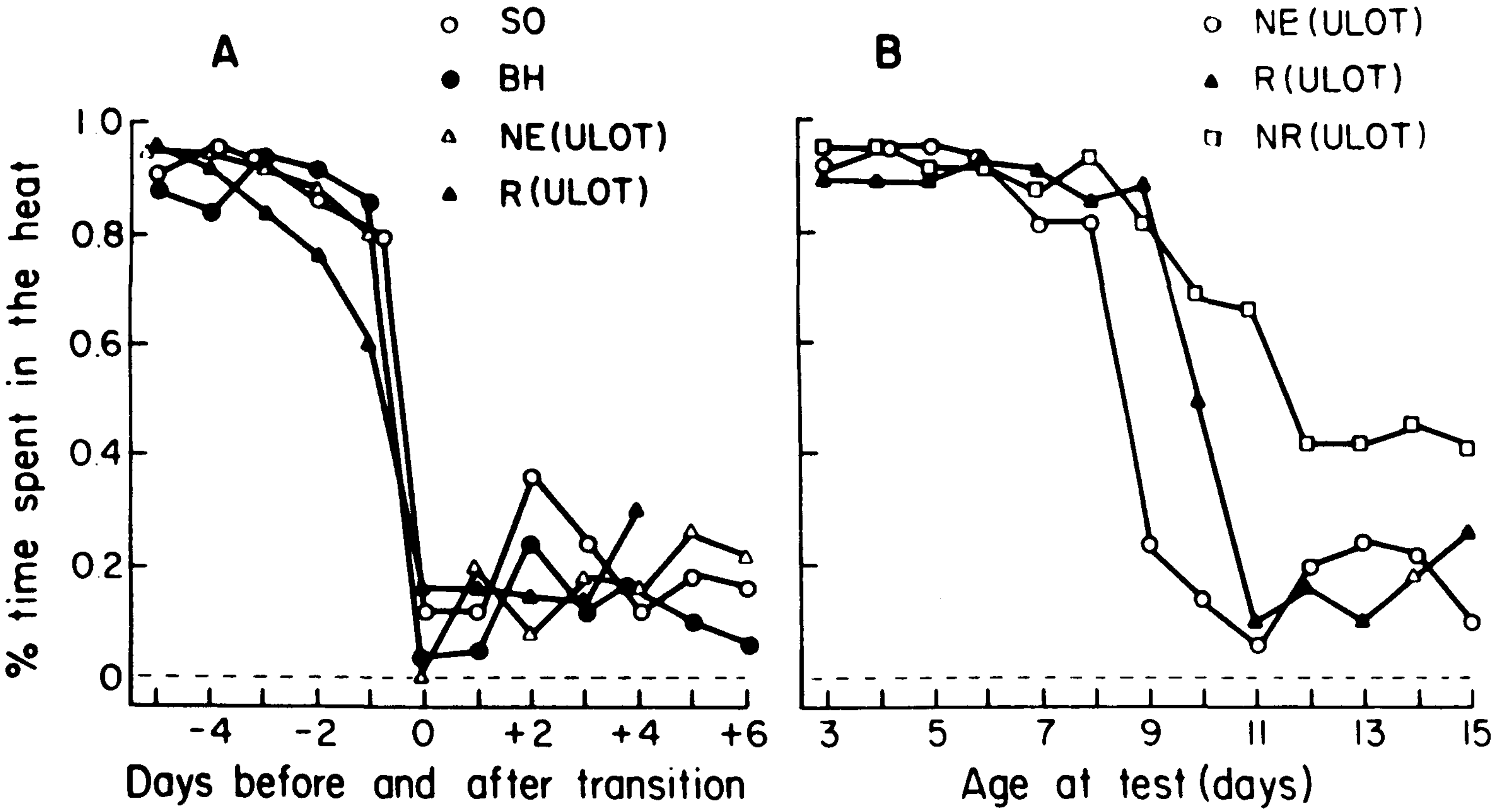

A
B
○ SO
● BH
△ NE(ULOT)
▲ R(ULOT)
○ NE(ULOT)
▲ R(ULOT)
□ NR(ULOT)
% time spent in the heat
1.0
0.8
0.6
0.4
0.2
0
-4
-2
0
+2
+4
+6
Days before and after transition
3
5
7
9
11
13
15
Age at test (days)

FIGURE 9

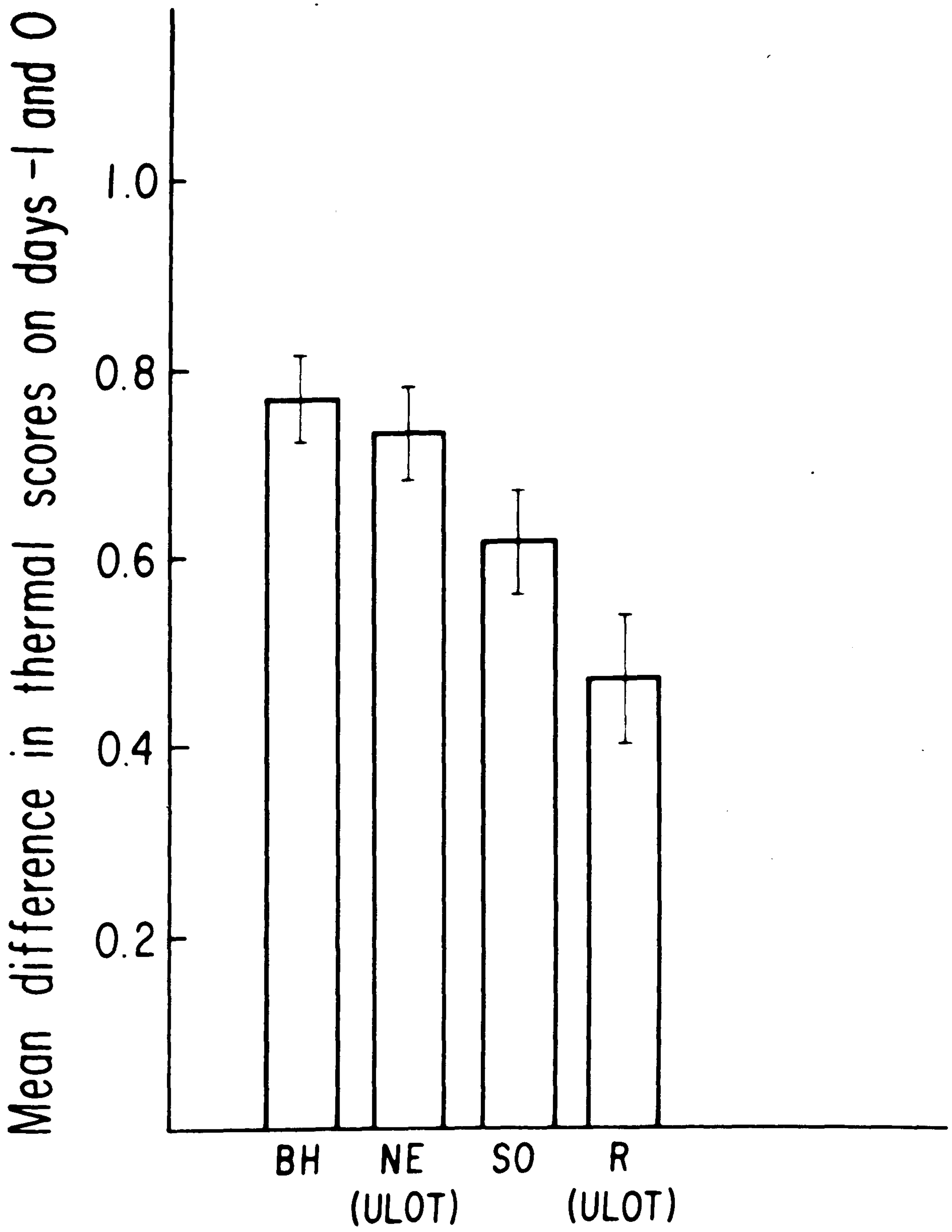

Mean difference in thermal scores on days -1 and 0
1.0
0.8
0.6
0.4
0.2
BH
NE
(ULOT)
SO
R
(ULOT)

