Perinatal Development
A Psychobiological Perspective

Edited by

Norman A. Krasnegor
Human Learning and Behavior Branch,
Center for Research for Mothers and Children
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, Maryland

Elliott M. Blass
Department of Psychology
Johns Hopkins University
Baltimore, Maryland

Myron A. Hofer
Department of Psychiatry
Columbia University College of Physicians and Surgeons
and Department of Developmental Psychobiology
New York State Psychiatric Institute
New York, New York

William P. Smotherman
Laboratory for Psychobiological Research
Departments of Psychology and Zoology
Oregon State University
Corvallis, Oregon

1987

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando  San Diego  New York  Austin
Boston  London  Sydney  Tokyo  Toronto
Neural and Behavioral Plasticity Induced by Early Olfactory Learning

MICHAEL LEON, ROBERT COOPERSMITH, SUZANNE LEE, REGINA M. SULLIVAN, DONALD A. WILSON, and CYNTHIA C. WOO

Department of Psychobiology
University of California
Irvine, California 92717

I. Introduction

A. Maternal Olfactory Attractant

Over a decade ago, we found that young Norway rats reliably (99%) come to approach the odor of their mother (Leon & Moltz, 1971, 1972). This behavior pattern has now been reported in more than a dozen species, ranging from crayfish (Little, 1975, 1976) to humans (MacFarlane, 1975; Russell, 1976). Norway rat pups are normally exposed to low concentrations of the maternal odor early in life (Leon, 1983). They orient toward the maternal odor (Altman, Sudarshan, Das, McCormick, & Barnes, 1971) and then approach it when they are mobile enough to leave the nest (Leon & Moltz, 1971). This olfactory preference facilitates mother–young reunions at a time when the mobile young still require their mother’s milk (see Leon, 1983, for a review).

B. Experiential Basis for Attraction to Maternal and Other Odors

The principal source of the odor is the cecotrophe portion of the mother’s anal excreta, (Leon, 1974). Cecotrophe is differentiated from the feces in the cecum, a large gastrointestinal structure located at the junc-
tion between the small and large intestines. Synthesis of the cecal odor depends on cecal bacterial function (Leon, 1974, 1975). Since enteric bacterial populations and their metabolic products differ with different diets (Draser et al., 1973; Lampen & Peterjohn, 1951; Porter & Rettger, 1940), it seemed possible that the cecal odor would also differ with different maternal diets. Indeed, it did. Leon (1975) found that pups raised with mothers on one diet were attracted only to mothers eating that diet. Pups must therefore acquire their attraction to the odor that they will approach postnataally, since no single "maternal" odor is invariably present in maternal anal excreta. Daily exposure to either a maternal or another odor induces a preferential approach response by the pups (Alberts, 1978; Brunjes & Alberts, 1979; Leon, 1974; Leon, Galef, & Behse, 1977).

C. Enhanced Olfactory Bulb Response

We considered the possibility that experience with a specific odor would induce the developing olfactory bulb to have a special, perhaps enhanced, response to that odor. If one accepts the reasonable assumptions that elevated glucose use reflects activity and that the uptake of the glucose analogue 2-deoxyglucose (2-DG) reflects elevated neuronal glucose use, then $^{14}$C-labeled 2-DG autoradiography is a powerful technique for localizing areas of differentially active cells (see Gallistel, Piner, Allen, Adler, Yadin, & Negin, 1982; Mata et al., 1980; Sokoloff, 1981; Yarowski & Ingvar, 1981). It also should be noted that the metabolic pathways of different cell types have not been identified in the developing olfactory bulb, and it is not completely clear what proportion of neural activity is accounted for by this method in each type of cell.

