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Dissociation of Behavioral and Neural Correlates of Early Associative Learning

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Abstract

Wistar rat pups were trained in an olfactory associative conditioning task on postnatal Day 6, 12, or 20. The training consisted of 20 pairings of a novel odor (peppermint) with footshock (1.5 mA, 1 s) with an intertrial interval of 3 min. Additional pups were trained in either unpaired or naive control conditions. On the day following training, pups were either tested for their behavioral response to the conditioned odor in a two-odor choice test, or injected with ^{14}C-2-deoxyglucose and exposed to the odor for examination of olfactory bulb neural responses to the odor. The results demonstrate that, although pups at all ages learned to avoid the odor, only pups trained during the first postnatal week had a modified olfactory-bulb glomerular-layer response to the odor. These results suggest that although olfactory memory is correlated with modification of olfactory bulb glomerular layer function in newborns, these changes are not required for normal memory in older pups.

INTRODUCTION

Memory for odor in the infant rat is correlated with specific changes in metabolic (2-deoxyglucose uptake; Coopersmith & Leon, 1984;Sullivan & Leon, 1986) and neural (mitral/tufted cell single-unit activity; Sullivan & Wilson, 1991;Wilson, Sullivan, & Leon, 1987) activity in odor-specific regions of the olfactory bulb. These modified olfactory bulb responses are hypothesized to signal to higher processing areas that an odor has some acquired significance, with information regarding the hedonic valence of that significance dependent on other brain regions (Sullivan & Wilson, 1991;Wilson & Sullivan, 1994). Thus, the modified olfactory bulb response may be the initial step in directing the animalâs behavioral response to that odor.

The modified 2-deoxyglucose (2-DG) response is characterized by enhanced focal 2-DG uptake in odor-specific regions of the olfactory bulb glomerular layer in response to the learned odor (Sullivan & Leon, 1986). The relationship between learned olfactory behavior and the enhanced olfactory bulb 2-DG response has been examined under a number of age, training, pharmacological, and physiological conditions (for review see Wilson & Sullivan, 1994). To this point, there has been a perfect correlation between learned behavior and the enhanced 2-DG response in pups less than 17 days old. That is, conditions under which behavioral change occurs are always associated with changes in 2-DG uptake and conditions under which behavioral change does not occur are never associated with changes in 2-DG uptake. The strength of this correspondence adds to the belief that changes in the glomerular layer, as evidenced by enhanced 2-DG uptake, are involved in memory storage for the learned odor in pups.

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The present experiment was an attempt to systematically further explore the relationship between early learning and 2-DG uptake by using odor aversion learning at different postnatal ages. Learned odor aversions produce changes in 2-DG uptake in the olfactory bulb indistinguishable from those produced by odor preference learning (Sullivan & Wilson, 1991). Odor aversion conditioning is an especially useful model, however, in that odor aversions can be learned throughout development and into adulthood (Camp & Rudy, 1988;Kucharski & Spear, 1984). Odor preference conditioning, on the other hand, shows developmental sensitive periods, depending on the stimulus used as the reinforcer (Sullivan & Wilson, 1994;Woo & Leon, 1987).

In the present study, pups were trained with odor-footshock pairings at postnatal Day 6, 12, or 20, and tested for learned odor aversions and olfactory bulb glomerular layer 2-DG uptake to the aversive odor on the following day. The results demonstrate that although pups acquired odor aversions at all ages, only postnatal Day 6 pups demonstrated modified olfactory bulb responses to the learned odor.

METHODS

Subjects

The subjects were 275 (n = 85 for behavior, n = 190 for 2-DG) male and female rat pups from 85 different litters (8-11 pups/behavior group 9-34 pups/2-DG group) born of Wistar rats (offspring of Hilltop Lab Animals, Scottsdale, PA) in the vivarium at the University of Oklahoma. No more than 1 male and/or female pup from a given litter was used in an experimental condition. High numbers of pups/group were used in the 2-DG test because we believed that, based on the literature (Woo & Leon, 1987;Hamrick, Wilson, & Sullivan, 1993) we might be searching for null effects in the older age groups. Thus, we wanted to maximize the power of our statistical tests. (Number of animals/group for both behavior and 2-DG tests are shown in the figures.) Dams were housed in rectangular polypropylene cages (34 Ã 29 Ã 17 cm) lined with wood chips in a temperature- (23ÂC) and light-(0800-2000 hr) controlled room. Ad-lib food and water were available at all times. Births were checked at 0800 and 1700 hr. The day of birth was considered to be postnatal Day 0. Litters were culled to 10-12 pups on postnatal Day 1.