FIGURE 10

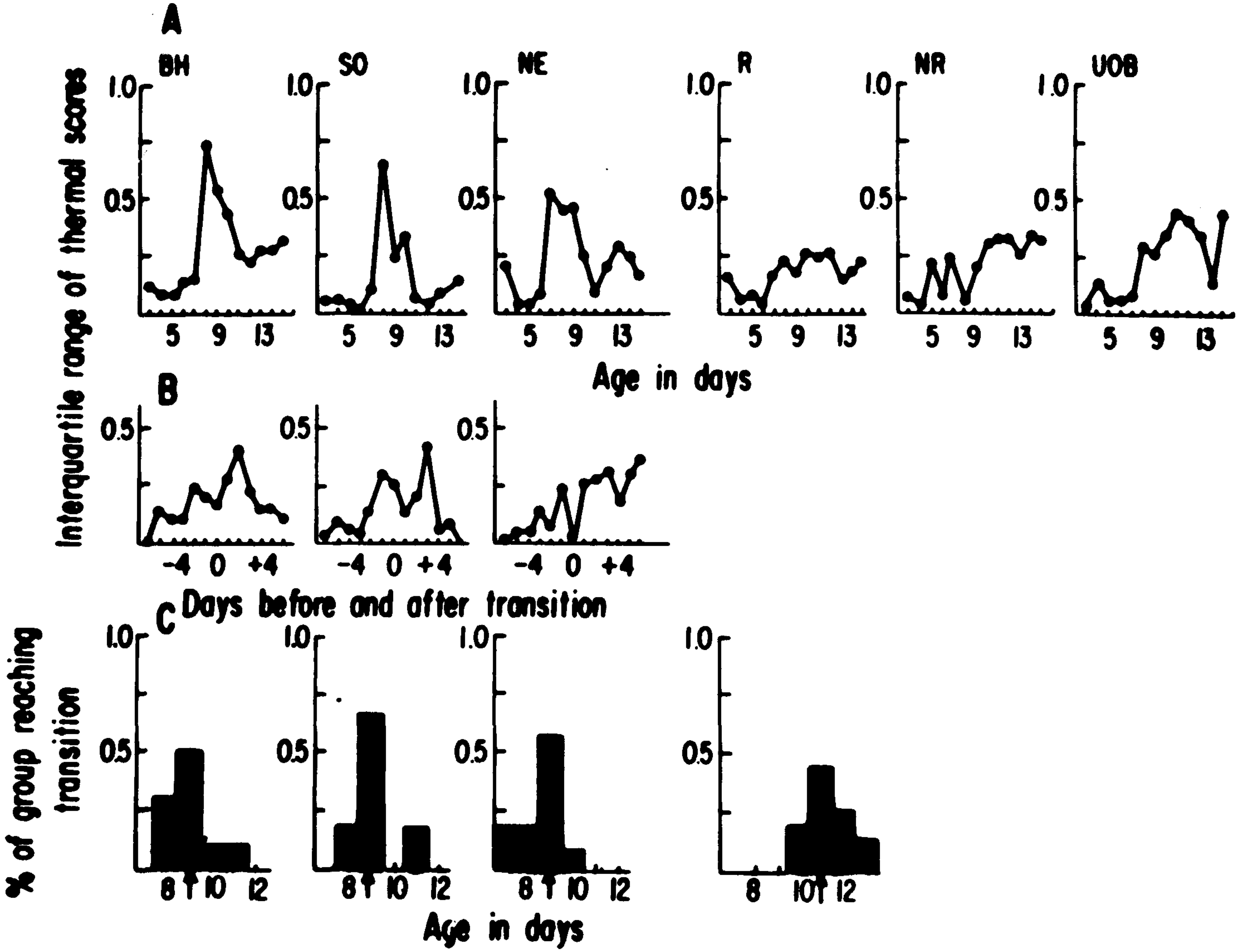
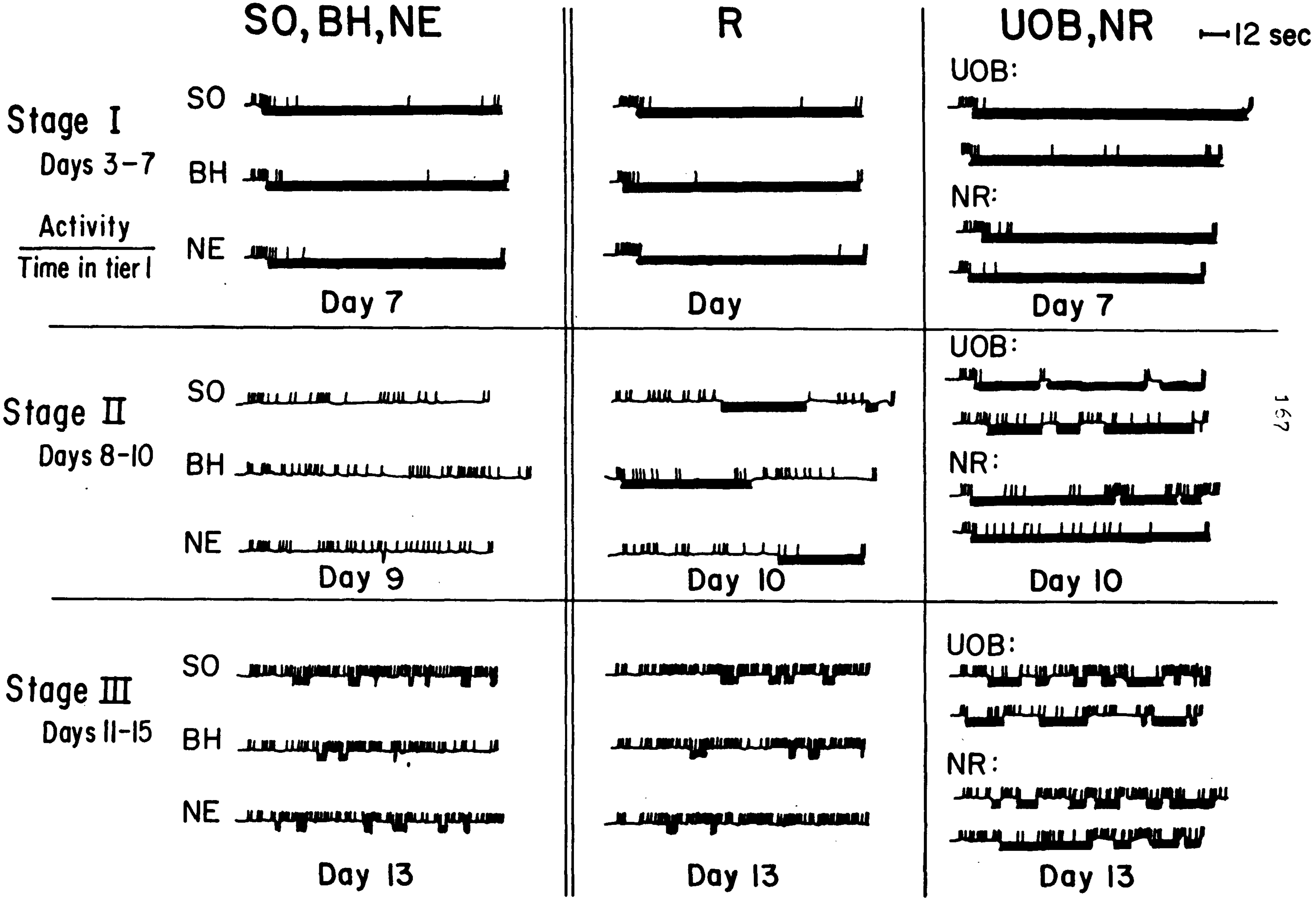

A
BH
SO
NE
R
NR
UOB
Interquartile range of thermal scores
1.0
0.5
5
9
13
Age in days
B
0.5
-4
0
+4
Days before and after transition
C
% of group reaching transition
1.0
0.5
8
10
12
Age in days

FIGURE 11

FIGURE 12


SO, BH, NE
R
UOB, NR
12 sec
Stage I
Days 3–7
Activity
Time in tier I
Stage II
Days 8–10
Stage III
Days 11–15
SO
BH
NE
UOB:
NR:
Day 7
Day
Day 7
Day 9
Day 10
Day 10
Day 13
Day 13
Day 13