An artificial odor was chosen as the olfactory stimulus, since its presentation could be controlled more precisely than the maternal odor. Peppermint was the odor of choice because, after repeated exposure, rat pups would approach it to the same extent as they would approach maternal odors (Leon et al., 1977). We used $^{14}$C 2-DG autoradiography to map the response of the olfactory bulbs of 19-day-old rat pups during a 45-min test exposure to peppermint odor after the pups had been exposed to either peppermint or fresh air for 18 days. The olfactory experience occurred during daily 10-min sessions when the pups were held in either the peppermint or fresh airstream. Pups received perineal stimulation while exposed to the odor during the 10-min exposures (but not during the 45-min test on Day 19) because similar stimulation has been shown to facilitate odor-preference acquisition in neonatal rats (Pedersen & Blass, 1982; Pedersen, Williams, & Blass, 1982; Sullivan, Hofer, & Brake, 1986).
Autoradiographs of 20-μm coronal sections of the olfactory bulb were made using standard techniques. During development of the autoradiographs, the tissue was accompanied by 14C standards that had previously been calibrated to 14C uptake in similar brain sections. To analyze the autoradiographs, we employed a digital image-processing system which digitized and stored the image of the brain on the autoradiograph by assigning an 8-bit gray level to each element of the pixel array. A calibration function was then constructed by plotting the gray values of the exposure standards against their previously determined 14C-labeled tissue equivalents. This curve was then fitted to a linear function. A new image of the brain section was then constructed from this calibration function. Concentrations of 14C could then be determined for any specified area of the section.

We found that both groups had a reliably high level of uptake in a specific area of the glomerular layer, lateral and 1.5–2.2 mm from the rostral pole. This uptake pattern presumably reflects a normal olfactory response to the peppermint odor present during the period of 2-DG uptake. The odor-experienced pups, however, had a significantly greater level of uptake (64%) in those areas (see Fig 1; Coopersmith & Leon, 1984). Neither the level of uptake in the periventricular core nor the uptake in other portions of the glomerular layer differed between groups, indicating that the response was not due to a generalized increase in neural activity.

Other investigators have found that when rats are exposed to different odors, the olfactory bulb reliably shows qualitatively different uptake patterns that are largely restricted to the glomerular layer (Astic & Saucier, 1982; Greer, Stewart, Kauer, & Shepherd, 1981; Greer, Stewart, Teicher, & Shepherd, 1982; Jourdan, Duveau, Astic, & Holley, 1980; Sharp, Kauer, & Shepherd, 1975; Stewart, Kauer, & Shepherd, 1979; Teicher, Stewart, Kaver, & Shepherd, 1980). We found that while there is no change in the qualitative pattern of 2-DG uptake after early experience with an odor, there is a quantitative difference in the relative response of the olfactory bulb to the same concentration of the same odor.

D. Lack of Differential Respiration

The possibility existed, however, that the familiar odor induced the animals to increase their respiration rate, thereby increasing the stimulus intensity, during the test exposure. The increase in olfactory bulb activity could then be attributable to an increase in stimulus intensity (Greer et al., 1981). No statistical differences were found between groups in the number of respirations or the distribution of respiratory frequencies either
Fig. 1. Mean relative \(^{14}\text{C}\) 2-DG uptake in specific glomerular areas that are lateral and 1.5–2.2 mm from the rostral pole of the olfactory bulb in peppermint-peppermint (odor-familiar) and air-peppermint (odor-unfamiliar) pups on postnatal Day 19. Means and SEMs are shown in these graphs. From Coopersmith & Leon (1984), Science, 225, 849–851. Copyright 1984 by the AAAS.

during the entire 45-min test exposure period or in any of eight shorter intervals (Fig. 2; Coopersmith & Leon, 1984; unpublished observations). These data suggest that the enhanced response may be due to a reorganization of the olfactory system itself rather than to a behavioral difference between groups.

E. The Enhanced Response Is Odor-specific

It is possible that familiarity with one odor would increase the olfactory bulb response to any odor. That is, the enhanced neural response may be nonspecific. We therefore familiarized pups with either peppermint or cyclohexanone and tested their 2-DG uptake patterns in response to peppermint while their respiration frequency was being analyzed on Day 19.

The 2-DG uptake of peppermint-familiar pups replicated almost exactly the uptake levels reported by Coopersmith and Leon (1984). In addition, peppermint-familiar pups showed significantly higher 2-DG uptake than
cyclohexanone-familiar pups in the same area of the bulb described above (Coopersmith, Henderson, & Leon, 1986). No differences were found in respiration frequency patterns between the groups. The enhanced neural response therefore is specific to the odor with which the pup is familiar, although there may also be a nonspecific component of the response.

