

BRESO 51072

## Modified behavioral and olfactory bulb responses to maternal odors in preweanling rats

Regina M. Sullivan\*, Donald A. Wilson\*, Rebecca Wong, Adrian Correa and Michael Leon

Department of Psychobiology, University of California, Irvine, CA 92717 (U.S.A)

(Accepted 26 December 1989)

*Key words:* Olfactory bulb; Maternal odor; Rat pup; [<sup>14</sup>C]2-Deoxyglucose; Odor preference; Learning

Rat pups acquire an attraction for maternal odors, which can vary with maternal diet. In the two experiments reported here, maternal diet was modified and both pup behavioral responses and pup olfactory bulb neural responses ([<sup>14</sup>C]2-DG uptake) to maternal odors were examined. In experiment 1, pups were reared from birth to postnatal day 19 with either a dam fed normal rat chow or a dam fed a sucrose based diet which suppressed her normal maternal odor. In experiment 2, pups were raised from birth to postnatal day 19 with either a dam fed the sucrose-based diet adulterated with peppermint, or the non-scented sucrose-based diet. Pups selectively expressed both a behavioral attraction and an enhanced olfactory bulb neural response to odors that they experienced in the nest.

### INTRODUCTION

Infant rats exhibit a behavioral preference for odors experienced in the nest<sup>1,14,15</sup>. Under normal circumstances, therefore, a preference is acquired for the odor of the pup's mother and littermates. The predominant maternal odor is produced in the caecum and defecated as caecotrophe<sup>14</sup>. The particular caecotrophe odor is determined by the maternal diet, thus allowing pups to distinguish their mother from other lactating females eating different diets<sup>13</sup>. In addition to the natural maternal odor, prolonged exposure to any odor within the nest, even artificial odors, influences pup responsiveness to that odor<sup>2,4,6-8</sup>.

Behavioral preferences can also be acquired outside of the nest, as long as appropriate stimulation is provided<sup>16,18,22</sup>. Under temporal constraints consistent with classical conditioning, pairings of an odor and reinforcers that mimic maternal care lead to both acquisition of a behavioral preference for that odor, and specific conditioned responses to that odor<sup>10-12,16-18</sup>. These results suggest that the stimulation pups receive from the mother in the nest has rewarding properties for pups<sup>17</sup>, and thus, the preference for maternal odor is learned.

This preweanling olfactory learning is associated with a modified olfactory bulb response to the conditioned odor. The modified olfactory bulb response is characterized by enhanced focal [<sup>14</sup>C]2-deoxyglucose (2-DG)

uptake<sup>5,19,20</sup> and by an altered single-unit response pattern of output neurons, mitral/tufted cells, near these 2-DG foci<sup>23,24</sup>. The acquisition of these olfactory bulb responses also conform to the constraints of classical conditioning<sup>21</sup>.

### EXPERIMENT 1

The modified olfactory bulb responses have not, however, been demonstrated in response to odors which are naturally found in the nest. The purpose of the present paper was, therefore, to determine whether olfactory bulb responses are modified to maternal odors that are experienced within the nest. Maternal odor was varied by allowing the dams to eat different diets. In the first experiment, the behavioral and olfactory bulb responses of rat pups were assessed to naturally occurring maternal odor. Pups were raised either with a mother with a normal maternal odor or with a mother who had her dominant maternal odor suppressed through a special diet<sup>14</sup>.

### Methods

*Subjects.* The subjects were 40 (16 for 2-DG, 24 for behavior) male and female 19–20-day-old rat pups from 14 different litters, born of Wistar (Hilltop Lab Animals, Scottsdale, PA) rats mated in the University of California at Irvine animal colony. Litters were culled to 8 pups on

\* Present address: Developmental Psychobiology Laboratory, Department of Psychology, University of Oklahoma, Norman, OK 73019, U.S.A.

Correspondence: R. Sullivan, Developmental Psychobiology Laboratory, Department of Psychology, University of Oklahoma, Norman, OK 73019, U.S.A.

postnatal day 1 (PN1). No more than 1 male and/or 1 female were used from a litter for a treatment/test condition.