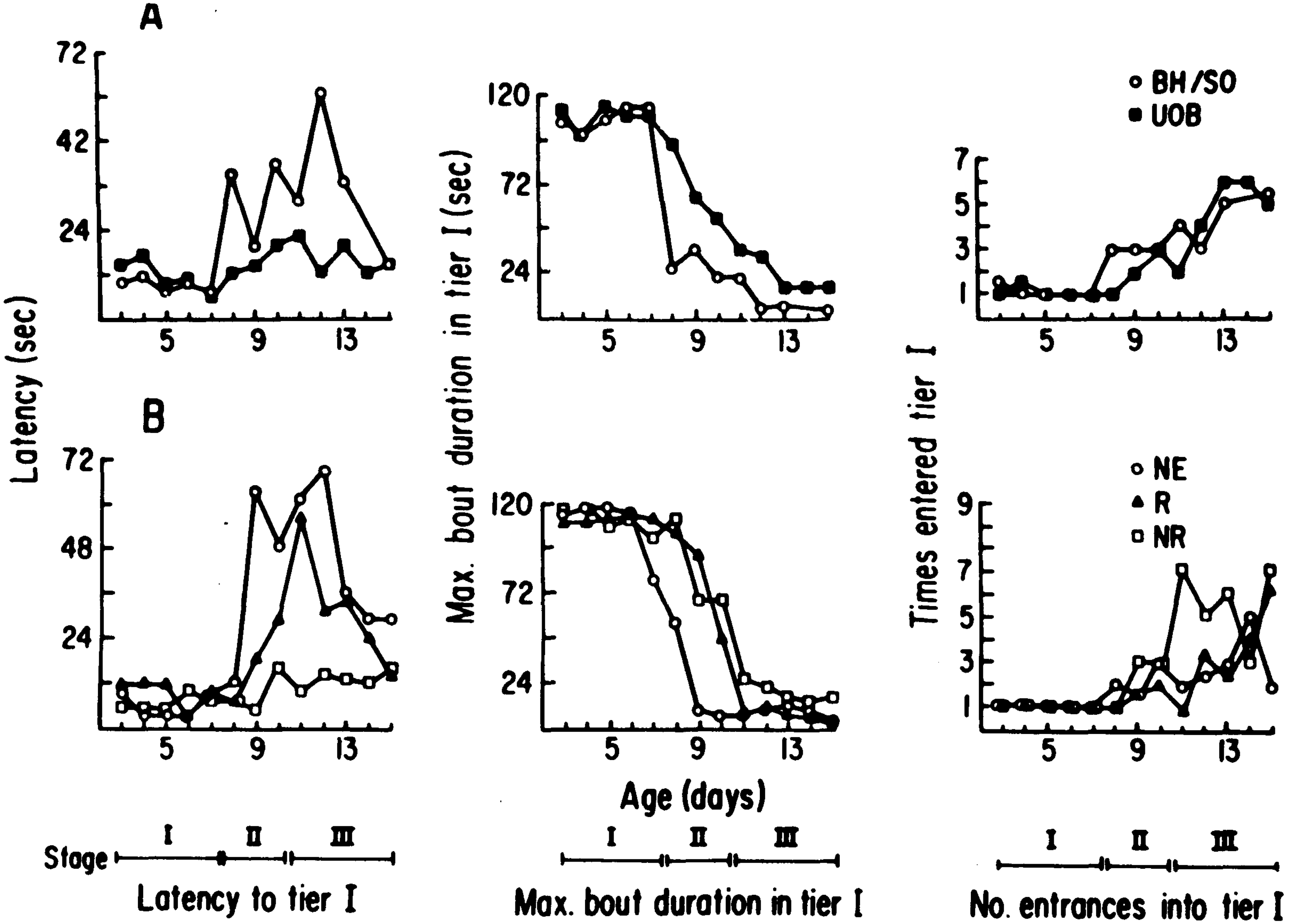

A
B
Latency (sec)
72
42
24
48
Max. bout duration in tier I (sec)
120
72
24
Times entered tier I
7
5
3
1
9
○ BH/SO
■ UOB
○ NE
▲ R
□ NR
5
9
13
Age (days)
Stage
I
II
III
Latency to tier I
Max. bout duration in tier I
No. entrances into tier I