We then studied the effects of cyclohexanone experience on subsequent responsiveness to cyclohexanone. When tested on Day 19 with cyclohexanone, cyclohexanone-familiar pups had an enhanced neural response in a glomerular area different from that identified for peppermint. The glomerular area with particularly high 2-DG uptake is medial and caudal to the identified peppermint area (2.5–3.0 mm from the rostral pole.
of the bulb). The cyclohexanone-familiar pups had an enhanced response
to cyclohexanone compared with that of cyclohexanone-unfamiliar (air-
exposed) pups. The enhanced response was also not accompanied by
changes in respiratory pattern.

F. The Enhanced Response is Long-Lasting

Rats exposed to peppermint odor and stroking from days 1–18 were
tested for 2-DG uptake in their olfactory bulb in response to that odor on
day 90. We found that such animals still had an enhanced glomerular
uptake of 2-DG compared with air/stroking controls (Coopersmith and
Leon, 1986). Exposure of adults to odor/stroking stimulation did not in-
duce a subsequent enhanced response to that odor (Woo and Leon, 1987).

G. Familiarity Alone Is Insufficient to Produce
the Enhanced Response

We then went on to find that simple odor exposure was insufficient to
induce both odor attraction and the enhanced 2-DG uptake in the olfac-
tory bulb. Recall that we routinely stroked the back or perineum of the
pups with a small brush to mimic maternal licking during both odor and
fresh air exposure because Pedersen et al. (1982) reported that similar
stimulation facilitated odor-preference acquisition in perinates. To deter-
mine the importance of such stimulation in our situation, we exposed
pups for 10 min/day to peppermint odor with stroking, odor without strok-
ing, or stroking only or left them with their mothers until the test.

On Day 19, all groups of pups were tested for their olfactory prefer-
ence; identical groups were exposed to peppermint odor following 2-DG
injection. As can be seen in Fig. 3, only pups that were simultaneously
stroked and exposed to peppermint had an increased preference and an
enhanced neural response to the odor (Sullivan & Leon, 1986). These data
suggest that simple odor familiarity is not sufficient to induce odor prefer-
ences or the enhanced glomerular response. Sullivan and Hall (1987) have
recently shown that stroking acts as a reinforcer. In fact, their data indi-
cate that stroking is as reinforcing to pups in learning situations as is milk.
These data therefore suggest that the enhanced neural response develops
after early olfactory learning rather than after other kinds of olfactory
experience.

Several laboratories have previously found that the development of
behavioral preferences for odors by young rats is greatly facilitated by
stimulation of some sort during odor exposure. Such manipulations in-
Fig. 3. Relative 2-DG uptake and behavioral preference for peppermint odor on Day 19 after being exposed to peppermint odor accompanied by perineal and back stroking, exposed to the odor alone, simply stroked, or left undisturbed during Days 1–18. From Sullivan & Leon (1986).

clude intraoral milk infusions (Brake, 1981; Johanson & Hall, 1979; Johanson, Hall, & Polefrone, 1984; Johanson & Teicher, 1980; Sullivan & Hall, 1987), stroking the ventral and dorsal areas of pups (Pedersen et al., 1982; Sullivan, Hofer, & Brake, 1986), stroking the perineal area (Coopersmith & Leon, 1984), tail pinching (Sullivan, Brake, Hofer, & Williams, 1986), amphetamine injection (Pedersen et al., 1982), morphine injection (Kehoe & Blass, 1986), prolonged isolation (Leon et al., 1977), huddling with conspecifics (Alberts & May, 1984), or placing the young in a warm ambient temperature (Alberts & May, 1984; Pedersen et al., 1982). These varied stimuli may mimic the stimuli, or the pup responses to the stimuli, provided by the mother during their interactions. Pups may normally develop olfactory preferences only when they are being cared for by the mother in the nest, thereby restricting the olfactory experience of the pups to maternal odors at the time in their lives when the pups must develop an olfactory preference only to their mother's odor.

