

Unique Neural Circuitry for Neonatal Olfactory Learning

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Imprinting ensures that the infant forms the caregiver attachment necessary for altricial species survival. In our mammalian model of imprinting, neonatal rats rapidly learn the odor-based maternal attachment. This rapid learning requires reward-evoked locus ceruleus (LC) release of copious amounts of norepinephrine (NE) into the olfactory bulb. This imprinting ends at postnatal day 10 (P10) and is associated with a dramatic reduction in reward-evoked LC NE release. Here we assess whether the functional emergence of LC $\alpha 2$ inhibitory autoreceptors and the downregulation of LC $\alpha 1$ excitatory autoreceptors underlie the dramatic reduction in NE release associated with termination of the sensitive period. Postsensitive period pups (P12) were implanted with either LC or olfactory bulb cannulas, classically conditioned with intracranial drug infusions (P14), and tested for an odor preference (P15). During conditioning, a novel odor was paired with either olfactory bulb infusion of a β -receptor agonist (isoproterenol) to assess the target effects of NE or direct LC cholinergic stimulation combined with $\alpha 2$ antagonists and $\alpha 1$ agonists in a mixture to reinstate neonatal levels of LC autoreceptor activity to assess the source of NE. Pups learned an odor preference when the odor was paired with either olfactory bulb isoproterenol infusion or reinstatement of neonatal LC receptor activity. These results suggest that LC autoreceptor functional changes rather than olfactory bulb changes underlie sensitive period termination.

Key words: olfactory bulb; locus ceruleus; norepinephrine; acetylcholine; phenylephrine ($\alpha 1$ agonist); idazoxan ($\alpha 2$ antagonist); memory; learning; infant rat; sensitive period; imprinting; development; mother–infant attachment

Introduction

The strong influences of infant experiences on adult life will remain elusive until we understand the unique functions of the neonatal brain that lay the foundation for future brain processing. Childhood experiences, especially within the context of attachment, have a strong impact on emergence of adult mental health and character traits (Bowlby, 1969; Glaser, 2000; Schore, 2001; Teicher et al., 2003). Our mammalian imprinting model explores this issue and has shown that the neonatal rat uses a unique, simplistic learning circuit to acquire the life-sustaining odor attachment to the caregiver. Specifically, without brain areas normally involved in adult learning (nonfunctional hippocampus, amygdala, frontal cortex), the infant must rely on a unique learning circuit involving the olfactory bulb and locus ceruleus (LC) (Rudy and Morledge, 1994; Wilson and Sullivan, 1994; Vermer et al., 1996; Stanton, 2000; Sullivan et al., 2000b; Sullivan, 2003).

In our rat mammalian imprinting model using odor–reward pairings, neonatal rats can rapidly learn an odor-based attachment to their mother. This sensitive period learning is dependent on a simplistic learning circuit involving the olfactory bulb and LC and results in a rapid strong attachment to the mother corre-

sponding with olfactory bulb metabolic and anatomical changes (Wilson et al., 1987; Woo et al., 1987; Wilson and Leon, 1988; Wilson and Sullivan, 1990; McLean et al., 1999; Sullivan, 2001). These learning-induced behavioral and neural changes require norepinephrine (NE) (LC lesion or blocking olfactory bulb NE prevents learning), and an odor preference is learned from odor–NE pairings (LC stimulation or olfactory bulb NE infusions) (Sullivan et al., 1992, 1994, 2000b; Yuan et al., 2003). Although the behavioral and neural changes are dependent on acquisition during the sensitive period, the odor is later important for the mate choice, sex, and maternal behavior of the adult (Pager, 1974; Coopersmith and Leon, 1986; Fillion and Blass, 1986; Woo and Leon, 1987; Moore et al., 1996; Fleming et al., 1999; Shah et al., 2002).

At postnatal day 10 (P10), as the sensitive period ends, pups develop the motor abilities to leave the nest (Bolles and Woods, 1965), and their learning abilities become more adult-like. First, the infant's learning abilities expand to permit passive avoidance, active avoidance, and inhibitory conditioning (Collier et al., 1979; Blozovski and Cudennec, 1980; Camp and Rudy, 1988; Myslivecek, 1997; Sullivan et al., 2000a). The developmental emergence of amygdala functioning seems to underlie these new learning abilities (Wilson and Sullivan, 1993; Sullivan et al., 2000a). Second, learning diminishes in concert with the loss of NE release to odor and reward (Rangel and Leon, 1995). Because the LC is the sole source of NE for the olfactory bulb, we assess here whether developmental changes in the LC may underlie pups' loss of rapid, robust odor-preference learning. The LC contains recurrent collaterals, along with corresponding LC NE autoreceptors to regulate LC function (Berridge and Waterhouse, 2003). Although the LC $\alpha 2$ autoreceptors are present in the neo-