FIGURE 13

FIGURE 14

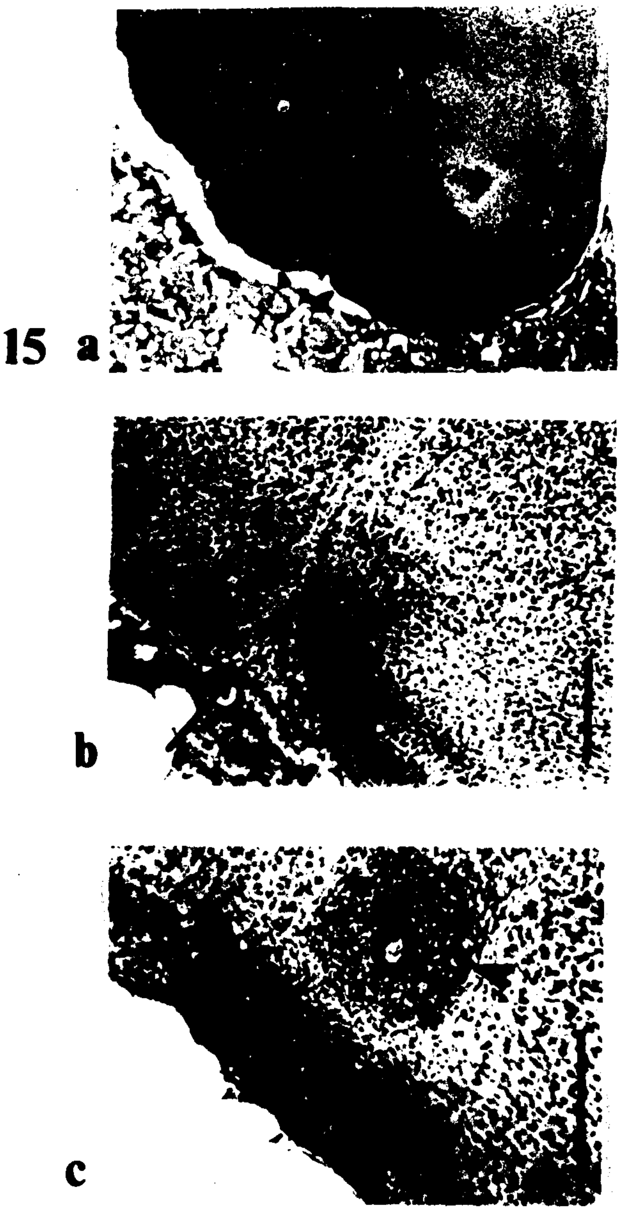


FIGURE 15

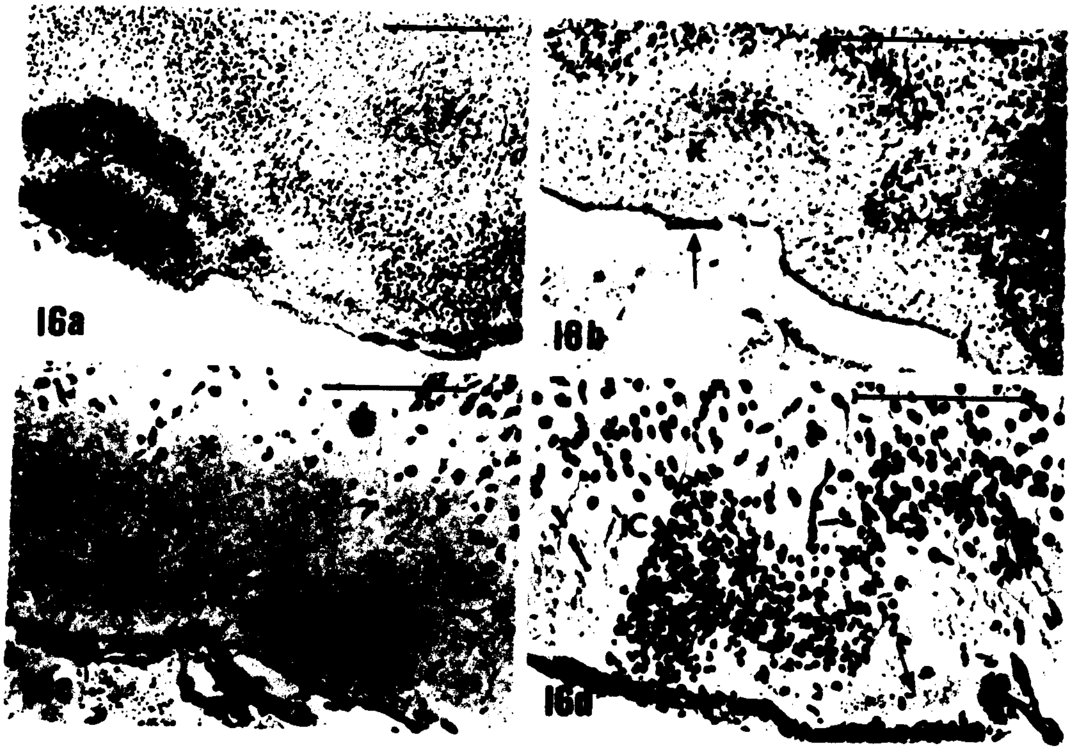


FIGURE 16

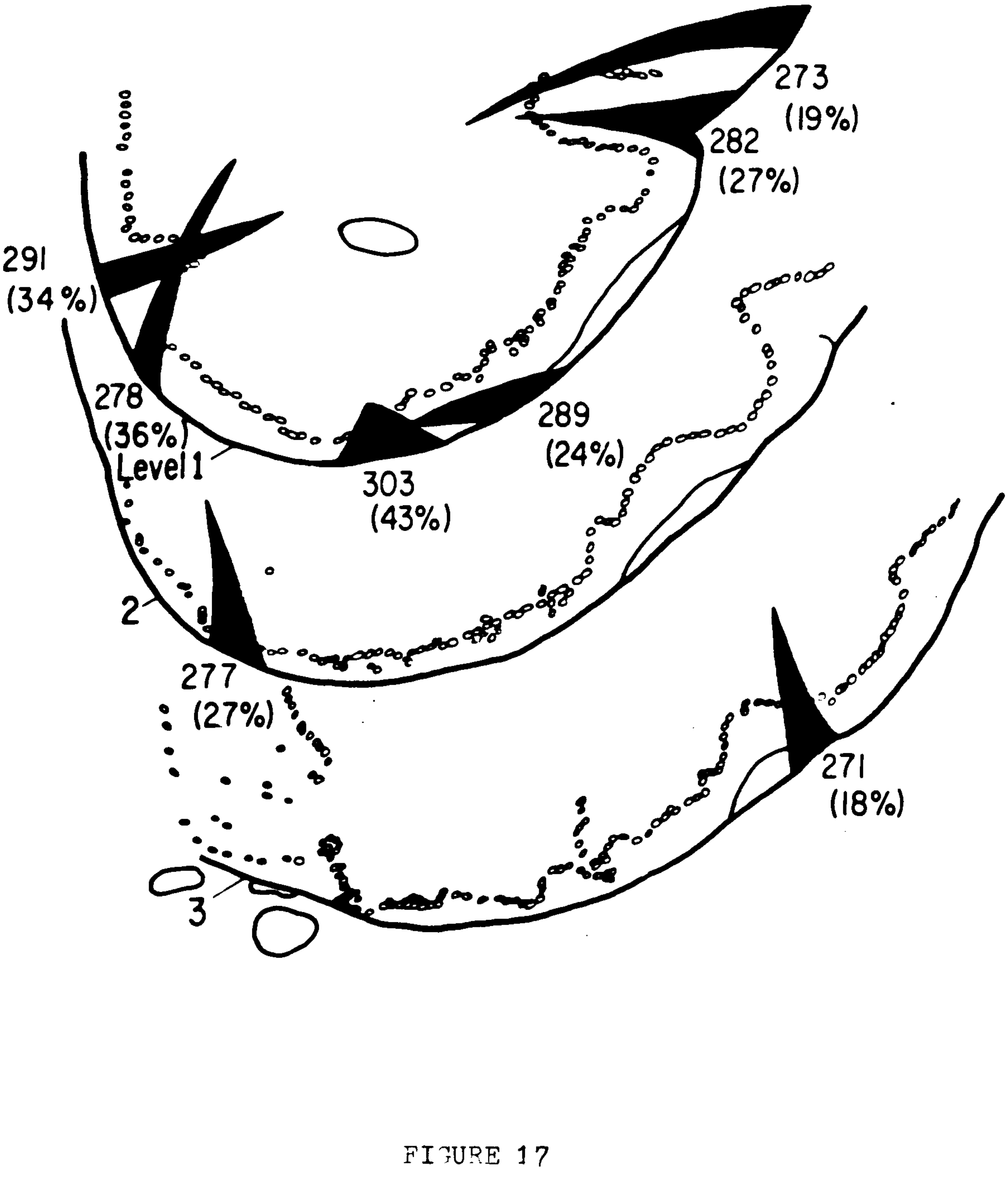