We next determined whether the enhanced glomerular response would be found after any type of olfactory learning. Specifically, we wondered whether odors experienced in conjunction with aversive consequences
would produce an enhanced response. We therefore trained pups to avoid peppermint odor on Day 18 by exposing them to either peppermint odor or fresh air for 30 min and then subjecting them to toxicosis (Cooper-smith, Lee, & Leon, 1986). Five min after the beginning of the odor exposure, pups were injected IP with LiCl or with saline. All pups were given a 2-DG test with peppermint odor on Day 19. The efficacy of the aversion training was assessed with another pup from the same litter by its avoidance of the odor during a 3-min odor preference test.

The enhanced response is absent to familiar odors experienced with aversive consequences, even if that odor is behaviorally relevant to the pup. Those pups experiencing toxicosis in the presence of the odor avoided it, spending 26% of the test time in the presence of peppermint odor, while both control groups had a neutral response to peppermint (47%). Odor/toxicosis pups had significantly less 2-DG uptake in the identified glomerular areas than the odor/no toxicosis pups and were no different from the air/toxicosis pups. There is a slight, but significant, enhancement of 2-DG uptake in pups after only one brief experience with peppermint that was not followed by toxicosis. Again, differences in the pattern of respiration did not account for the difference in 2-DG uptake. The depressive action of LiCl on the brain also did not seem to account for the data collected 24 hr after toxicosis. While these pups did not have a number of olfactory experiences equivalent to the pups that developed an attraction to the odor over 18 days, they did learn a behavioral avoidance of the odor, and this learned response was not accompanied by the enhanced neural response.

II. Mechanisms for the Development of the Enhanced Glomerular Response

A. Structural Changes

While we are continuing to investigate the experiential circumstances under which the enhanced glomerular response develops, we also have become interested in determining its mechanism. Indeed, it has been our hope that the phenomenological results would either suggest or rule out possible mechanisms. Both the magnitude of the enhancement and the fact that it lasts into adulthood suggested that we might see a major structural change associated with the area in which the enhanced neural responses are observed. We first wanted to examine the glomerular area to determine whether the glomeruli associated with the enhanced 2-DG uptake had any obvious structural modification. We stained the brain
sections with the Goshgarian modification of the Palmgren stain to delineate the fibers in the glomerular layer (Woo, Coopersmith, & Leon, 1987).

We also reacted the bulbs of odor-familiar and odor-unfamiliar pups for the mitochondrial enzymes, succinic dehydrogenase (SDH) and cytochrome oxidase (CO) following a 2-DG test with odor presentation (Woo, Coopersmith, & Leon, 1987). Every third section was used for 2-DG autoradiography, with the other two sections used to react for SDH and CO. The SDH and CO sections were then aligned with the autoradiographs, using the image analysis system.

Figure 4 shows our striking finding. The peppermint-familiar pups had enlarged glomeruli in the area generating the increased activity while the same area of odor-unfamiliar pups had no modified glomerular complex. In fact, areas of increased 2-DG uptake invariably have a modified glomerular complex, whereas we have not observed such structures in any odor-unfamiliar pup in the identified areas of the glomerular layer. The modified glomerular complexes have a characteristic orientation, protruding from the glomerular layer into the external plexiform layer.

It should be noted that we observed these clusters in other, particularly medial, parts of the bulb which are not associated with the focal areas of 2-DG uptake. These complexes may reflect the fact that the pups are exposed to other odors. Wherever there is an area of enhanced 2-DG uptake, however, there is a modified glomerular complex.

Morphometric analysis of these glomerular areas confirmed the observations of a structural modification in this lamina. The glomerular layer underlying the focuses of high 2-DG uptake in peppermint-familiar animals was about 30% wider than that in peppermint-unfamiliar animals. Baseline measurements from areas adjacent to these focuses were virtually identical in peppermint-familiar and peppermint-unfamiliar animals. While the number of glomeruli did not differ between groups, the cross-sectional area of peppermint-familiar pups was about 21% greater than for peppermint-unfamiliar pups. If one assumes that the glomeruli are ball shaped, such an increase would result in a 33% increase in glomerular size. This phenomenon, coupled with the concentration of two or three glomeruli in a small area, may account for the enhanced 2-DG uptake that we observed in the autoradiographs.