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nate (receptor autoradiography and mRNA) (Winzer-Serhan et al., 1999), they do not appear to function until approximately P10, at least in whole animals receiving sensory stimulation during extracellular single-cell LC recordings (Kimura and Nakamura, 1987; Nakamura et al., 1987; Nakamura and Sakaguchi, 1990). Specifically, extracellular recording of single LC neurons in response to sensory stimuli (1 sec air puff or electric shock) elicits prolonged excitation (20–30 sec) in neonates, whereas the same stimulation produces only a response duration measured in milliseconds in older pups. This developmental response difference appears to be attributable to the functional emergence of $\alpha 2$ inhibitory noradrenergic autoreceptors, resulting in the LC auto-inhibition within milliseconds of activation and the functional attenuation of LC $\alpha 1$ excitatory noradrenergic autoreceptors, which prolong LC neonatal responses (Nakamura et al., 1987; Pieribone et al., 1994; Scheinin et al., 1994). On the basis of these data, we hypothesized that developmental LC autoreceptor changes may be responsible for the failure of older pups to learn the rapid, robust odor preference simply because the LC releases insufficient amounts of NE to support learning. To test this hypothesis, we attempted to reinstate neonatal LC autoreceptor activity in postsensitive period pups through infusions of an autoreceptor agonist–antagonist mixture and assessed whether imprinting-like odor-preference learning could be reinstated. Moreover, because the olfactory bulb is the site of the learning-induced changes, we assessed the role of NE within the olfactory bulb by intrabulbar infusions of an NE β -agonist. Our results suggest that the developmental change in LC autoreceptors rather than the olfactory bulb may underlie, at least in part, sensitive period termination.

Materials and Methods

Subjects. The subjects were male and female Long–Evans rat pups born and bred in our colony at the University of Oklahoma (originally from Harlan Farms). Animals were housed in polypropylene cages ($34 \times 29 \times 17$ cm) lined with wood chips and were kept in a temperature (23°C)- and light (7:00 A.M. to 7:00 P.M.)-controlled room. Food and water were available *ad libitum*. The day of birth was considered P0, and litters were culled to 10 on P1. No more than one male and one female from a litter were used in each experimental condition. All procedures followed University of Oklahoma and National Institutes of Health standards for animal treatment.

Cannula implantation. On P12, pups were anesthetized by inhalation (with metofane until tailpinch reflex was eliminated) and placed in an adult stereotaxic apparatus adapted for use with infants. Stainless-steel cannulas (30 gauge tubing) were implanted bilaterally in the olfactory bulb or LC through holes drilled in the overlying skull. Stereotaxic coordinates derived from the atlas of Paxinos et al. (1991) were used as a reference and adapted through pilot work (Sullivan et al., 2000b) for implanting cannulas into the LC (caudal, 1.40 mm; lateral, ± 0.60 mm from lambda). The cannulas were lowered 5.5 mm from the surface of the skull, which placed the tip near the LC. The cannula assembly was fixed to the skull with dental cement, and anchor wires (one for the olfactory bulb, two for the LC) were placed ~ 3 mm from the cannula holes (Gilbert and Cain, 1980). To ensure patency of the cannulas, guide wires were placed in the lumen of the tubing until training. Olfactory bulb cannulas were implanted under visual guidance. After recovery from surgery (generally within 30 min), pups were returned to the litter and dam until training 2 d later.

Drug infusion protocol and conditioning. On P14, pups were placed in training chambers and bilateral cannulas were connected via polyethylene 10 tubing to a syringe pump (Harvard Apparatus, South Natick, MA) driving two microliter syringes (Hamilton, Reno, NV). The cannulas were filled (6 sec for olfactory bulb cannulas and 12 sec for LC cannulas at $0.5 \mu\text{l}/\text{min}$) with either drug (described below) or vehicle. During both the 10 min behavioral adaptation period and the 10 min training period,

drug or vehicle was infused at $0.1 \mu\text{l}/\text{min}$ for a total infusion volume of $2.0 \mu\text{l}$ as described previously (Sullivan et al., 1992, 2000b). Pups were given a 10 min adaptation period to permit acclimation to the training chamber and to allow drug distribution within the targeted brain area. During the subsequent 10 min, brain infusions continued, and pups were presented with citral odor ($0.25 \mu\text{l}$; Sigma, St. Louis, MO), which served as the conditioned stimulus (CS). After training, pups were disconnected from the syringe pump and returned to the nest until testing the next day. Throughout adaptation and training, pups were observed continuously to ensure that all pups were healthy and responding normally to experimental stimuli. Pups exhibiting abnormally high (i.e., aversive) or low (i.e., lethargic) behavioral responsiveness were eliminated from the experiment during adaptation or conditioning.