FIGURE 17

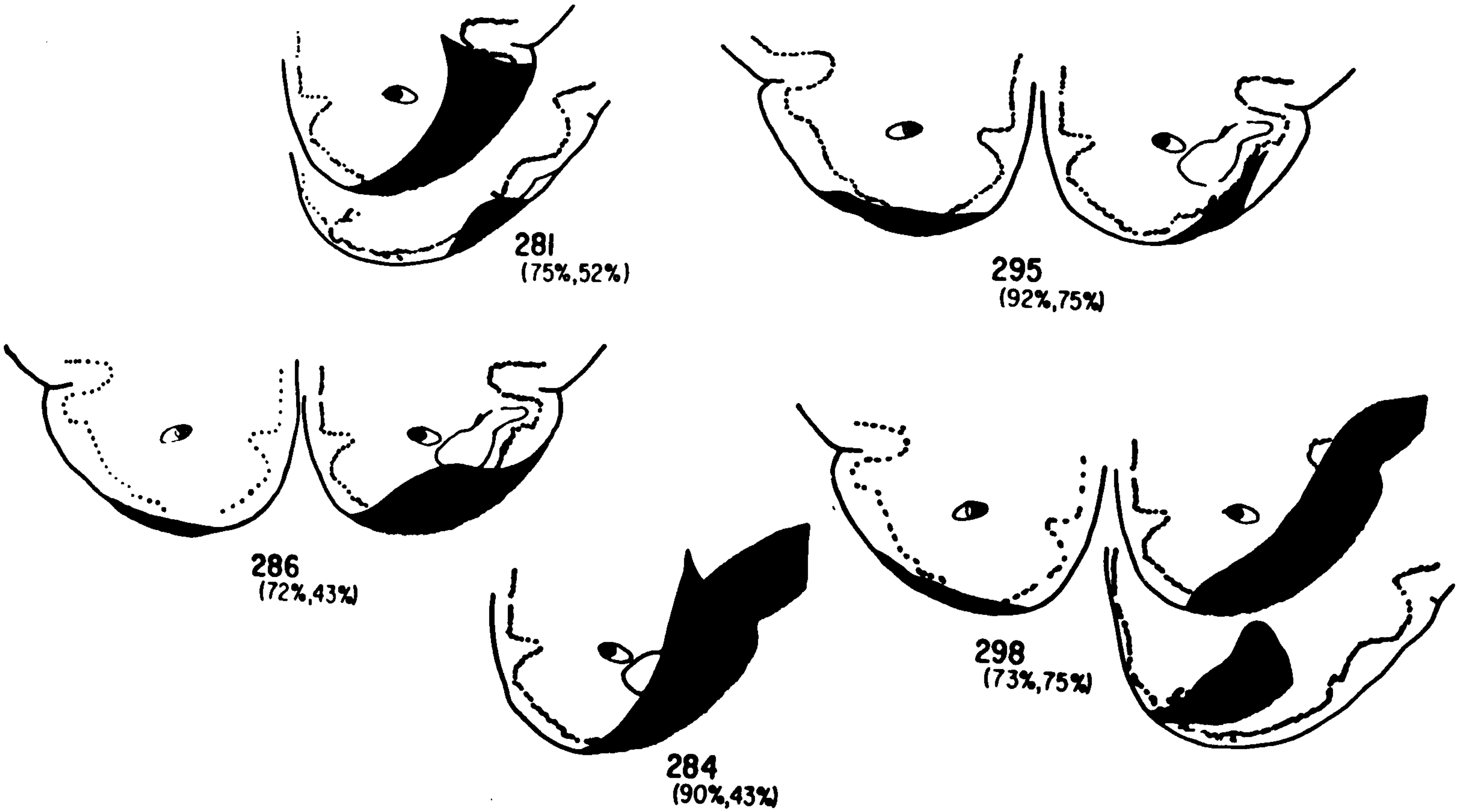


FIGURE 18

FIGURE 19

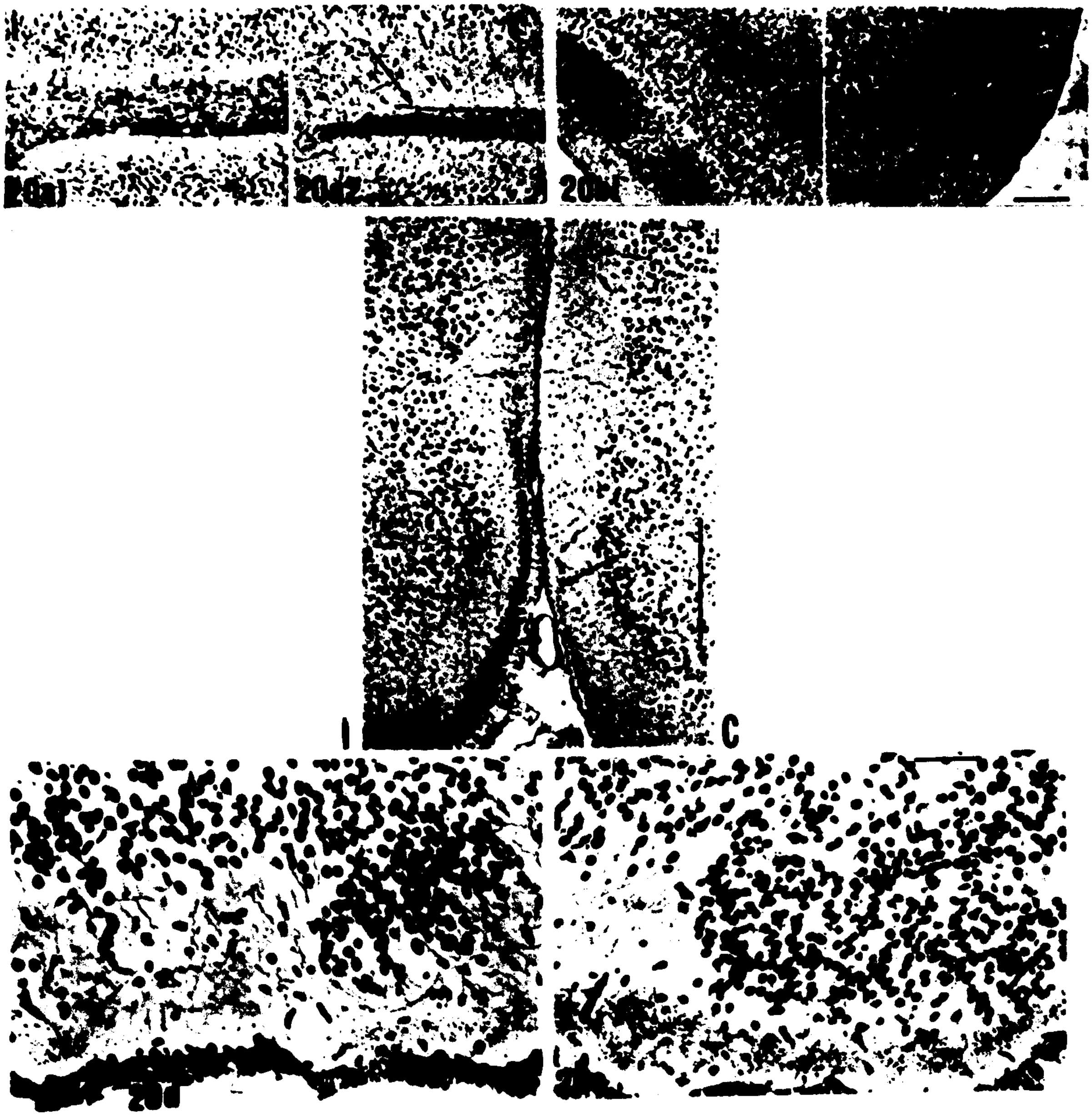
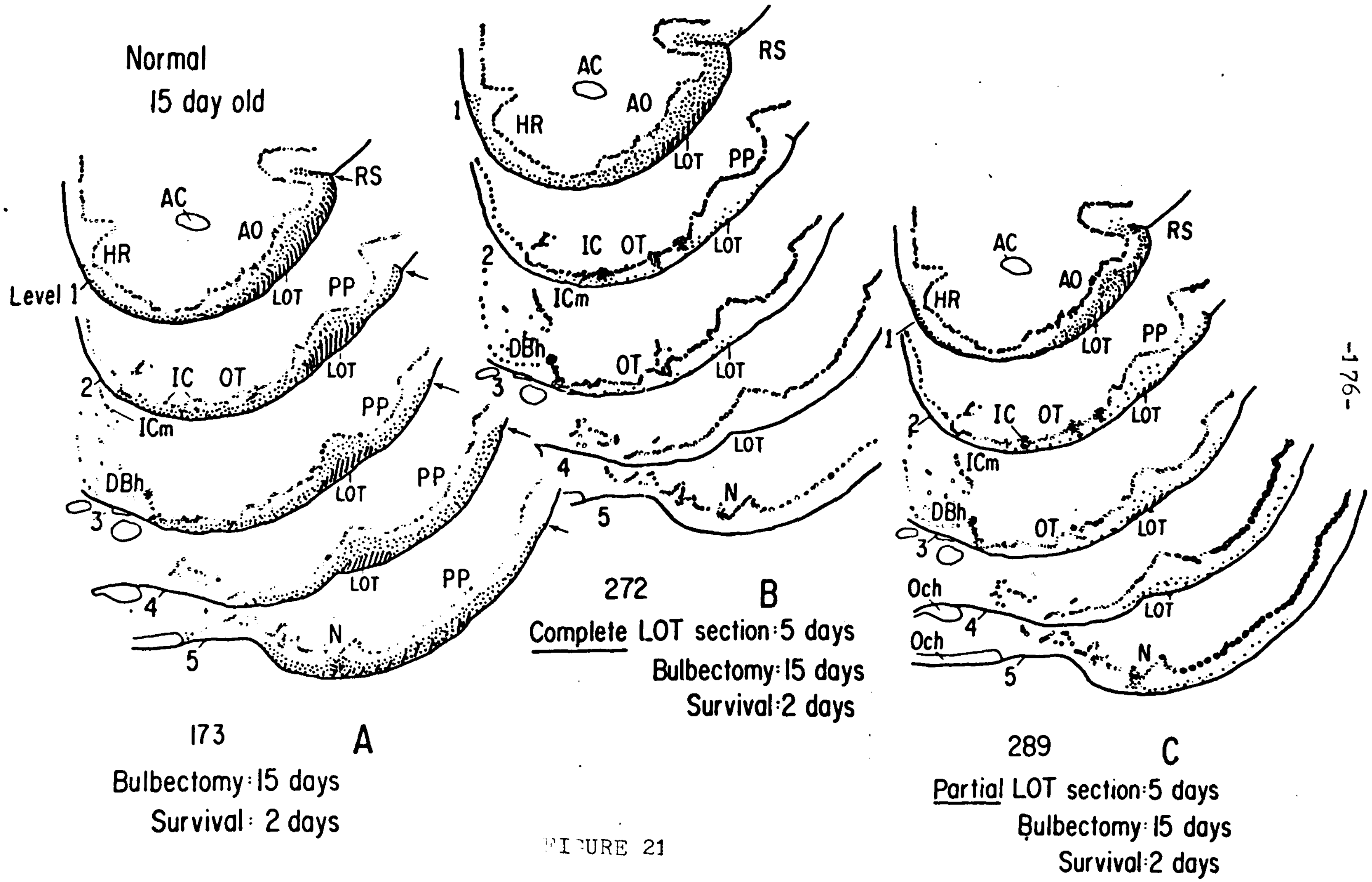