There are some data that lend at least some indirect support to this hypothesis. First, there is enormous cell death in the olfactory bulb early in life. During the first few weeks postpartum, approximately half of the mitral cells die (Rosselli-Austin & Altman, 1979). Without olfactory stimulation in early life, though, more mitral cells and even more tufted cells die (Brunjes & Borror, 1983; Laing & Panhuber, 1978; Meisami & Safari, 1981). Restriction of young animals to a single odor produces different
patterns of mitral cell degeneration (Doving & Pinching, 1973; Laing & Panhuber, 1978; Pinching & Doving, 1974) and reduced sensitivity to other odors (Laing & Panhuber, 1978; but see Dalland & Doving, 1981). Other cell types within the olfactory bulb may also die if they are not stimulated at some minimal level. At the other extreme, high levels of olfactory stimulation by a particular odor may prevent many neurons from dying. The glomeruli formed by these neurons may crowd each other into the external plexiform layer. This process of selective cell death, possibly regulated by differential peripheral input, has been sug-
gested to participate in the development of neural circuitry in other brain systems (Cowan, 1973; Guillery, 1972).

It is also possible that the modified glomerular complexes are the result, not of a failure to die, but of a differential arborization of the glomeruli that are there, since developing mitral cells arborize as they grow (Scheibel & Scheibel, 1975). Differential dendritic arborization with differential stimulation has been shown in other brain areas (see Greenough & Volkmar, 1973; Globus, Rosenzweig, Bennett, & Diamond, 1973).

Graziadei and his co-workers (Graziadei, Levine, & Monti Graziadei, 1978; Graziadei & Monti Graziadei, 1978; Graziadei & Samanen, 1980) have shown that incoming olfactory receptor axons will induce the formation of glomeruli in incompletely lesioned olfactory bulbs and even in cortical tissue. Increased local receptor stimulation by peppermint may allow more axons to penetrate and/or induce the formation of glomeruli in the olfactory bulb in the identified portions of the bulb. Indeed, some receptor axons normally penetrate into the external plexiform layer early in development (Hinds & Hinds, 1976; Monti Graziadei, Stanley, & Graziadei, 1980) and could induce the formation of enlarged glomeruli if they are stimulated.

B. Do Mitral Cells Mediate the Enhanced Glomerular Response?

To this point, our implicit assumption has been that the enhanced glomerular response is caused by differential mitral cell activity. Specifically, we have assumed that an increase in glomerular activity increases the firing rate of the mitral cells, which then relay this increased activity to the piriform cortex. This assumption, however, appears to be wrong.

We recorded single-unit activity from mitral cells associated with the identified glomerular areas of peppermint-familiar and peppermint-unfamiliar Day 19 pups (Wilson, Sullivan, & Leon, 1985; 1987). Mitral cells \((N = 111)\) were isolated and identified by location and/or antidromic stimulation from the piriform. We found that mitral cells of peppermint-familiar pups had significantly fewer excitatory and significantly more suppressive responses to peppermint than the mitral cells of odor-unfamiliar pups. No differences in response pattern were found to orange odor in these mitral cells associated with peppermint-responsive glomeruli (Fig. 5). Again, odor experience without reinforcing tactile stimulation was not effective in changing olfactory system responses (Wilson et al., 1987).

These data suggest that early olfactory experience alters the neural signal emanating from the olfactory bulb. The signal appears to be a reversed pattern of excitatory and suppressive responses transmitted to
the olfactory cortex. Such an inhibition may serve as a unique stimulus for recognition of an odor that has acquired attractive qualities for the pups. Since this is, to our knowledge, the first time that anyone has recorded unit activity from mitral cells in areas activated by a specific odor, these data should have important implications for the understanding of olfactory coding.

