

BRESO 60478

## Short Communications

# The role of olfactory bulb norepinephrine in early olfactory learning

R.M. Sullivan<sup>a</sup>, D.R. Zyzak<sup>a</sup>, P. Skierkowski<sup>b</sup> and D.A. Wilson<sup>a</sup>

<sup>a</sup> *Developmental Psychobiology Laboratory, Department of Psychology, University of Oklahoma, Norman, OK 73019 (USA)*  
and <sup>b</sup> *Radiation Safety Office, Department of Environmental Safety Services, University of Oklahoma, Norman, OK 73019 (USA)*

(Accepted 4 August 1992)

**Key words:** Noradrenaline; Propranolol; Associative learning; Development of learning; Olfaction

Wistar rat pups were implanted with bilateral olfactory bulb cannulas on postnatal day 5 (PN5). On PN6, pups were trained in an olfactory classical conditioning task with peppermint odor as the CS and tactile stimulation/stroking as the UCS. Pups were randomly assigned to either PAIRED, BACKWARD or ODOR-only conditions. Half the pups in each group received intrabulbar infusions of 100  $\mu$ M propranolol and half received intrabulbar infusions of saline during the training session. Propranolol infusions blocked acquisition of the learned odor preference expressed by PAIRED saline-infused pups. Diffusion of the infusate was checked in additional pups by infusing [<sup>3</sup>H]NE and performing LSC analysis. Infusate concentration did not significantly differ between the anterior and posterior halves of the bulb, but were sharply lower in the olfactory peduncle and more posterior areas. The results suggest that olfactory bulb NE is critical for early olfactory learning.

In the mature central nervous system, norepinephrine (NE) has been shown to modulate both neural plasticity and behavioral memorial processes<sup>2,4–6,10,13</sup>. During development, NE plays a critical, enabling role in sensory system plasticity<sup>8,13</sup>. Recent work has also demonstrated that NE is necessary and sufficient during acquisition for olfactory associative learning in newborn rat pups<sup>14,18</sup>. NE is not required, however, for expression of learned odor behaviors once they have been acquired<sup>15</sup>.

Although these previous studies of early olfactory learning manipulated NE through systemic injections of  $\beta$ -receptor agonists and antagonists, several factors suggest that NE is modulating early learning at least partially in the olfactory bulb itself. First, there is a large NE projection from the locus coeruleus to the olfactory bulb<sup>12</sup>. Second, this NE input modulates neural excitability in the olfactory bulb<sup>7</sup> as early as the first postnatal week<sup>20</sup>. Third, NE mediates a component of the olfactory bulb response to non-olfactory, rewarding stimulation<sup>19</sup>. Fourth, olfactory bulb NE has been shown to be critical for neurobehavioral changes induced by olfactory learning in mature rabbits<sup>6</sup>.

The present experiment examined the role of olfactory bulb NE in early olfactory associative learning. Rat pups were implanted with bilateral olfactory bulb cannulas on postnatal day 5 (PN5), and trained in an olfactory classical conditioning paradigm on PN6 during intrabulbar infusions of either saline or the NE  $\beta$ -receptor antagonist propranolol. The results suggest that blockade of NE  $\beta$ -receptors limited to the olfactory bulb prevents acquisition of learned olfactory responses in neonates.

Wistar rat pups, born in our colony were used as subjects. No more than one male and one female were used per condition from each litter. On PN5, pups were cold anesthetized and placed in a stereotaxic apparatus. Stainless steel cannulas (30 gauge tubing) were implanted bilaterally in the granule cell layer or periventricular core of the olfactory bulbs through holes drilled in the overlying skull. The cannula assembly was attached to the skull with dental acrylic and anchor wires as described in detail elsewhere<sup>19</sup>. To insure patency of the cannulas, guide wires were placed in the lumen of the cannulas until training. To prevent the dam from removing the cannula assembly, the cannula

and head cap were painted with Nailbiter (Sally Hansen). Following recovery from surgery, pups were returned to the litter and dam until training.