FIGURE 20

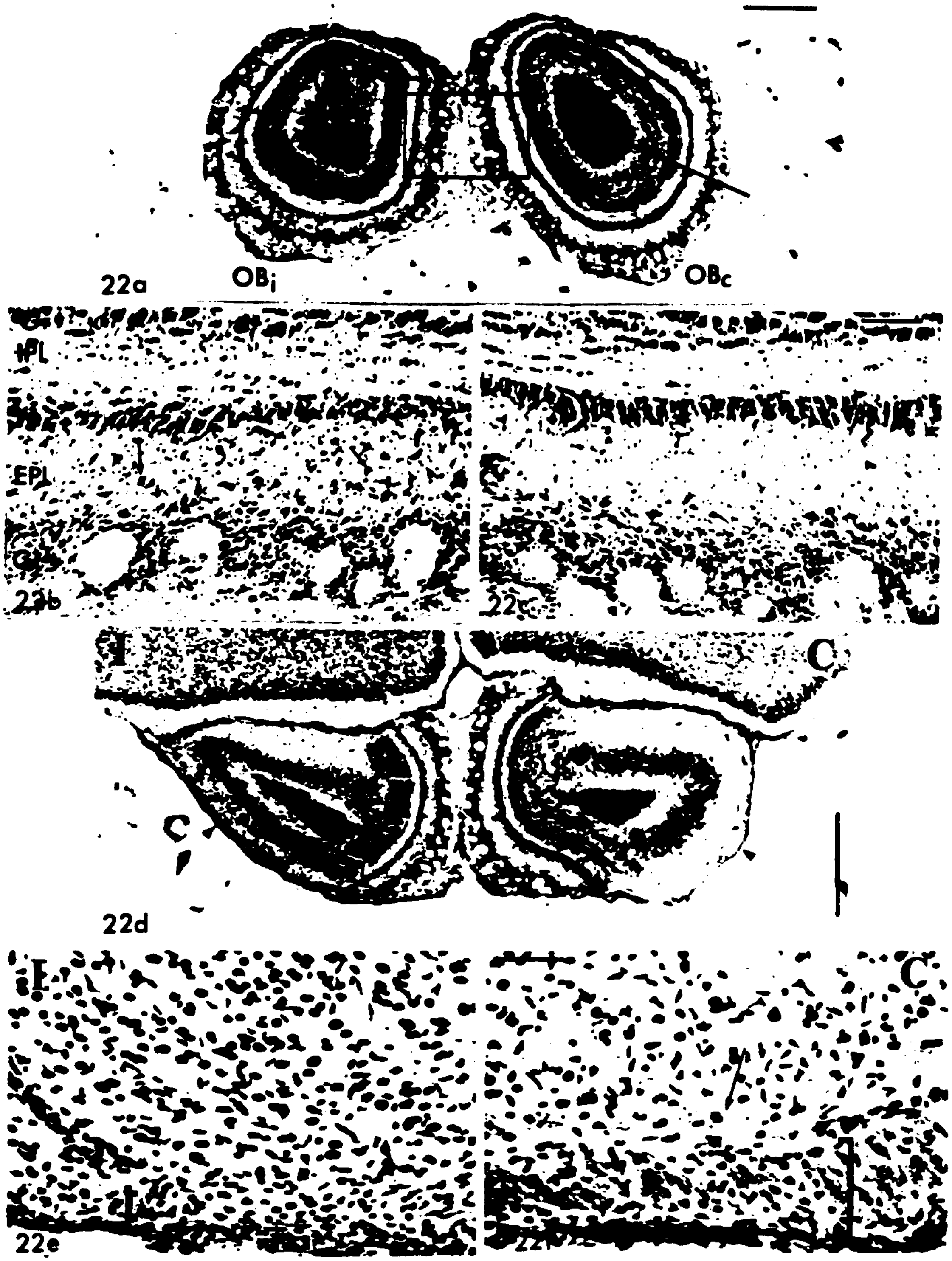


173 A
Bulbectomy: 15 days
Survival: 2 days

272 B
Complete LOT section: 5 days
Bulbectomy: 15 days
Survival: 2 days

289 C
Partial LOT section: 5 days
Bulbectomy: 15 days
Survival: 2 days

FIGURE 21


OB$_i$
OB$_c$
22a
IPL
EPL
22b
22c
I
C
C
22d
I
C
22e
22f

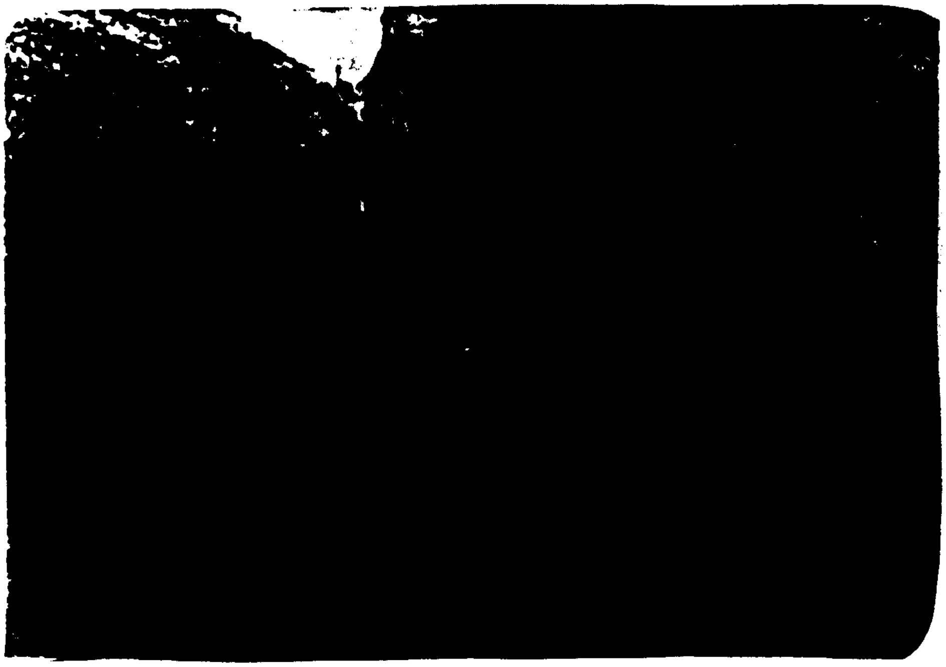


FIGURE 22g

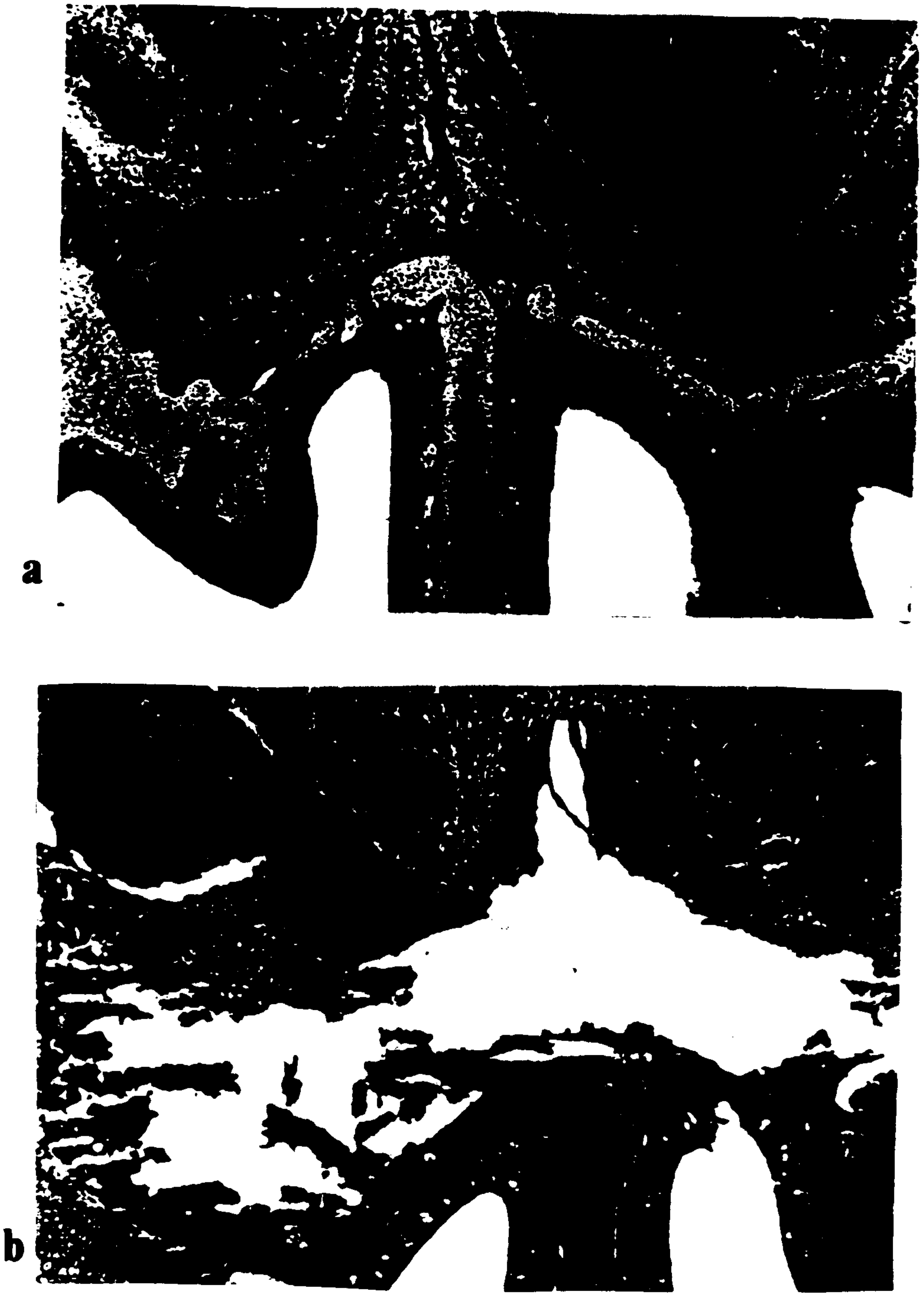


FIGURE 23 a-b

FIGURE 23 c-e

FIGURE 23 f-i

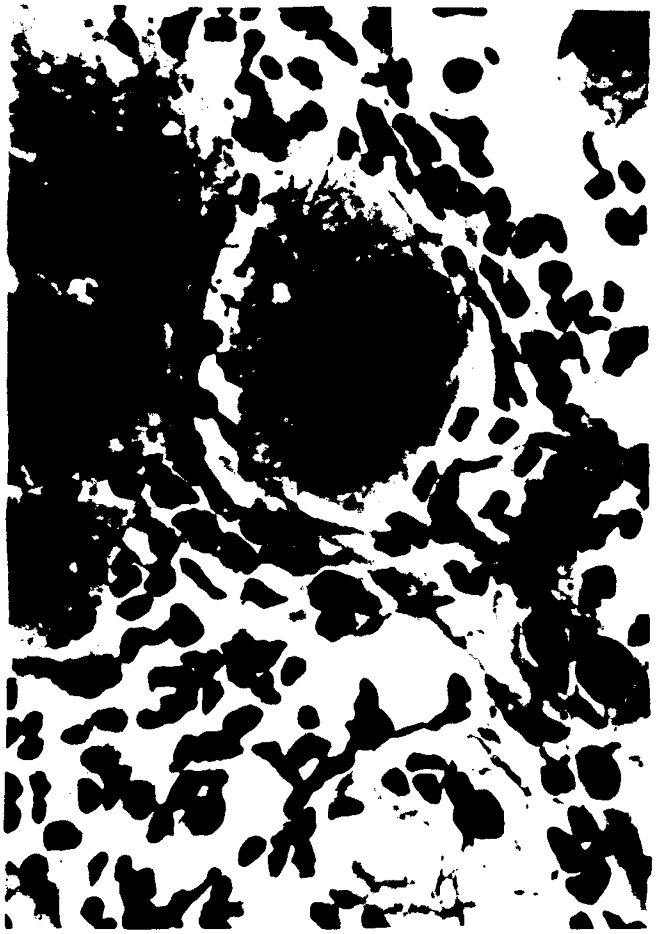


FIGURE 23j

FIGURE CAPTIONS

FIGURE 1. The ventral surface of a normal 17 day old hamster pup brain as traced from a photograph with the major olfactory projection areas indicated. The level of schematics shown in this paper is indicated on the right.

FIGURE 2. Schematic of the appartus used to measure thermal orientation on an air temperature thermal gradient. Pups were started in tier 4 (cool end) and scored for time spent in tier 1 (warm end). Insert shows the range of air temperatures measured at the cool end, midpoint and warm end of the gradient during a daily test session.

FIGURE 3. Median thermal scores of pups in the 4 treatment groups, representing the percent of time spent in the warm end of the thermal gradient on daily 2 min tests.

FIGURE 4. Mean thermal indices of the 4 treatment groups representing thermal scores averaged over days 8 to 10 (left) and days 11 to 13 (right). Verticle lines indicate ± 1 SEM.

FIGURE 5(a) Distribution of thermal indices of the ULOT group on days 8 to 10 and the cutoff points used to separate pups showing an effect of the lesion from those pups showing no lesion effect (NE). Distributions of thermal indices in the UOB and BH/SO groups are represented by the mean ± 1 SD on the lines below the graph. (b) The distribution of thermal indices on days 11 to 13 of those pups within the ULOT group who showed an effect of the lesion on days 8 to 10. The cutoff points used to separate these pups into recoverers (n = 16) and nonrecoverers (n = 8) are shown and the UOB and BH/SO distributions on days 11 to 13 are represented below the graph by the mean ± 1 SD.

FIGURE 6(a) and (b) Daily thermal scores of 4 representative pups in the SO (top), BH (middle), and NE (bottom) groups. In (a) on the left side of the figure, scores are shown as a function of age. In (b) on the right side, scores have been recalculated as a function of the day of the transition from thermal orientation (i.e., the first day on which a thermal score lower than 30% occurred).

FIGURE 7(a) Daily median thermal scores of control and NE groups shown as a function of age. (b) Daily median thermal scores have been recalculated as a function of the transitional day to emphasize the similar abrupt shift from high to low levels of thermal responsiveness that was observed in these groups.

FIGURE 8 (a) and (b) Daily thermal scores of 4 representative pups in the UOB and NR groups show that strong thermal responsiveness persisted at older ages and no transitional day was observed in these groups.
(c) and (d) Daily thermal scores of 4 representative pups in the recovery group shown as a function of age in (c), and as a function of the transitional day in (d).

FIGURE 9(a) Daily median thermal scores of the recovery (R) group show a more gradual pattern of decline in thermal orientation when compared to the decline of SO, BH, and NE groups. (b) The median daily thermal scores of the three ULOT subgroups, NE, R and NR are shown as a function of age to illustrate the similarity in the relationship between these three groups and that observed between UOB, ULOT and BH/SO groups. (Compare to Figure 3.)

FIGURE 10. A comparison of the differences in the mean rate of decline of thermal scores between day -1 and day 0 for those treatment groups whose scores were analyzed as a function of the transitional day. Vertical lines indicate ± 1 SEM.

FIGURE 11(a) The magnitude of the interquartile range of thermal scores shows a marked expansion when calculated as a function of age, only in those groups in which an abrupt decline in thermal responsiveness was observed (e.g., BH, SO and NE). (b) When thermal scores are grouped as a function of the transitional day, no expansion of the interquartile range is observed. (c) The percentage of pups whose thermal scores fell below 30% is shown by day of age for BH, SO, NE and R groups. The modal age at which thermal scores decreased to this level is indicated by arrows.