Although neonatal odor exposure selectively alters subsequent mitral cell responsiveness to the odor, the changes are not what one would predict if the enhanced glomerular activity was localized in the mitral cell dendrites or in the olfactory neuron axons synapsing in mitral cell glomeruli. Clearly, mitral cell activity did not increase according to expectations.

There are, however, at least two circumstances under which odor familiarity might actually induce greater mitral cell responsiveness but which would make detection of this increase difficult with the sampling technique used here. First, tonic levels of mitral cell excitation in specific glomeruli may be greater in odor-familiar pups than in odor-unfamiliar pups. This could make excitatory responses more difficult to detect and inhibitory responses easier to detect. A comparison of baseline firing rates, however, makes this explanation unlikely, since there was no difference in baseline firing rates between the familiar and unfamiliar groups (Wilson et al., 1985).

A second possibility is that a few mitral cells may have been intensely excited by the familiar odor, and this intense excitation induced an equally powerful surrounding region of inhibition via inhibitory granule
cells. This inhibitory surround could suppress neighboring, weakly excited mitral cell responses and increase the number of inhibitory responses likely to be recorded. One might expect, then, that those few excitatory responses that were recorded in odor-familiar pups would, on average, be more intense than excitatory responses in unfamiliar pups. We therefore compared the magnitude of excitatory responses to peppermint for the odor-familiar and odor-unfamiliar groups. The magnitude of excitation was calculated as \(((\text{spikes/second during odor}/[\text{baseline spikes/second}]) \times 100\). The magnitude of excitatory responses to peppermint revealed no difference between groups, suggesting that this possibility was unlikely to produce the observed mitral cell response (Wilson et al., 1985).

These results suggest that activity in neurons other than mitral cells may be responsible for the enhanced glomerular 2-DG uptake to familiar odors. Thus, a familiar odor may increase the activity of glomerular-layer neurons (either periglomerular and/or external tufted cells), which may then decrease mitral cell activity. The decrease in mitral cell activity to attractive familiar odors may result in a unique, sharpened signal to the piriform cortex.

C. Glomerular-Layer Neurons

Our data demonstrate that mitral cell responses to an odor are altered by the pup’s previous experience with that odor. The direction of this change in mitral cell responses (decreased excitation, increased inhibition), however, differs from what might be expected if the enhanced glomerular uptake of 2-DG reflects increased activity in the mitral cell dendrites. It seems likely, therefore, that another class of neuron increases its firing rate to attractive familiar odors, and these neurons directly or indirectly suppress mitral cell activity.

Macrides, Schoenfeld, Marchand, and Clancy (1985) have recently suggested that the external tufted cells become particularly active during olfactory stimulation and that the activity of these cells may be reflected in increased 2-DG uptake. They go on to suggest that the increased activity of tufted cells may mediate the sort of contrast enhancement or gain-setting functions in olfactory processing that we have found. A subset of tufted cells form topographically organized intra bulbar associational connections with the opposite side of the ipsilateral bulb (Macrides et al., 1985). That is, laterally situated tufted cells project to the internal plexiform layer of the medial side of the same bulb. A close examination of the spatial pattern of 2-DG uptake to odor reveals a similar lateral-medial relationship (Jourdan et al., 1980; Skeen, 1977; Wilson et al., 1987).
Another population of tufted cells synapse on granule cells in the ipsilateral internal plexiform layer (Orona et al., 1984). Thus, an increase in activity of this population of external tufted cells could lead to increased local mitral cell inhibition. Superficial tufted cells are far more responsive to olfactory nerve stimulation and have a greater tendency to have inhalation-related firing patterns than mitral cells and internal tufted cells (Onoda & Mori, 1980; Schneider & Scott, 1983).

All of these characteristics make the superficial tufted cells likely candidates to mediate the glomerular 2-DG patterns observed in both odor-unfamiliar and odor-familiar animals. In fact, the mutant mouse strain PCD (Purkinje cell degeneration) has no mitral cells but still has normal patterns of 2-DG uptake in response to an odor (Greer & Shepherd, 1982). The observed glomeruli were smaller than those normally seen in mice with their mitral cell complement. These data are consistent with the idea that the tufted cells had formed these 2-DG-responsive glomeruli.