*Postnatal odor treatment.* The pups were reared in one of two odor rearing conditions: (1) normal maternal odor – the dam was fed ad lib Purina rat chow and water and housed in the main colony room throughout pre- and postpartum periods; or (2) suppressed maternal odor – starting 2–3 days prior to parturition and extending for the duration of the experiment, dams were provided ad lib a sucrose-based, fat-sufficient diet (Teklad Diet TD69446, Madison, WI) which suppresses the production of the caecotrophe and dominant maternal odor<sup>13</sup>. The only constituent carbohydrate in the diet is sucrose which is absorbed above the level of the caecum. The resident microbial population of the caecum is thereby deprived of nutrient and cannot act on the contents of the gut to produce the dominant maternal odor<sup>13,14</sup>.

On PN19, pups were removed from the dam and given one of two tests: (1) behavioral Y-maze test (soiled shavings from a normal maternal odor cage vs soiled shavings from a suppressed maternal odor cage) to assess the development of a preference for the maternal odor; or (2) an injection of [<sup>14</sup>C]2-DG (20  $\mu$ Ci/100 g; Sigma) followed by exposure to the shavings from a normal maternal odor cage. Both the normal maternal odor and suppressed maternal odor shavings were taken from a cage that had not been cleaned for 1–2 weeks, housing a 19–21-day postpartum female.

*Behavior test.* The testing apparatus consisted of an opaque Plexiglas Y-maze with a start box and two goal boxes, each at the end of a 23 cm alley. Immediately prior to testing, 150 ml of shavings from a normal maternal odor cage was placed in one goal box, and 150 ml of shavings from a suppressed maternal odor cage was placed in the other goal box. The shavings were placed under a wire mesh screen floor which prevented pups from coming into direct contact with them. Just prior to the 5-trial test, pups were given experience with the Y-maze which contained the shavings. A pup was placed into the start box and permitted to run down one of the alleys (the other alley was blocked off). The pup was returned to the holding cage for 10 s, placed in the start box again and permitted to run down the other alley. The order in which pups experienced the two goal boxes and the placement of the odors in the goal boxes were counterbalanced for each of the rearing conditions.

A 5-trial test was begun by placing a pup in the start box, facing away from the two odor sources. The door was then opened permitting access to the odor sources and the odor chosen by the pup was noted. All pups made a choice within 30 s. Between trials, the pup was placed in a holding cage for 10 s and the floor of the

Y-maze was wiped clean with water and dried.

*2-DG autoradiography.* For the odor/2-DG test, pups were injected with [<sup>14</sup>C]2-DG and placed in a glass canister (29  $\times$  11 cm) which contained 200 ml of shavings from a normal maternal odor cage. Pups were placed on a wire mesh screen 1–2 cm above the soiled shavings. To record respiration, the test canister was fitted with a pressure transducer connected to a respiration monitor (Columbus Instruments).

Following odor exposure, pups were decapitated and their brains were quickly removed and frozen in Freon at  $-40^{\circ}\text{C}$ . The frozen brain was equilibrated to  $-17^{\circ}\text{C}$  in a cryostat for 45 min. The olfactory bulb was cut coronally in 20  $\mu\text{m}$  sections. Each section was immediately picked up on a coverslip and placed on a slide warmer at  $60^{\circ}\text{C}$  for 5–10 min. Coverslips were glued to a sheet of cardboard and exposed to Kodak SB-5 X-ray film for 10 days at  $25^{\circ}\text{C}$  in an exposure cassette. A set of [<sup>14</sup>C]-methylmethacrylate standards (Amersham), previously calibrated to <sup>14</sup>C-uptake in 20  $\mu\text{m}$  brain sections, was exposed with each sheet of film. Autoradiographs were developed using standard techniques.

The autoradiographs were analyzed using a computer-based digital image processor (Imaging Research Inc.) that allowed pseudocolor imaging and quantitative optical densitometry. To quantify 2-DG uptake, the [<sup>14</sup>C]-standards that were exposed with the brain sections were calibrated to that of their previously-determined [<sup>14</sup>C]-tissue equivalent. The computer then linearized this function so that the grey values of the autoradiograph could be interpolated into <sup>14</sup>C-levels, and hence 2-DG uptake. Comparisons were made of relative 2-DG uptake in easily-identifiable, odor-specific focal regions of the glomerular layer, as previously described<sup>5,19</sup>.

### Results

Pups acquired a relative preference for the odor which was present during development (Fig. 1). Specifically, pups raised with a dam with normal odor (fed rat chow) exhibited a preference for the odor of normal maternal shavings compared to pups raised with a suppressed-odor dam (fed sucrose-based diet;  $t = 8.68$ ,  $P < 0.001$ ). On the other hand, pups raised with a dam with the normal maternal odor suppressed exhibited a preference for the shavings from a dam which had the normal maternal odor suppressed.