FIGURE 12. Individual polygraph records of daily 2 min tests on the thermal gradient contrast the pattern of change in activity level (indicated by upward deflections) and time spent in the warm extreme (indicated by the thickened bar below the baseline) for each of the treatment groups. Left column: These records illustrate the three stage sequence seen in pups from SO, BH, and NE groups: I) Pups enter tier 1 with short latencies and settle quietly for the remainder of the test (days 3 - 7); II) Pups slowly explore the grid and show little tendency to enter tier 1 (days 8 - 10); III) Pups move rapidly about the grid and do not settle when they enter tier 1 (days 11 - 15). Middle column: These

records are from three pups who showed recovery from the effects of LOT section. The transition from thermal orientation to exploratory behavior occurred gradually over a period of a few days in which these two behaviors overlapped (e.g., day 10). By stage III these pups showed no evidence of thermal orientation and like control groups, spent the entire test investigating the grid. Right column: Records from two pups in the UOB group and two pups in the NR group all indicate a persistence in thermal orientation at older ages. Pups continue to enter tier 1 with short latencies and to settle quietly while in the heat during stages II and III. Tests on day 13 show that several heat bouts are interspersed with periods of rapid exploration. Two upward deflections mark the start and end of each test.

FIGURE 13(a) Median measures of thermal orientation of the UOB group and the combined BH/SO groups show that with age the UOB group continues to enter tier 1 with short latencies (left) and to remain for longer heat bouts than the BH/SO group (middle). No difference in the frequency of entrances into the tier 1 was observed (right). (b) The pattern of change in median response measures of NE and NR subgroups resembles those of control and UOB groups shown in Fig. 13a. The profile of pups showing recovery (R) initially resembles the NR group and then shifts towards the profile of the NE group at older ages.

FIGURE 14 (a) - (d). Photomicrographs of frontal sections through portions of the olfactory cortex 2 days after bulbectomy at 15 days of age. Degeneration argyrophilia forms a continuous, dense band in the superficial plexiform layer of the olfactory cortex: Fink-Schneider technique. The arrow in (a) indicates the rhinal sulcus which marks the dorsolateral extent of the projection throughout the cortex. Arrowheads indicate the position of the lateral olfactory tract, located in (a) at the lateral portion of the anterior olfactory nucleus, and in (b) in the tract sulcus separating the olfactory tubercle and prepiriform cortex. Calibration bar shown in (a) is 500 µm and applies also to (b) - (d). (e) Silver particles in the tract sulcus show the different appearance and laminar arrangement of fibrous degeneration in lamina 1α and terminal degeneration in Ia. Few silver particles are seen in the deeper laminae Ib and II. The arrow indicates one of several normal fibers from the association system which are seen throughout the plexiform layer after complete bulbectomies: Fink-Schneider technique: bulbectomy 15 days of age; survival 5 days. Calibration bar indicates 50 µm.

FIGURE 15. Photomicrographs of sections taken from the lesion site in three pups from the recovery group, to illustrate the range of damage observed in this group. (a) Case 279 had the most partial LOT transection of the R group. The cut removed a wedge of the plexiform layer through the LOT, sparing a pocket of fibers at the lateral margin of the tract and an even smaller number of fibers at the medial margin of the tract (indicated by arrowheads). Fink-Schneider technique: bulbectomy 15 days of age; 2 day survival. Calibration bar 500 μm. (b) Case 272 had a complete LOT section at the level of the rostral tubercle. Arrow indicates the path of the knife which passed through the tract sulcus. Fink-Heimer technique: bulbectomy 15 days of age; 2 day survival. Calibration bar 500 μm. (c) Case 275 was one of the two cases in the recovery group with a deep transection that destroyed most of the lateral portion of the anterior olfactory nucleus, in addition to destroying the LOT. Arrowhead indicates granule cells, usually located deep in the anterior olfactory nucleus as seen in Fig. 15a. Fink-Heimer technique: bulbectomy 15 days of age; 5 day survival. Calibration bar 250 μm.