Conditioning

Associative olfactory conditioning consisted of a l-hr training session on postnatal Day 6, 12, or 20. Pups were randomly assigned to one of the following conditions: (a)Paired-a 10-s presentation of a novel peppermint (Schilling) odor (flow-dilution olfactometer; 1: 10-saturated odor: air, 1 L/min flow rate) with a 1.5-mA, constant current, foot shock presented during the last 1 s of the odor presentation. There were 20 stimulus presentations with an intertrial interval (ITI) of 3 min; (b) Unpaired-a 10-s odor presentation with a 3-min ITI. The l-s, 1.5-mA shock was pseudorandomly delivered during the IT1 but not within 10 s of the odor presentation; or (c) Naive-pups were placed in the training chamber but received neither stimulus. Following training, pups were returned to the nest and reunited with the dam.

Behavioral Testing

The behavior test consisted of a Plexiglas Y-maze (start box: 8.5 cm width, 10 cm length, 8 cm height; choice arms: 8.5 Ã 24 Ã 8 cm) that required pups to choose between two odors: the conditioned peppermint odor and a familiar pine odor (type of shavings used in nest). Both odors were delivered via a flow-dilution olfactometer (same flow rate and concentration as training) into the ends of the choice arms. Pups were placed in the start box and allowed 60 s to choose an arm. The pups were removed from the maze for 10 s between trials and received five trials. The maze was wiped clean with water between trials. Older pups were required to

walk a further distance down the choice arm than younger pups in order for a choice to have been considered: Pups were required to walk 4, 8, and 12 cm down the alley respectively for postnatal Days 6, 12, and 20 pups.

2-DG Autoradiography

For the 2-DG test, pups were injected with ^{14}C 2-DG (20 uCi/lOO g) 5 min prior to odor-only delivery and placed in a chamber similar to that used during training. Following the 45-min odor exposure (flow-dilution olfactometer delivery; cycle of 3 min on, 1 min off; same concentration and flow rate as training), pups were decapitated and their brains quickly removed and frozen in 2-methylbutane at -45ÂC. The frozen brain was equilibrated to-1}} 7ÂC in a cryostat for 45 min and the olfactory bulb was cut coronally in 20 Î sections. Each section was immediately picked up on a cover slip and placed on a slide warmer at 60ÂC for at least 5-10 min. Coverslips containing the brain sections were then glued to a sheet of cardboard and exposed to Kodak SB-5 x-ray film for 8 days at room temperature in an exposure cassette. A set of ^{14}C labeled methylmethacrylate standards (Amersham), previously calibrated to ^{14}C uptake in 20 Î brain sections, was exposed with each sheet of film. Autoradiographs were developed using standard techniques.

Odor presentation during 2-DG uptake produces an odor-specific spatial pattern of focal 2-DG uptake in the olfactory bulb glomerular layer, regardless of the animalâs previous experience with that odor (Astic & Saucier, 1982;Jourdan, Duveau, Astic, & Holley, 1980;Sharp, Kauer, & Shepherd, 1977;Stewart, Kauer, & Shepherd, 1979). However, the density of uptake within the focal pattern is modified by experience (Coopersmith & Leon, 1984;Sullivan & Leon, 1986). The autoradiographs were analyzed using a computer-based digital image processor (MCID, Imaging Research Inc.) that allowed pseudocolor imaging and quantitative optical densitometry. To quantify 2-DG uptake, the computer constructed a calibration curve that related the gray value of ^{14}C standards that were exposed with the brain sections to that of its previously determined ^{14}C tissue equivalent. The computer then translated the density measures into ^{14}C levels, and hence 2-DG uptake by the tissue. The autoradiographs were visually scanned for the presence of odor-specific glomerular layer foci, which are generally 2-3 times above background glomerular-layer uptake and thus, easily observed. Five readings were taken from the center of the focus and 5-10 readings taken from the periventricular core of the olfactory bulb. Focal glomerular-layer activity was expressed relative to uptake in the periventricular core, which has been shown to not be modified by experience (Coopersmith & Leon, 1984;Sullivan & Leon, 1986).