Olfactory bulb infusions. Pups with bilateral cannulas in the olfactory bulbs received either isoproterenol ($50 \mu\text{M}$; Sigma), an NE β -receptor agonist, or vehicle (saline) (Sullivan et al., 1989).

LC infusions. Pups with cannulas implanted into the LC received one or more of the following compounds: acetylcholine (2 mM acetylcholine chloride; Sigma), which activates the LC (Adams and Foote, 1988); phenylephrine (1 mM L-phenylephrine; Sigma), which is an $\alpha 1$ agonist that stimulates the excitatory LC autoreceptors (Mouly et al., 1995); or idazoxan ($2 \mu\text{M}$; Sigma), which is an $\alpha 2$ antagonist that blocks the inhibitory LC autoreceptors (Devauges and Sara, 1990; Ivanov and Aston-Jones, 1995; Sullivan et al., 2000b).

Systemic injections. To confirm the noradrenergic basis of LC manipulation effects on odor learning, a separate group of LC-infused pups was injected systemically with the β -receptor antagonist propranolol ($20 \text{ mg}/\text{kg}$, i.p.) 30 min before training (Sullivan et al., 1989).

Behavior tests. On P15, pups were given one of two tests, depending on the experiments. Both tests, which have been widely used and dependably assess infant learning, are described below. No drugs were infused during testing, and drugs present during training had left the system the previous day (Goodman and Gilman, 1985).

Two-odor choice test. This test measures the amount of time pups spent over a familiar odor (clean pine nest shavings) versus the CS citral odor used during conditioning. The test apparatus consisted of a Plexiglas arena (24 cm long \times 14 cm wide) with a wire mesh floor that enabled pups to smell the odor beneath them. The floor was divided into two parts, separated by a 2 cm midline. One side contained the odor CS ($0.20 \mu\text{l}$ of citral placed on a $5 \times 5 \text{ cm}$ Kimwipe), and the other side contained a familiar pine odor (160 ml of wood chips). Pups were placed on the midline between the two odors (direction was counterbalanced), and the amount of time each pup spent over each odor was monitored for three 60 sec trials, with 10 sec between each trial. Between each trial, the floor was cleaned with distilled water and then dried (Cornwell-Jones, 1981; Sullivan et al., 1989).

Y-maze. This test requires pups to choose between two arms of a Plexiglas Y-maze (start box, 8.5 cm width, 10 cm length, 8 cm height; choice arms, $8.5 \times 24 \times 8 \text{ cm}$), one containing the citral odor CS and the other containing the familiar odor of pine shavings. Pups were placed in the start box (direction was counterbalanced), and after 5 sec, the door to each alley was opened, at which time pups were given 60 sec to choose an arm. A response was considered a choice when a pup's entire body was past the entrance to the alley. Occasionally (three pups, only one trial each), pups did not make a choice within 60 sec, and in these cases, the pups were removed and returned to the maze to repeat the trial. Pups received five trials, with 30 sec between trials (Sullivan and Wilson, 1991).

Drug diffusion. To characterize the extent of drug diffusion within and outside of the LC, additional pups were used. On P12, pups were anesthetized by urethane and placed in a stereotaxic apparatus. Holes were drilled through the skull at 1.4 mm posterior to lambda and $\pm 0.60 \text{ mm}$ from the midline. A $10 \mu\text{l}$ Hamilton syringe was lowered 5.5 mm from the surface of the skull, which placed the tip near the LC. The pups were infused with $2 \mu\text{l}$ of a saline solution of [^3H]NE ($56.9 \text{ Ci} \cdot \text{mmol}^{-1} \cdot \mu\text{m}^{-1}$, $0.37 \text{ Ci} \cdot \text{mmol}^{-1} \cdot \mu\text{l}^{-1}$; DuPont NEN, Boston, MA), and 20 min after infusion, the brains were quickly removed and frozen in methyl butane at -45°C . Brains were sliced in $20 \mu\text{m}$ coronal sections. The slides were apposed to a tritium storage phosphor screen