FIGURE 16. Prior partial and complete LOT transections can be distinguished by the size and arrangement of degeneration particles at levels caudal to the cut. (a) Silver particles in the normal olfactory bulb projection form two distinct laminae in the tract sulcus; coarse, fibrous degeneration is located subjacent to the pia while fine, terminal degeneration forms a continuous bond extending medially, laterally and deep to the degenerating fibers. Fink-Schneider technique: bulbectomy 15 days of age; survival 5 days. Calibration bar 500μm. (b) Caudal to a prior partial LOT cut, coarse silver particles appear in the typical location subjacent pia (arrow) and represent spared fibers at the lateral margin of the tract. Fine silver particles representing terminal degeneration, form a well defined lamina Ia of normal width and location, lateral and deep to the tract. Fink-Heimer technique: bulbectomy 15 days of age; survival 2 days. Calibration bar 500μm. (c) and (d) At levels caudal to a prior complete LOT transection only fine degeneration is present, usually arranged in discontinuous clusters. (c) A pocket of fine degeneration occurs in the tract sulcus subjacent to the pia. Fink-Heimer technique: bulbectomy 15 days of age; survival 2 days. Calibration bar 125μm. (d) A pocket of fine degeneration (arrow) occurs close to an Isle of Calleja in the olfactory

tubercle. Fink-Schneider technique: bulbectomy 15 days of age; survival 5 days. Calibration bar 125 µm. Astericks in (a) - (c) mark the position of the tract sulcus.

FIGURE 17. Summary of the lesions of 8 pups in the NE group. Two cases (289 and 271) had partial damage to the LOT, sparing a considerable portion of the fibers in the tract while all other lesions did not involve the tract. Thermal indices on days 8-10 are indicated in parentheses below the case numbers. (A ninth case observed in this group but not shown, had a bulbectomy that extended beyond the site of the early transection. The pattern of the projection suggested that this pup also had a partial transection since sparse degeneration was present throughout most of the cortex).

FIGURE 18. Summary of the lesions of 5 pups in the NR group. These pups showed continued thermal orientation throughout the period of testing. Most lesions were deep transections that destroyed cortical layers underlying the tract, and in some cases produced bilateral LOT damage. Thermal indices on days 8-10 followed by days 11-13 are indicated in parentheses below the case numbers.

FIGURE 19. The appearance of the olfactory bulb projection at levels rostral to complete LOT transection. Case 272 - Lamina Ia ipsilateral (I) to the tract section is wider and contains a denser projection throughout the anterior olfactory nucleus and hippocampal rudiment than is seen on the contralateral (C) side. In (I) degeneration extends above the rhinal sulcus (arrowhead) and into the dorsal portion of the hippocampal rudiment. In contrast, lamina Iα, containing the tract fibers, is narrower and less densely packed with fibers than the contralateral tract. Fink-Schneider technique: bulbectomy 15 days; survival 2 days. Calibration bar 250 μm.

Figure 20. A comparison of the olfactory bulb projection ipsilateral (I) and contralateral (C) to LOT transection, 10 days after surgery. (a1) - (a2) The projection in (I) has densely invaded the sulcal neocortex (asterick). The arrow indicates the rhinal sulcus, separating neocortex from paleocortex. Calibration bar 125 μm. (b1) and (b2) The plexiform layer is narrower in (I) than in (C). In (I) the width of lamina Ia is increased while that of lamina Iα is decreased so that the combined width of these laminae is the same on both sides of the brain. Lamina Ib shows severe shrinkage in (I). Calibration bar 125 μm. (c) The medial projection is wider in (I) and continues up the medial

wall of the hemisphere well beyond the dorsal limit of the contralateral projection (see arrows). Calibration bar 250 μm. (d) and (e) The projection to the tubercle at levels caudal to the cut appears as pockets of particles in (I) while in (C) it forms a continuous band. Calibration bar 50 μm. (a) - (e) Fink-Schneider technique: bulbectomy 15 days; survival 2 days.

FIGURE 21. Schematic summary of degeneration argyrophilia in the olfactory cortex 2 days after bulbectomy at 15 days of age. (a) In the normal 15 day old, coarse and fine degeneration occur in a laminar pattern with fibrous degeneration located subjacent to the pia and fine degeneration forming a continuous band which extends deep, medially and laterally to the tract. (b) Ten days after complete LOT section (lesion occurred between levels 1 and 2), there is an increase in the laminar thickness and density of terminals in regions rostral to the cut (see level 1), as well as an invasion of the projection into the sulcal neoncortex and the cortex along the medial wall dorsal to the hippocampal rudiment. At caudal levels, pockets of fine degeneration extend as far as the mid tubercle (level 3). (c) Ten days after partial LOT section (lesion damaged the medial margin of the tract rostral to and at level 1) there is an increase in the density of

the projection at level 1 and a slight invasion of the sulcal cortex and dorsal hippocampal rudiment. More caudally, the projection still forms a continuous but sparse band and a pocket of coarse degeneration is present in the tract sulcus. By level 4, the projection begins to separate from the pial surface and the band of degeneration is very narrow. By level 5, the band of degeneration no longer reaches the rhinal sulcus. The projection disappears gradually behind level 5, and the caudalmost olfactory cortex contains no projection. Levels shown on the bottom left of each schematic correspond to levels shown on the right of Figure 1.

FIGURE 22. Changes observed 5 days after LOT section in the olfactory bulb and tract rostral to the section. (a) OB_i is smaller than OB_c and its mitral cell layer appears less densely stained. The inner core of granule cells (arrow) also appears to be less dense and the external plexiform layer is densely filled with cells in OB_i. These changes can be seen more easily in Fig. 22g. The region inside the box is shown in Fig. 22b and c. Cresylechtviolet stain. Calibration bar 500 μm. (b) and (c) The area within the box of Fig. 22a has been enlarged to show the reduced density of mitral cells and disorganization of the mitral cell layer in OB_i.

The external plexiform layer of OB_i contains many more large neurons (arrow) as well as small glial cells than OB_c. The width of IPL shows some expansion in OB_i. Cresylechtviolet stain. Calibration bar 50μm. (d) At the rostral anterior olfactory nucleus, an intense glial reaction is present in the plexiform layer adjacent to the tract (arrowhead in (I)). Arrowheads indicate the position of the enlargements shown in Fig. 22e and f. Cresylechtviolet stain. Calibration bar 500μm. (e) and (f) The lateral olfactory tract is severely reduced in (I) as compared to (C) (see brackets) at levels rostral to the lesion. This section is an enlargement of a section adjacent to Fig. 22d, but stained with the Fink-Schneider technique. Normal fibers present in lamina Ib of (C) are not present in (I). No degeneration debris is seen in Ib, 5 days after tract section. Calibration bar 50μm. (g) The density and width of the mitral cell layer in OB_i is reduced and EPL is densely filled with cells. IPL shows some expansion on the side of the lesion. Cresylechtviolet stain. (Xerox color reproduction of color photomicrograph). Calibration bar 50μm.

FIGURE 23. The appearance of the normal neonatal olfactory bulb and the remaining olfactory peduncle at various intervals after neonatal bulbectomy at 5 days of age. (a) - (e) and (g) - (i) are horizontal sections which include portions of the nasal cavity, cribiform plate and brain; (f) is cut in the frontal plane. (a) The appearance of the nasal cavity and olfactory bulbs of a normal 5 day old hamster pup. Arrow indicates fascicles of olfactory axons within the cribiform plate. (b) 8 hrs after olfactory bulbectomy, the nasal cavity contains necrotic debris. Fascicles of olfactory axons are seen within the cribiform plate at arrows. (c) 6 days after bulbectomy, olfactory fascicles begin to form a loose plexus just inside the cribiform plate adjacent to the zone of granule cells which caps the remaining peduncle. (d) The tract culcus is devoid of axons after bulbectomy. Compare to (e) which shows the normal LOT in the tract sulcus. (f) After neonatal bulbectomy, the prepiriform cortex shows an intensification of the fiber band in lamina Ib, 20 days after surgery. (g) - (i). 20 days after bulbectomy at 5 days of age, the irregular plexus of the olfactory nerve abuts the zone of granule cells and in deeper portions of the plexus, glomerular formations are seen. (j) Large neurons (arrow) are seen immediately adjacent to glomeruli and their

dendritic arbors can be seen ramifying through the glomeruli. (a) - (j) Fink-Schneider technique. (Xerox color reproductions of color photomicrographs).