The regions of focal glomerular layer 2-DG uptake to the odor of lactating females fed rat chow appeared much more dispersed and weaker than those described for single artificial odors in rat pups<sup>3,5,19,20</sup>. This variability may be due to the relatively low concentration of odor in the soiled beddings compared to artificial odorants. Focal glomerular layer uptake sites were most reliably found in

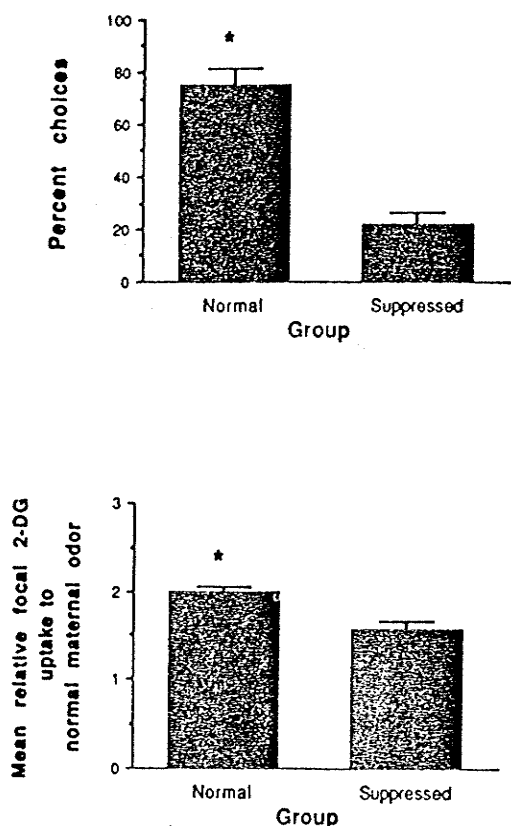


Fig. 1. Behavioral (top) and focal olfactory bulb 2-DG uptake (bottom) responses to normal maternal odor in PN19 pups raised with mothers fed either a normal diet (Normal) or a sucrose-based diet that suppressed the dominant maternal odor (Suppressed). Asterisks correspond to statistical significance between groups.

an anterior cluster 0.3–1.0 mm from the rostral pole of the bulb and in a posterior group 3.5–5.0 mm from the rostral pole of the bulb. The locations of focal uptake sites did not appear to differ between groups. However, pups that were raised with a dam with a normal maternal odor exhibited an enhanced olfactory bulb 2-DG uptake within these specific glomerular layer foci upon presentation of the normal maternal odor ( $t = 3.67$ ;  $P < 0.01$ ). Pups raised with a dam with the maternal odor suppressed did not exhibit an enhanced olfactory bulb 2-DG uptake in response to exposure to normal maternal odor.

Since respiration may alter the amount of odor pups receive and hence 2-DG uptake, respiration was recorded during the 2-DG uptake test. However, no differences in respiration were detected between the two odor treatment groups. Additionally, previous work, in which pups were artificially respired during 2-DG uptake, demonstrated that differential respiration did not underlie the enhanced 2-DG response to learned odors in pups<sup>21</sup>.

## EXPERIMENT 2

The results of the first experiment demonstrate that rat

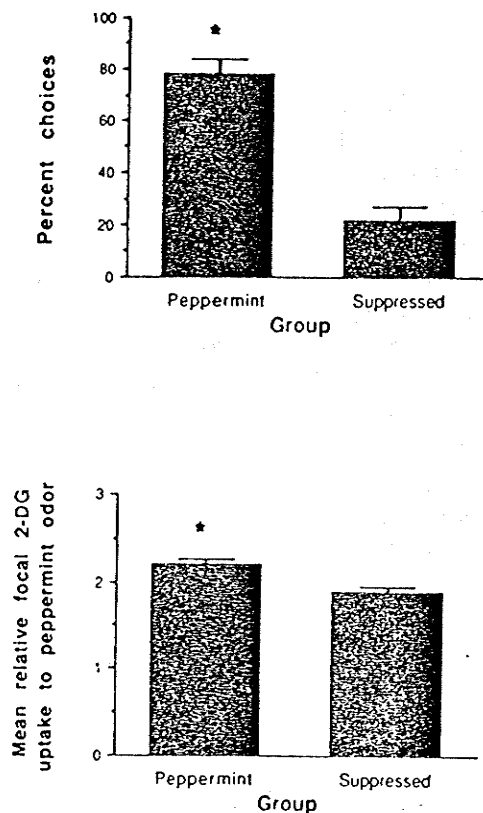


Fig. 2. Behavioral (top) and focal olfactory bulb 2-DG uptake (bottom) responses to peppermint odor in PN19 pups raised with mothers fed either a sucrose-based diet adulterated with peppermint (Peppermint) or a sucrose-based diet that suppressed the dominant maternal odor (Suppressed). Asterisks correspond to statistical significance between groups.