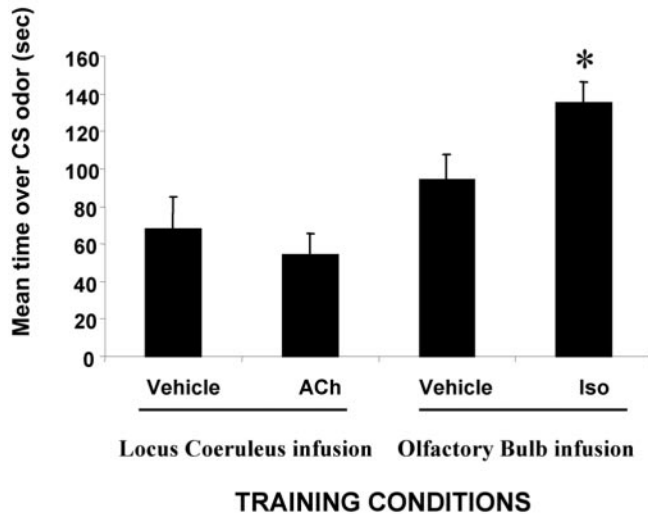


Figure 1. Mean \pm SEM time spent over CS odor during the two-odor choice test. Training infusion into the olfactory bulb of the NE receptor agonist isoproterenol (Iso) significantly increased the learned relative odor preference compared with vehicle group. Training infusion into the locus coeruleus of ACh, an LC stimulant, did not produce a significant change in odor preference compared with vehicle groups ($n = 7-8$ per group). The asterisk represents significant differences from all other groups ($p < 0.05$).

(Amersham Biosciences, Arlington Heights, IL). After 14 d of exposure, the screen was scanned at a pixel density of $50 \mu\text{m}$ (5000 dots per cm^2) with a Storm 820 PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Phosphorimaging of the slides results in a tagged image file format (Tucker et al., 2002).

Histology. After behavioral testing, pups were overdosed with urethane and perfused with saline and 4% formalin. Brains were removed and postfixed in 4% formalin and 30% sucrose. Sections ($40 \mu\text{m}$) were cut, and cresyl violet staining was used to verify LC cannula placements.

Results

Behavioral observations of pups during training

Behavior was observed during training to ensure that the experimental manipulations did not adversely affect pups. Two pups were excluded from the study for exhibiting hyperactivity during training, and four other pups were excluded for weight loss between surgery and training. Pups gained weight over the course of the experiment, with initial weights of 28.67 gm (SE, ± 1.62) and 27.96 gm (SE, ± 0.94) for olfactory bulb and LC cannula pups, respectively. After testing, olfactory bulb and LC cannula pups weighed 33.71 gm (SE, ± 1.18) and 32.45 gm (SE, ± 1.02), respectively.

There were no statistical weight differences at either training or testing between groups for each cannula placement. Pup treatment weight gain did not change over the course of the experiment, although pups that did not gain weight after surgery were discontinued from the experiment (saline vs drug treatment at surgery, training, and testing (ANOVA; $F_{(3,76)} = 0.198$; NS).

Postsensitive period LC activation does not support odor learning

In contrast to neonatal rat pups during the sensitive period (Sullivan et al., 2000b), preweaning (P14) rat pups cannot learn an odor preference by simple activation of the LC during an odor presentation. As shown in Figure 1, only pups that received isoproterenol infused into the olfactory bulb exhibited a preference for the citral odor CS. ACh infusion into the LC, which produces an odor preference in younger neonatal pups, was insufficient to

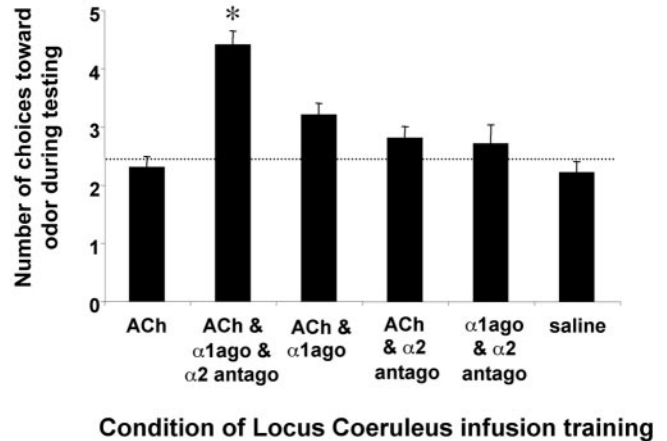


Figure 2. Mean \pm SEM number of choices toward the CS odor during the Y-maze test. Training infusion of ACh and the $\alpha 1$ agonist phenylephrine ($\alpha 1$ ago) and the $\alpha 2$ antagonist idazoxan ($\alpha 2$ antago) increased the learned relative odor preference compared with the other groups ($n = 3-7$ per group). The asterisk represents significant differences from all other groups ($p < 0.001$). The dotted horizontal line represents a chance line.