RESULTS

As shown in Figure 1, pups that received paired odor-shock presentations during training developed a relative aversion for the conditioned odor, ANOVA, 3 Ã 3 (Training x Age) interaction, $F(4,76) = 0.24$, n.s.; main effect of age, $F(2,76) = 0.41$, n.s.; main effect of training, $F(2,76) = 68.41$, $p < .001$). Post-hoc Fisherâs PLSD revealed that Paired conditioned pups chose the conditioned odor side significantly less often than either the Unpaired or Naive at all ages, $p < 0.05$.

However, as illustrated in Figure 2, only at postnatal Day 6 did Paired pups exhibit enhanced focal glomerular-layer 2-DG uptake to the aversive odor compared to their age-matched conditioning control groups, ANOVA, Training Ã Age interaction, $F(4, 181) = 1.0$, n.s.; main effect of age, $F(2,181) = 4.58$, $p < 0.01$; main effect of training, $F(2,181) = 4.73$, $p < 0.01$. Post-hoc Fisherâs PLSD revealed that only the postnatal Day 6 paired group differed from each of the postnatal Day 6 control groups, $p < 0.05$. The postnatal Day 12 and postnatal Day 20 Paired groups were not statistically different from their same aged controls.

DISCUSSION

The present results demonstrate a dissociation between learned olfactory behaviors and enhanced olfactory-bulb glomerular-layer 2-DG uptake responses. Despite similar expressed learned behaviors at all postnatal ages tested, only postnatal Day 6 pups showed modified glomerular-layer activity to the learned odor. Thus, these results confirm a sensitive period in at least one form of olfactory bulb plasticity (focal glomerular-layer 2-DG uptake) and raise questions about the role of 2-DG changes in olfactory memory.

The Role of Focal Glomerular Changes in Olfactory Memory

Are focal glomerular-layer changes critical for early olfactory memory? For memories acquired after the 2nd or 3rd postnatal weeks in the rat, the answer appears to be no. As the present and previous studies have demonstrated (Coopersmith, Lee, & Leon, 19876; Hamrick et al., 1993), glomerular-layer 2-DG uptake patterns are not modified by experience after the first postnatal week despite obvious learned changes in behavior. The glomerular layer, thus, appears to be sensitive to and is molded by experience in the first postnatal week, but then becomes fixed as it matures into the 2nd postnatal week. In fact, this time frame corresponds well with the reported ontogeny of olfactory bulb glomeruli and juxtaglomerular neurons, both of which show a rapid increase in number over the first 10 postnatal days in the rat (Altman & Das, 1966;Frazier & Brunjes, 1988;Meisami, 1979). If and/or how these glomerular-layer changes are involved in storing or expressing olfactory memories acquired during the 1st postnatal week, however, remains unclear.

In addition to glomerular-layer change, early olfactory learning also changes response patterns of olfactory-bulb output neurons, mitral/tufted cells (Sullivan & Wilson, 1991;Wilson, Sullivan, & Leon, 1987). It is currently not known if these neurons also demonstrate a sensitive period. However, response patterns of mitral cells in the olfactory bulb of adult female sheep are modified by olfactory experience associated with partuition and recognition of newborns (Kendrick, Levy, & Keverne, 1992). Furthermore, work with a variety of paradigms in adult rats and rabbits suggests that pharmacological manipulation of the olfactory bulb during olfactory experience can impair or facilitate olfactory memories (Mouly, Kindermann, Gervais, & Holley, 1993;Gray, Freeman, & Skinner, 1986). Thus, it appears that although the glomerular layer may demonstrate an early sensitive period for plasticity, other local circuits in the olfactory bulb may maintain their plasticity throughout life.

Sensitive Periods in Ontogeny of Olfactory Learning

Are sensitive periods in olfactory memory at the behavioral level mediated by the sensitive period in olfactory-bulb glomerular-layer plasticity? Newborn rats are uniquely primed to acquire olfactory preferences. During the 1st postnatal week, association of a myriad of stimuli with an odor will produce a learned approach response to that odor. For example, pairing an odor with tactile stimulation will produce a relative preference for that odor in pups during the 1st postnatal week, but produce no response in older pups (Sullivan & Wilson, 1994). A predisposition to learn an approach response to odors is critical for newborn rats, whose primary source of stimulation is from the mother.