It is also possible that the periglomerular cells increase their firing rate to odors and produce the increase in 2-DG uptake in the identified glomeruli. These cells receive input directly from the olfactory receptor neurons and via dendrodendritic synapses with mitral and tufted cells, and they appear to mediate interglomerular inhibition (Macrides & Davis, 1983). Increased activation of these cells by external tufted cell glomeruli may then decrease mitral cell firing.

Both external tufted cells and periglomerular cells are small neurons located in the glomerular region. Although these cell types can be distinguished by their size and presence or lack of dendritic spines, they cannot be definitively distinguished by extracellular electrophysiological criteria alone, since no periglomerular cells, and only a subset of external tufted cells, project out of the bulb (Getchell & Shepherd, 1975; Macrides et al., 1985; Onoda & Mori, 1980; Scott, 1981).

If there is normally a die-off of tufted cells, and these neurons are even more sensitive to olfactory deprivation than mitral cells (Meisami & Safari, 1981), the increased activation of specific tufted cells by odor experience in the identified areas might prevent their death. The increased number of tufted cells responding to the familiar odor would then activate granule cells in the internal plexiform layer and inhibit neighboring mitral cells. If this model is correct, then one might expect to see increased numbers of tufted cells in association with the modified glomerular complexes. One might also expect increased activity of granule cells in line with these glomeruli in the internal plexiform layer of odor-familiar pups. We investigated the first possibility by using a silver fiber stain and counterstaining for Nissl substance to be able to localize cell bodies near the modified glomerular complexes. In some sections, we found that there
were groups of what may be tufted cells in association with the modified glomeruli. We are currently quantifying these observations.

Close examination of the 2-DG autoradiographs suggested that there may have been an increased uptake in the internal plexiform or granule cell layer in odor-familiar pups. To determine whether there is increased activity in the granule cells in the ipsilateral internal plexiform layer, we used a technique that could give us a measure of relative activity with cellular resolution. We reasoned that if the granule cells or the tufted cells mobilize stored glycogen into glucose during increased firing, we should be able to detect the presence of the active form of the enzyme that promotes this reaction and thereby gain a measure of differential cellular activity. Such neurons exist, for example, in the spinal cord (Woolf, Chong, & Rashdi, 1985) and have been shown in somatosensory cortical whisker barrels in mice (Wallace, 1983). We aligned 2-DG autoradiographs with alternate sections stained for glycogen phosphorylase in odor-familiar and odor-unfamiliar pups. In only odor-familiar pups exposed to peppermint did we see increased activity in the internal plexiform layer aligned with the identified glomerular areas (Fig. 6). A restricted line of increased activity can be seen aligned with the modified glomerular complex only in peppermint-familiar pups tested with peppermint odor (Coopersmith & Leon, in press).

As it happens, astrocytes are known to contain and use glycogen (Phelps, 1972; Sotelo & Palay, 1968), and it was possible that the cells that we identified were not neurons. Indeed, Benson, Burd, Greer, Landis, and Shepherd (1985) have reported that glia have the greatest uptake of 2-DG of the cells identified in the olfactory bulb using a high-resolution technique. To distinguish between these possibilities, we used immuno-histochemical procedures for marking the presence of glial fibrillary acidic protein (GFAP), a protein present only in astrocytes (Bignami & Dahl, 1977; Ludwin, Kosek, & Eng, 1976; Schachner, Hedley-Whyte, Hsu, Schoonmaker, & Bignami, 1977). We obtained excellent staining for GFAP and observed that the presumed astrocytes were in the same area as the highest levels of cells with glycogen phosphorylase activity (Coopersmith, Anderson, Cotman, & Leon, unpublished observations). These data support the idea that glia in the bulb have an important, energy-dependent role in the coding of olfactory cues.