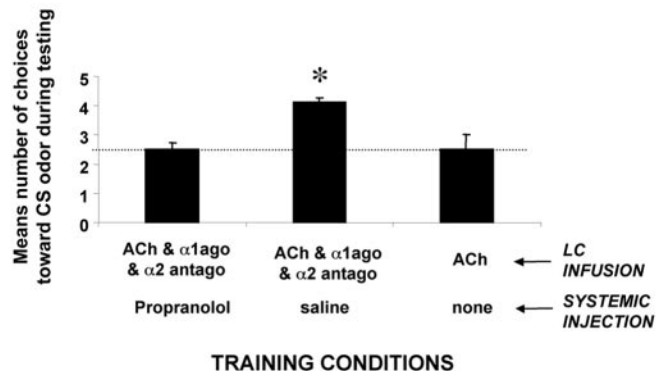


Figure 3. Mean \pm SEM number of choices toward the CS odor during the Y-maze test. A pretraining injection of propranolol (20 mg/kg) significantly reduced the learned relative odor preference expressed by pups receiving infusion of ACh and the $\alpha 1$ agonist phenylephrine ($\alpha 1$ ago) and the $\alpha 2$ antagonist idazoxan ($\alpha 2$ antago) compared with the control groups. The asterisk represents significant differences from all other groups ($p < 0.03$). The dotted horizontal line represents a chance line.

produce an odor preference in these preweaning pups (ANOVA; $F_{(3,26)} = 6.834$; $p < 0.005$). *Post hoc* Fisher tests revealed that olfactory bulb isoproterenol pups spent significantly more time over the CS odor than did the other groups ($p < 0.05$). It should be noted that there was a difference between the ACh LC group and the vehicle olfactory bulb groups ($p < 0.05$), but neither of these two groups differed from the vehicle LC group. Preweaning rat pups learn an NE-induced odor preference by reinstating the neonatal LC. As shown in Figure 2, only those pups that received activation of the LC by ACh concurrently with the blockade of LC inhibitory autoreceptors ($\alpha 2$ antagonist, idazoxan) and activation of the LC excitatory autoreceptors ($\alpha 1$ agonist, phenylephrine) during a citral odor presentation exhibited a subsequent preference for the citral odor ($F_{(5,24)} = 14.332$; $p < 0.0001$). Pups that received any combination of less than three of these drugs or vehicle did not exhibit learning as determined by *post hoc* Fisher tests (each group spent significantly less time over the CS; $p < 0.001$). As illustrated in Figure 3, a systemic blockade of NE receptors during the training prevented the reinstatement of the NE-dependent odor learning seen during the neonatal sensitive