On PN6, pups were placed in individual glass training chambers and their cannulas connected via PE10 tubing to a Harvard syringe pump driving two Hamilton microliter syringes. Pups were randomly assigned to one of two drug conditions. The cannulas were rapidly filled with either 100  $\mu\text{M}$  DL-propranolol, a NE  $\beta$ -receptor antagonist or saline (cannula volume was 1  $\mu\text{l}$ ). Propranolol or saline was then delivered at a rate of 0.1  $\mu\text{l}/\text{min}$  for a 10 min habituation period. This habituation period allowed the pups to habituate to the training chamber and allowed the concentration of the infusate to increase in the olfactory bulbs. Infusion was then continued during the 10 min training session for a total infused volume of 2  $\mu\text{l}/\text{bulb}$ .

Pups were assigned to one of three training conditions. Pups in all groups received 10 min of peppermint odor (1:10 concentration of saturated vapor, 2 l/min flow rate) which was either explicitly paired (PAIRED,  $n = 8$  saline, 7 propranolol) with tactile stimulation (vigorously stroking all parts of the body with a sable hair brush), explicitly unpaired (BACKWARD,  $n = 6$  saline, 5 propranolol) with tactile stimulation (pups were stroked for 10 min followed by 10 min of odor), or presented with the odor alone (ODOR-ONLY,  $n = 6$  saline, 5 propranolol). Following training, pups were disconnected from the syringe pump and returned to their litter until testing.

On PN7, acquisition of a conditioned odor preference was assessed with a Y-maze (start box: 7 cm long  $\times$  9 cm wide; alleys: 22 cm long  $\times$  9 cm wide at 45° angle to the startbox). No drugs were infused during testing. To increase pup mobility, pups were separated from their mother for 1 h prior to testing. Peppermint odor and the odor of pine wood chips used for bedding in the litter were presented down opposite arms of the Y-maze. The pup was placed in the start box allowed to choose one arm. Each pup was given 5 testing trials. Behavior testing was performed by an observer blind with respect to conditioning group. The number of choices toward the conditioned odor was determined and compared across training and drug groups with a 2  $\times$  3 ANOVA and post-hoc comparisons.

Pups infused with saline during training expressed a relative preference for the conditioned odor compared to control groups (Fig. 1). Infusion of 100  $\mu\text{M}$  propranolol into the olfactory bulbs during training blocked this conditioned odor preference (training  $\times$  drug interaction,  $F_{2,31} = 7.71$ ,  $P < 0.002$ ). Post-hoc comparisons showed that PAIRED-saline pups chose the conditioned odor significantly more than any other group

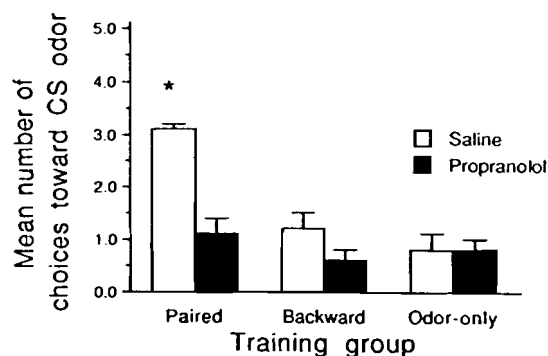


Fig. 1. Mean number of choices toward the peppermint CS odor in a olfactory Y-maze. Intrabulbar infusions of propranolol during training blocked acquisition of the learned odor preference expressed by saline controls. Asterisk represents significantly different from all other groups ( $P < 0.05$ ).

(Tukey,  $P < 0.05$ ). Histological examination in several pups revealed cannula tip placements in the granule cell layer or periventricular core. No differences in placements were noted between drug groups.

In order to determine the extent of drug diffusion within and outside of the olfactory bulbs, 8 additional pups were implanted with bilateral cannula on PN5. On PN6, these pups were infused with a saline solution of [ $^3\text{H}$ ]NE (56.9 mCi/ $\mu\text{M}$ , 0.37  $\mu\text{Ci}/\mu\text{l}$ ; NEN Research Products) at a rate of 0.1  $\mu\text{l}/\text{min}$  for 20 min, as described above. After infusion, the brains were quickly removed and dissected for analysis of [ $^3\text{H}$ ]NE distribution. The olfactory bulbs were divided into anterior and posterior halves. Two additional sections of 0.5–1.0 mm thickness were taken immediately posterior to the bulbs (labelled peduncle and cortex in Fig. 2).