A model of how olfactory experience may affect neural coding for familiar odors is summarized in Fig. 7. Differential olfactory experience increases the size and configuration of glomeruli in the olfactory bulb, perhaps because there is an increase in the number of tufted cells avoiding an early death in that area. The additional tufted cells would increase granule cell activation in the internal plexiform layer, which would inhibit
Fig. 6. Glycogen phosphorylase activity in the olfactory bulbs of peppermint-familiar pups in response to peppermint odor. Arrows point to enzyme activity associated with granule cells deep to the modified glomerular clusters that display enhanced 2-DG uptake. 20 μm coronal sections. Scale bar = 100 μm. From Coopersmith & Leon (in press).
Fig. 7. A model for the development of the enhanced neural response and its consequences for the neural coding of familiar odors. (A) Odor-unfamiliar pups may have many of their external tufted cells die, and the remaining cells may not be able to inhibit neighboring mitral cells via granule cell activation. (B) Early olfactory learning may allow an increased number of external tufted cells to survive in the stimulated area. Their increased activation of granule cells in response to the attractive odor would then inhibit neighboring mitral cells.

neighboring mitral cells. It is also possible that an increase in periglomerular cell inhibition could mediate the decrease in mitral cell activity. It is interesting to note that high-resolution 2-DG autoradiography demonstrated increased activity in response to olfactory stimulation in each of the cell types that we suggest may be involved in mediating early olfactory learning (Lancet, Greer, Kauer, & Shepherd, 1982).

Recall that there is also a subset of external tufted cells that form point-to-point, reciprocal connections between opposite regions of the medial and lateral bulb (Schoenfeld, Marchand, & Macrides, 1985), a pattern that correlates with 2-DG uptake. It seems possible that increased tufted cell input to granule cells from the contralateral side of the same bulb could also mediate the observed decrease in mitral cell activity.

III. Prospectus

We expect the future to bring a focus on normally occurring individual differences in the brain that underlie differences in behavior. Since the fields of behavioral ecology and of personality development have focused around the concepts of individual differences, an appreciation of such differences in neurobehavioral analyses may bring these areas closer to a reductionistic analysis. It should also be noted that the differences may be largely inherent or may be based on specific postnatal experiences. This research raises the possibility that there can be different courses for normal brain development.
The second focus for future research may well be the neurobiology of the learning that occurs in mammalian neonates. The neural basis for such learning may be relatively simple and may allow much progress to be made on the question. The developing mammal learns a few very important things about its environment that are critical for survival. Young mammals may be specially adapted for this sort of learning and may have the rather large changes that we have found in the brain as their consequence. Such large and permanent changes make the system amenable for analysis in the same way as the use of animals with few neurons make some types of neurobiological analyses possible. This developmental approach has allowed progress to be made in similar questions in birds and fish (Cooper & Hasler, 1973; Nottebohm, 1980).

The third prospect for the future is an increase in the appreciation of the olfactory system for providing an opportunity to understand plastic changes during development and in adulthood. Both olfactory neurons and olfactory bulb granule cells continue to be formed into adulthood (Graziadei & Monti Graziadei, 1978; Hinds, Hinds, & McNelly, 1984; Hinds & McNelly, 1981; Kaplan & Hinds, 1977). These plastic changes may represent an unusual capability of the olfactory system for change. The olfactory system also appears to subserve the type of complex learning used by humans (Nigrosh, Slotnick, & Nevin, 1975; Slotnick & Kaneko, 1981; Slotnick & Katz, 1974; Staubli, Ivy, & Lynch, 1984). Haberly (1985) and Lynch (1986) argue persuasively that the basis for this capacity may reside in the particular kind of neural network that constitutes the piriform cortex. The olfactory system may therefore even hold the key to understanding the neurobiology of human cognition.

Acknowledgments

This work has been supported, in part, by grant NS21484 from the National Institute of Neurological and Communication Disorders and Stroke and BNS 8606786, as well as an equipment grant BNS 80-2310 from the National Science Foundation to M.L., who holds Research Scientist Development Award MH00371 from the National Institute for Mental Health. R.M.S. is supported by postdoctoral fellowship HD06818 from the National Institute for Child Health and Human Development. We thank Foteos Macrides for his insightful discussions.

References