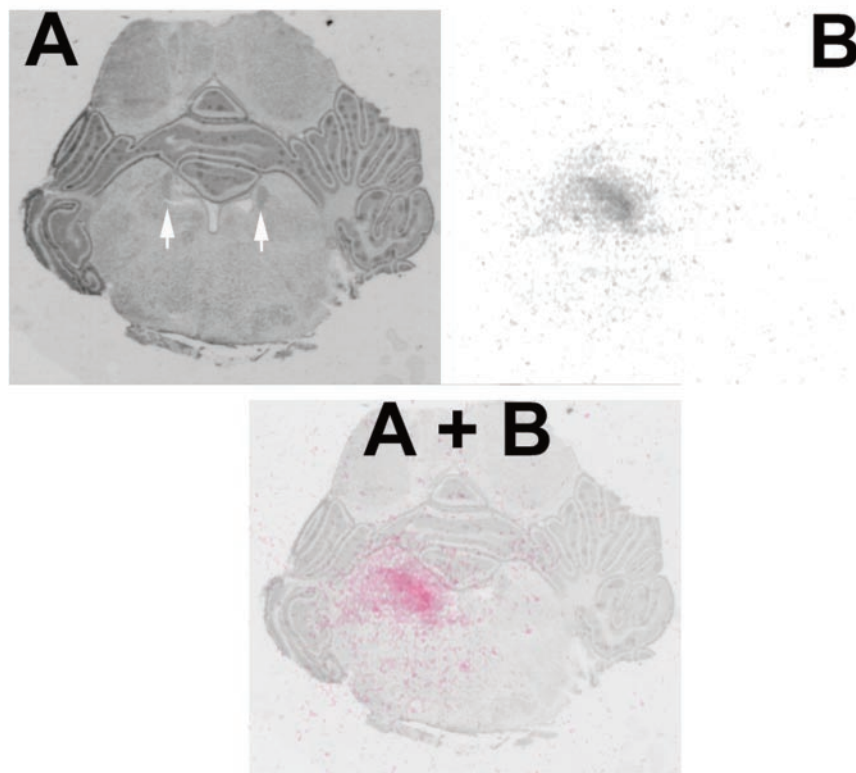


Figure 4. *A*, Section from a P14 pup counterstained with cresyl violet. The white arrows mark the locus ceruleus bilaterally. Actual cannula tip placement is outside the plane of this section. *B*, The same section as in *A* at the same magnification and orientation, characterizing the extent of [³H]NE drug diffusion within the LC. *A + B*, Color overlay of [³H]NE diffusion on the histological section showing drug diffusion over the region of the locus ceruleus.

period (ANOVA; conditioning group \times odor; $F_{(2,17)} = 18.338$; $p < 0.0001$; Fisher *post hoc* tests indicate that the pups injected with propranolol and infused concurrently with acetylcholine, idazoxan, and phenylephrine are significantly different from each of the other groups; $p < 0.03$).

Drug diffusion

As demonstrated in Figure 4, the volume of drugs infused into the LC for data illustrated in Figure 2 diffused <1 mm from the LC.

Histology

Cannula tip placements for cannulas directed at the LC are shown in Figure 5. All tip placements were <1 mm from the LC except for one pup, which was excluded from the experiment. This pup, which received an infusion of ACh concurrently with phenylephrine and idazoxan, did not exhibit a subsequent preference for the citral odor, unlike pups receiving the same infusion into the LC.

Discussion

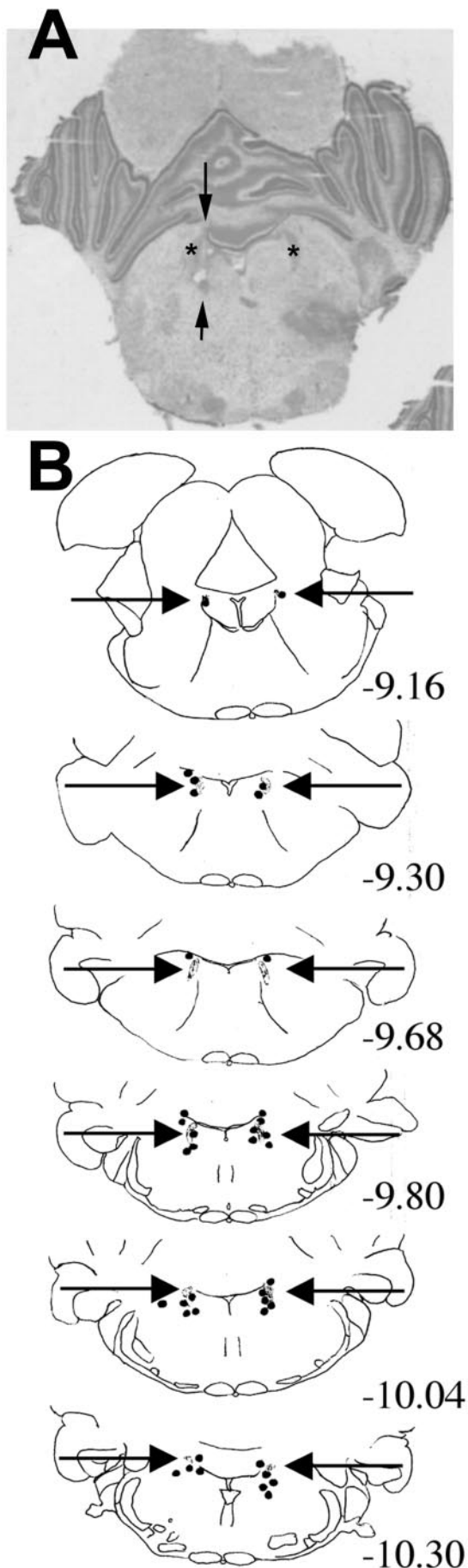
These results suggest that the developmental emergence of autoinhibition and attenuation of autoexcitation in the LC may be responsible, at least in part, for the termination of the infant rat's sensitive period for learning. Our results have shown that preweanling (P14) pharmacological LC manipulations intended to reinstate neonatal sensitive period LC function (attenuating LC autoinhibition and amplifying LC autoexcitation) are sufficient to reinstate the neonatal NE-dependent odor learning. Specifically, in postsensitive period P14 pups, we reversed the LC characteristics that emerge at P10 by blocking the LC α_2 inhibitory autoreceptors and activating the LC α_1 excitatory autorecep-