Individual tissue sections were assayed for radioactivity as follows. Samples were minced, weighed and dissolved in 200  $\mu\text{l}$  of Soluene 350 tissue solubilizer

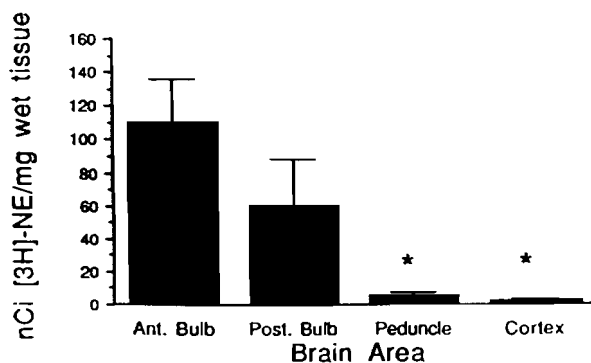


Fig. 2. Distribution of [ $^3\text{H}$ ]NE infused into the olfactory bulbs after 20 min (0.1  $\mu\text{l}/\text{min}$ ).  $^3\text{H}$ -activity did not significantly differ between anterior and posterior halves of the bulb (cannula located between halves) but was significantly reduced outside of the bulbs. Asterisks represents significantly different from olfactory bulb sections ( $P < 0.05$ ).

(Packard Instrument Co.) by heating at 50°C overnight. Ten ml of CytoScint (ICN Biomedicals) scintillation cocktail was added and radioactivity measured in a Beckman LS5801 Liquid Scintillation Counter (10 min, 1%-2 $\sigma$  counting error). H# standardization was utilized to determine absolute decay rates. Data were expressed as nCi of [<sup>3</sup>H]NE per mg of wet tissue and compared across brain regions with an ANOVA.

As shown in Fig. 2, with the infusion rates and durations used in the present experiment, infusate concentration did not significantly differ between large areas of the olfactory bulb, and did not appreciably extend beyond the olfactory bulb (ANOVA,  $F_{3,63} = 7.07$ ,  $P < 0.001$ ). Post-hoc analyses showed that while [<sup>3</sup>H]NE/mg tissue did not significantly differ between anterior and posterior olfactory bulb sections, both olfactory bulb sections had significantly greater content than either non-olfactory bulb section (Fisher,  $P < 0.05$ ).

These results suggest that olfactory bulb NE is necessary for acquisition of a learned odor preference in neonatal rat pups, and extend previous work which demonstrated modulation of early learning by systemic administration of NE agonists and antagonists<sup>14,18,19</sup>.

Failure to acquire a learned odor preference during intrabulbar infusions of propranolol is most likely *not* due to: (1) non-specific bulb damage from the cannula implantation or infusion, since saline infused PAIRED animals learned normally; (2) non-specific actions of propranolol (e.g. local anesthetic effects), since Gray et al.<sup>6</sup> have reported normal olfactory bulb evoked potentials and presynaptic fiber volleys following propranolol infusion (100  $\mu$ M) into the rabbit olfactory bulb; (3) propranolol-induced anosmia since Doty et al.<sup>3</sup> have reported normal olfactory behavioral thresholds following olfactory bulb NE depletion with 6-hydroxydopamine. Furthermore, propranolol does not interfere with *expression* of a previously learned olfactory preference in infant rats<sup>15</sup>, suggesting sufficient olfactory discrimination abilities in the presence of propranolol (although all testing in the present study was performed 24 h after acute propranolol infusion).

These results suggest that the action of NE within the olfactory bulb is critical for early olfactory associative learning. Thus, although behaviorally activating reinforcers and systemic manipulation of the NE system may have a myriad of consequences, ranging from changes in mucus secretions and perhaps function of the olfactory epithelium to changes in activity in a variety of central structures, one critical locus for NE action in early learning is the olfactory bulb. These findings are consistent with those reported for the role of olfactory bulb NE in olfactory learning in mature

rabbits<sup>6</sup> and accessory olfactory bulb NE in olfactory learning in mice<sup>4</sup>.

It should be emphasized, however, that the present results do not rule out the influence of other olfactory bulb neurotransmitter systems<sup>1,11</sup> or other brain regions<sup>9,16</sup> in early olfactory learning. Rather, NE may play a necessary modulatory role in olfactory bulb function during acquisition and the immediate post-training period<sup>17</sup> that allows long-term changes to occur in the olfactory bulb and/or elsewhere.