pups acquire both modified behavioral and olfactory bulb responses to the natural odor of their mother. It has previously been demonstrated that similar responses can be acquired to artificial odors through associative conditioning<sup>10-12,16-18</sup>. The second experiment examined pup neurobehavioral responses to artificial odors associated with normal maternal care. The mothers were fed an artificially scented diet during the preweaning period, and pup neurobehavioral responses to this artificial odor were examined.

## Methods

**Subjects.** The subjects were 34 male and female rat pups from 12 different litters born and raised as in experiment 1. No more than 1 male and/or 1 female was used in a particular treatment/test condition.

**Postnatal odor treatment.** Pups were reared in one of two conditions, both of which had the dominant maternal odor suppressed from the day of parturition: (1) suppressed maternal odor – pups were reared with a dam fed a sucrose-based diet as described in experiment 1; or (2) peppermint maternal odor – pups were reared with a dam fed a peppermint adulterated sucrose-based diet (200 ml

sucrose diet/0.2 ml Schilling peppermint extract; renewed daily). The peppermint adulterated food artificially scents the nest through the mother's excretions<sup>6,13</sup> and through some spillage.

**Testing.** On PN19, pups were given one of the following tests: (1) a Y-maze test providing a choice between soiled shavings of a peppermint-fed dam or soiled shavings of a suppressed odor dam; or (2) an injection of 2-DG followed by exposure to peppermint odor. The peppermint odor was presented through a flow dilution olfactometer at a concentration of 1:10 of saturated vapor at a flow rate of 2 l/min. Additional details of both the behavior test and the 2-DG autoradiography are described in experiment 1.

### Results

As in experiment 1, pups demonstrated a behavioral preference for the odor that they were reared with (Fig. 2). Pups reared with peppermint-fed dams demonstrated a significant preference for peppermint compared to pups reared without peppermint ( $t = 5.56$ ,  $P < 0.01$ ). Similarly, while all pups demonstrated focal glomerular layer 2-DG uptake to peppermint odor along the lateral aspect of the bulb and 1.5–2.2 mm from the rostral pole of the bulb, focal uptake in pups that had been reared by peppermint-fed dams was enhanced compared to controls ( $t = 2.87$ ,  $P < 0.05$ ). There were no significant differences in respiration in response to peppermint between the two groups.