tors. The pharmacologically neonatized LC, combined with LC cholinergic stimulation paired with a novel odor, was sufficient to produce an odor preference that was blocked by systemic blockade of NE β receptors.

Our results also suggest that the olfactory bulb remains plastic even after the sensitive period has terminated, because simply increasing olfactory bulb NE is sufficient to produce the rapid NE-dependent odor preference. Indeed, increasing NE via LC stimulation or direct olfactory bulb infusions showed that the olfactory bulb maintains its ability to produce rapid acquisition of an odor preference. These results complement previous results on the important role of NE in developmental plasticity other than learning, such as that which occurs in the developing visual system (Kasamatsu and Pettigrew, 1979; Kasamatsu et al., 1979; Bear and Singer, 1986; Shirokawa et al., 1989).

These results are in sharp contrast to the effects of NE on learning in adults, in which NE appears to have a modulatory effect on acquisition and on attention consolidation (Gold and Van Buskirk, 1975; Liang et al., 1990; Selden et al., 1990; McGaugh et al., 1996; Roozendaal et al., 1999; Feenstra et al., 2001). Specifically, similarly to neonates, neural correlates of learning could be achieved by large NE infusions directly into the olfactory bulb, hippocampus, and auditory cortex of the adult rat, suggesting that the mature brain retains at least some potential for NE-dependent plasticity, provided that sufficient levels of NE are available (Gray et al., 1986; Harley et al., 1996; Chaulk and Harley, 1998; Harley, 1998; Edeline, 1999).

The differences in the role of NE in neonatal and adult learning appear to be attributable to developmental differences in the response of the LC to sensory stimuli. The infant LC and adult LC are both activated by sensory stimuli, but the LC of the adult is less likely than the LC of the infant to respond to non-noxious stimuli (Foote et al., 1980; Kimura and Nakamura, 1985; Nakamura and Sakaguchi, 1990; Selden et al., 1990; Harris and Fitzgerald, 1991; Harley and Sara, 1992; Aston-Jones et al., 1994; Sara et al., 1995; Vankov et al., 1995; Mansour et al., 2003). Additionally, the LC of the adult habituates after repeated (or even single) stimulus presentations, whereas the LC of the infant fails to habituate (Nakamura et al., 1987; Nakamura and Sakaguchi, 1990; Vankov et al., 1995). Even more dramatic is the response duration of the neonatal LC compared with the preweanling–adult LC, with a 1 sec presentation of tactile stimulation causing a response of a few milliseconds in the LC of the adult but a 20–30 sec response in the LC of the infant (Nakamura et al., 1987). These developmental differences in LC activity are reflected in dramatic age differences in stimulus-evoked release of NE in the olfactory bulb, with neonatal pups (sensitive-period age pups) releasing significantly more NE than slightly older pups (postsensitive period pups) (Rangel and Leon, 1995). This developmental decrease in the sensory-evoked release of NE in the olfactory bulb



occurs despite the extensive increase in NE centrifugal fibers innervating the bulb at this developmental stage (McLean and Shipley, 1991). Together with the present results, these findings suggest that developmental changes in the LC may be responsible for the increasingly subtle role of NE in learning and memory as rats mature.

Throughout the lifespan, attachments continue to be formed within the context of mate selection, mating, and care of the young. Although the neural circuitry learning for these behaviors is more complex than that seen in the neonate, the importance of NE in learning re-emerges (Levy et al., 1990; Brennan and Keverne, 1997; Insel and Young, 2001). There is some evidence to suggest that the LC changes to accommodate the rapid learning required in these reproduction-related learning situations. Specifically, similarly to the LC of the neonatal rat pup, spontaneous activity is greatly diminished during pregnancy, although the amount of NE released is greatly enhanced (Nakamura et al., 1988).