Although the time course of this predisposition closely matches the sensitive period of glomerular layer plasticity, we believe this link may be coincidental. Rather, we believe the inclination toward odor learning may be based on the responsiveness of brainstem reward mechanisms in the newborn (for review see Sullivan & Wilson, 1994). For example, the noradrenergic nucleus locus coeruleus, which is activated by reward stimuli in the neonate (Nakamura, Kimura, & Sakaguchi, 1987;Wilson & Sullivan, 1991), and is critical for neonatal olfactory learning (Sullivan, Wilson, Lemon, & Gerhardt, 1994;Sullivan, Wilson, & Leon,

1989), shows a marked decrease in the range of stimuli to which it will respond during the 2nd postnatal week in the rat. This sharpening of locus coeruleus neural-response patterns with maturation may function to limit associative learning in the maturing neonate.

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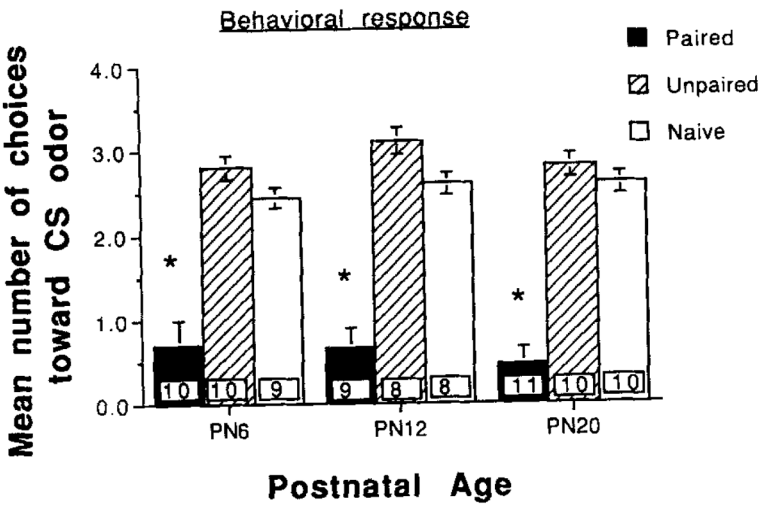

Fig 1.

Behavioral responses to odors learned at Days 6,12, and 20. Conditioned pups demonstrated a significant avoidance of the learned odor at all ages. Number of pups/group is shown inset in each bar. Asterisks represent significant differences from age-matched control groups,$p < 0.05$.

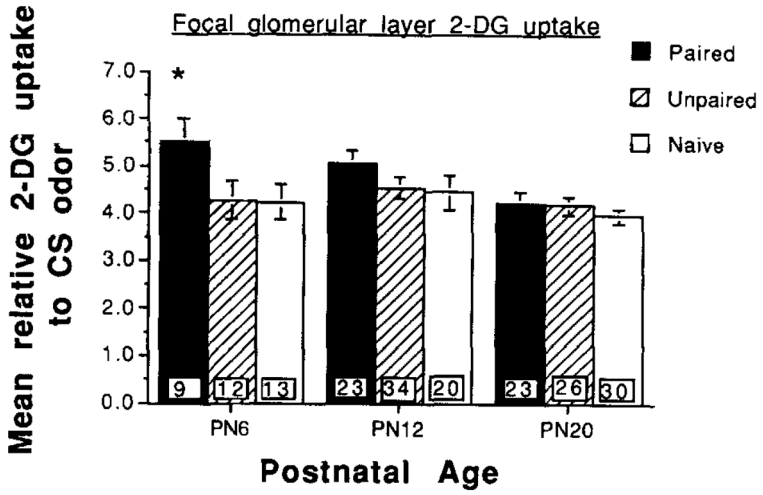

Fig 2.

Olfactory bulb neural response (focal 2-DG uptake) to odors learned at Days 6, 12, and 20. Conditioned pups demonstrated a significant enhancement of focal 2-DG uptake to the learned odor when tested at Day 6, but not at other ages. Number of pups/group is shown inset in each bar. Asterisk represents a significant difference from age-matched control groups, $p < 0.05$.