### REFERENCES

- Alberts, J.R., Olfactory contributions to behavioral development in rodents. In R.L. Doty (Ed.), *Mammalian Olfaction, Reproductive Processes and Behavior*, Academic Press, New York, 1976.
- Alberts, J.R. and May, B., Nonnutritive, thermotactile induction of filial huddling in rat pups, *Dev. Psychobiol.*, 17 (1984) 161–181.
- Astic, L. and Saucier, D., Ontogenesis of the functional activity of rat olfactory bulb: autoradiographic study with the 2-deoxyglucose method, *Dev. Brain Res.*, 2 (1982) 243–256.
- Caza, P.A. and Spear, N.E., Short-term exposure to an odor increases its subsequent preference in preweanling rats: a descriptive profile of the phenomenon, *Dev. Psychobiol.*, 17 (1984) 407–422.
- Coopersmith, R.M. and Leon, M., Enhanced neural response to familiar olfactory cues, *Science*, 225 (1984) 849–851.
- Duveau, A. and Godinot, F., Influence of the odorization of the rearing environment on the development of odor-guided behavior in rat pups, *Physiol. Behav.*, 42 (1988) 265–270.
- Galef, B.G., Jr., Acquisition and waning of exposure-induced attraction to a nonnatural odor in rat pups, *Dev. Psychobiol.*, 15 (1982) 479–490.
- Galef Jr., B.G. and Kaner, H.C., Establishment and maintenance of preference for natural and artificial olfactory stimuli in juvenile rats, *J. Comp. Physiol. Psychol.*, 94 (1980) 588–595.
- Hofer, M.A., Shaping forces within early social relationships. In N.A. Krasnegor, E.M. Blass, M.A. Hofer and W.P. Smotherman (Eds.), *Perinatal Development*, Academic Press, New York, 1987, pp. 251–274.
- Johanson, I.B. and Hall, W.G., Appetitive conditioning in neonatal rats: conditioned orientation to a novel odor, *Dev. Psychobiol.*, 15 (1982) 379–397.
- Johanson, I.B., Polefrone, J.M. and Hall, W.G., Appetitive conditioning in neonatal rats: conditioned ingestive responding to stimuli paired with oral infusions of milk, *Dev. Psychobiol.*, 17 (1984) 357–381.
- Johanson, I.B. and Teicher, M., Classical conditioning of an odor preference in 3-day-old rats, *Behav. Neural Biol.*, 29 (1980) 132–136.
- Leon, M., Dietary control of maternal pheromone, *Physiol. Behav.*, 14 (1975) 311–319.
- Leon, M., Development of olfactory attraction by young Norway rats. In D. Muller-Schwarze and R.M. Silverstein (Eds.), *Chemical Signals*, Plenum Press, New York, 1980.
- Rosenblatt, J.S., Olfaction mediates developmental transition in the altricial newborn of selected species of mammals, *Dev. Psychobiol.*, 16 (1983) 347–375.
- Sullivan, R.M., Brake, S.C., Hofer, M.A. and Williams, C.L., Huddling and independent feeding of neonatal rats is enhanced by a conditioning change in behavioral state, *Dev. Psychobiol.*, 19 (1986) 625–635.
- Sullivan, R.M. and Hall, W.G., Reinforcers in infancy: classical conditioning using stroking or intra-oral infusions of milk as a UCS, *Dev. Psychobiol.*, 21 (1988) 215–223.
- Sullivan, R.M., Hofer, M.A. and Brake, S.C., Olfactory-guided orientation in neonatal rats is enhanced by a conditioned change in behavioral state, *Dev. Psychobiol.*, 19 (1986) 615–623.
- Sullivan, R.M. and Leon, M., Early olfactory learning induces

### DISCUSSION

The results presented here suggest that variations in maternal diet influence both pup odor preferences and pup olfactory bulb neural response patterns to those odors. Odors within the preweanling nest have previously been demonstrated to be primarily determined by maternal diet<sup>13,14</sup>. The present results suggest that postnatal experience with these odors produces both a behavioral preference and an enhanced olfactory bulb response.

It is hypothesized that the mechanisms of these modified neurobehavioral responses to maternal/nest odors are similar to those controlling classically conditioned olfactory responses outside the nest. Conditioning of odor preferences or olfactory bulb responses outside the nest is dependent on association of the conditioned odor with a reward or unconditioned stimulus<sup>16,18,19,24</sup>. A variety of stimuli have been shown to function as reward to pups, ranging from tactile stimulation to intra-oral milk infusions<sup>10–12,16–18</sup>. Within the nest, these stimuli are provided by the dam<sup>9</sup>. Thus, the pup's association of maternal odors with stimulation provided during maternal care can result in a learned preference for those odors, and a concomitant change in pup brain function.

**Acknowledgements.** This work was supported by the PEW Charitable Trusts and Grant DC00480 to R.M.S., Grant BNS8608786 to D.A.W. and M.L. and Grants HD24236 to M.L. who holds Research Scientist Development Award MH00371.

- an enhanced olfactory bulb response in young rats, *Dev. Brain Res.*, 27 (1986) 278-282.
- 20 Sullivan, R.M. and Leon, M., One-trial olfactory learning enhances olfactory bulb responses to an appetitive conditioned odor in 7-day-old rats, *Dev. Brain Res.*, 35 (1987) 301-311.
- 21 Sullivan, R.M., Wilson, D.A., Kim, M.H. and Leon, M., Behavioral and neural correlates of postnatal olfactory conditioning: I. Effects of respiration on conditioned neural responses, *Physiol. Behav.*, 44 (1988) 85-90.
- 22 Sullivan, R.M., Wilson, D.A. and Leon, M., Associative processes in early olfactory preference acquisition: neural and behavioral consequences, *Psychobiology*, 17 (1989) 29-33.
- 23 Wilson, D.A. and Leon, M., Spatial patterns of olfactory bulb single-unit responses to learned olfactory cues in young rats, *J. Neurophysiol.*, 59 (1988) 177-1782.
- 24 Wilson, D.A., Sullivan, R.M. and Leon, M., Single-unit analysis of postnatal olfactory learning: modified olfactory bulb output responses patterns to learned attractive odors, *J. Neurosci.*, 7 (1987) 3154-3162