The large release of NE during neonatal rat learning appears to underlie the neural changes uniquely associated with learning during the sensitive period. During acquisition, the NE from the LC maintains the responsiveness of mitral cells (primary output neurons of the olfactory bulb), whereas the mitral cells of control animals (backward, odor only) quickly habituate (Wilson et al., 1987). During consolidation, a cascade of molecular events is implicated in odor-preference learning in neonatal rats. An important interaction between serotonin and NE permits increasing mitral cell cAMP and cAMP response element-binding protein phosphorylation (Yuan et al., 2003; Zhang et al., 2003). This causes modifications in the olfactory bulb that last into adulthood, including enhanced odor-evoked glomerular layer 2-DG uptake and Fos-like immunoreactivity (Coopersmith and Leon, 1984; Sullivan and Leon, 1986; Johnson et al., 1995), modified odor response patterns of mitral-tufted cells (Wilson et al., 1987), and morphological changes in the glomerular layer (Woo et al., 1987). The acquisition of these olfactory bulb learning-induced changes is limited to the sensitive period (Woo et al., 1987; Sullivan and Wilson, 1991).

As described above, as the pup's sensitive period ends, rapid odor-preference conditioning ends. However, other types of learning emerge, such as fear conditioning, inhibitory conditioning, and passive avoidance (Collier et al., 1979; Blozovski and Cudennec, 1980; Camp and Rudy, 1988; Myslivecek, 1997; Sullivan et al., 2000a). The functional emergence of the amygdala seems to be responsible for the emergence of the learned fear system (odor, 0.5 mA shock) (Sullivan et al., 2000a). Moreover, the functional emergence of the pup's ability to release corticosterone in response to shock and other stressors may be related to the ability of the amygdala to function in the fear conditioning situation (odor, 0.5 mA shock) (Moriceau and Sullivan, 2003, 2004). Thus, developmental changes in pups' learning abilities likely reflect the myriad of developmental brain changes occurring during early development. We hypothesized that the chang-

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Figure 5. *A*, Representative location of cannula tip near the locus ceruleus. The cannula tip placement is marked by the arrows. The location of the locus ceruleus is illustrated by an asterisk. *B*, Locations of cannula tips (dots) in rats used for the experiment shown in Figure 2. The locus ceruleus is illustrated in gray and marked by the horizontal arrows. Corresponding sections from the adult stereotaxic atlas of Paxinos et al. (1991) were determined, and the relative distance from bregma for each coronal section on the basis of the adult atlas is noted on the right.

ing learning system reflects the changes in developmental demands placed on pups as new behaviors such as walking emerge. Other developmental changes in learning have been documented to coincide with weaning and with the emergence of hippocampal-dependent spatial learning and potentiated startle (Hunt and Campbell, 1999; Richardson et al., 2000; Stanton, 2000).

Although some prenatal olfactory experience may contribute to the pup's olfactory attachment to the mother, postnatal experience in the form of classical conditioning appears necessary for attachment (Thoman et al., 1968; Galef and Sherry, 1973; Johanson and Hall, 1982; Pedersen et al., 1982; Alberts and May, 1984; Sullivan et al., 1986a,b). A similar role for somatosensory learning, particularly the facial whiskers, may also be important in early attachment (Polan and Hofer, 1998; Landers and Sullivan, 1999b). Both somatosensory and olfactory learning are NE dependent (Landers and Sullivan, 1999a). Because of the late maturation of the auditory and visual systems in the rat, dependence on odor and somatosensory learning may be critical for survival (Moye and Rudy, 1985, 1987; Hunt and Campbell, 1999; Richardson et al., 2000). Learning in the olfactory system seems to be particularly important in several behaviors critical for survival (i.e., nipple attachment, huddling, orientation to the dam), and damage to the olfactory system or the source of the odor stimuli greatly reduces survival (Singh and Tobach, 1975; Hofer, 1976). Together, these results suggest that a unique learning circuitry in the neonate has evolved to ensure that infants form a rapid, robust attachment to the mother (Sullivan et al., 2000b; Hofer and Sullivan, 2001). This unique neonatal learning circuit may be particularly important in the infant rat, in which structures important to adult learning have not yet developed [i.e., the amygdala (Sullivan et al., 2000a), the hippocampus (Rudy and Morledge, 1994), and the cerebellum (Freeman and Nicholson, 2000)]. The present results are a further indication that the LC is a fundamental component of this unique learning circuit, and that the special balance of NE autoreceptors within the developing LC helps define a sensitive period that is critical for infant learning and survival.

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