This research was supported by grants from NIH DC00489 and NSF BNS9110506 to R.M.S. and NSF BNS8819189 to D.A.W. The authors would like to thank Matt Arrington and Jason Evans for technical assistance. D.R.Z.'s present address is Dept. Psychology, Univ. North Carolina, Chapel Hill, N.C. 27599.

- Coopersmith, R., Weihmuller, F.B., Kirstein, C.L., Marshall, J.F. and Leon, M., Extracellular dopamine increases in the neonatal olfactory bulb during odor preference training, *Brain Res.*, 564 (1991) 149–153.
- Devauges, V. and Sara, S.J., Memory retrieval enhancement by locus coeruleus stimulation: evidence for mediation by  $\beta$ -receptors, *Behav. Brain Res.*, 43 (1991) 93–97.
- Doty, R.L., Ferguson-Segall, M., Lucki, I. and Kreider, M., Effects of intrabulbar injections of 6-hydroxydopamine on ethyl acetate odor detection in castrate and no-castrate male rats, *Brain Res.*, 444 (1988) 95–103.
- Gervais, R., Holley, A. and Keverne, E.B., The importance of central noradrenergic influences on the olfactory bulb in the processing of learned olfactory cues, *Chem. Senses*, 13 (1988) 3–12.
- Gervais, R. and Pager, J., Olfactory bulb excitability selectively modified in behaving rats after local 6-hydroxydopamine treatment, *Behav. Brain Res.*, 9 (1983) 165–179.
- Gray, C.M., Freeman, W.J. and Skinner, J.E., Chemical dependencies of learning in the rabbit olfactory bulb: acquisition of the transient spatial pattern change depends on norepinephrine, *Behav. Neurosci.*, 100 (1986) 585–596.
- Jahr, C.E. and Nicoll, R.A., Noradrenergic modulation of dendrodendritic inhibition in the olfactory bulb, *Nature*, 297 (1982) 227–229.
- Kasamatsu, T. and Pettigrew, J.D., Depletion of brain catecholamines: failure of ocular dominance shift after monocular occlusion in kittens, *Science*, 194 (1976) 206–209.
- Kucharski, D. and Hall, W.G., Developmental change in the access to olfactory memories, *Behav. Neurosci.*, 102 (1988) 340–348.
- McGaugh, J.L., Hormonal influences on memory, *Annu. Rev. Psychol.*, 34 (1983) 297–323.
- McLean, J.H., Darby-King, A., Sullivan, R.M. and King, S.R., Serotonin and olfactory learning in the neonatal rat, *Soc. Neurosci. Abstr.*, 18 (1992) 1201.
- McLean, J.H. and Shipley, M.T., Postnatal development of the noradrenergic projection from locus coeruleus to the olfactory bulb in the rat, *J. Comp. Neurol.*, 304 (1991) 467–477.
- Singer, W., Tretter, F. and Yinon, U., Central gating of developmental plasticity in kitten visual cortex, *J. Physiol.*, 324 (1982) 221–237.
- Sullivan, R.M., McGaugh, J. and Leon, M., Norepinephrine induced plasticity and one-trial olfactory learning in neonatal rats, *Dev. Brain Res.*, 60 (1991) 219–228.
- Sullivan, R.M. and Wilson, D.A., The role of norepinephrine in the expression of learned olfactory neurobehavioral responses in infant rats, *Psychobiology*, 19 (1991) 308–312.
- Sullivan, R.M. and Wilson, D.A., Neural correlates of conditioned odor avoidance in infant rats, *Behav. Neurosci.*, 105 (1991) 307–312.

- 17 Sullivan, R.M. and Wilson, D.A., The role of norepinephrine in consolidation of early olfactory memories. *Soc. Neurosci. Abstr.*, (1992) in press.
- 18 Sullivan, R.M., Wilson, D.A. and Leon, M., Norepinephrine and learning-induced plasticity in infant rat olfactory system, *J. Neurosci.*, 9 (1989) 3998-4006.
- 19 Wilson, D.A. and Sullivan, R.M., Olfactory associative conditioning in infant rats with brain stimulation as reward. II. Norepinephrine mediates a specific component of the bulb response to reward. *Behav. Neurosci.*, 105 (1991) 843-849.
- 20 Wilson, D.A. and Leon, M., Noradrenergic modulation of olfactory bulb excitability in the postnatal rat, *Dev. Brain Res.*, 42 (1988) 69-75.