

Short communication

Norepinephrine and associative conditioning in the neonatal rat somatosensory system

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Abstract

Neonatal rats, aged postnatal days 3–4, were trained in a somatosensory associative conditioning task involving temporal correlation of facial vibrissa stimulation and aversive shock. This training resulted in a subsequent conditioned behavioral activation/arousal response to vibrissa stimulation alone, compared to non-learning control pups trained with random vibrissa–shock presentations. The acquisition of the conditioned response was blocked by systemic injections of the NE β -receptor antagonist propranolol in a dose-dependent manner. In a second study, vibrissa stimulation was paired with systemic injections of the NE β -receptor agonist isoproterenol. Association of vibrissa stimulation with β -receptor activation resulted in subsequent conditioned responses to vibrissa stimulation alone, in a dose-dependent manner. Together, these results suggest that early associative somatosensory conditioning requires and involves NE in a manner similar to that previously demonstrated for early olfactory learning. © 1999 Elsevier Science B.V. All rights reserved.

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Norepinephrine (NE) and the noradrenergic locus coeruleus appear to play a unique role in behavioral and neural plasticity early in development. While under most circumstances NE is modulatory for plasticity in the mature mammalian forebrain, NE is involved in, and necessary for a variety of experience-dependent changes in developing sensory systems.

For example, associative olfactory learning in newborn rats pups requires NE input from the locus coeruleus to the olfactory bulb during odor exposure for a memory of that odor to be formed. During the first postnatal week, rat pups learn both a conditioned behavioral activation and an approach response to odors associated with a variety of reinforcers such as milk, tactile stimulation or warmth [17]. Blockade of NE β -receptors within the olfactory bulb [20] or lesions of the locus coeruleus [18] prevent normal associative olfactory conditioning in these rat pups. Conversely, pairing an odor with systemic or intra-bulbar infusion of the NE β -receptor agonist isoproterenol ([16];

Sullivan et al., in prep.), or with pharmacological or electrical stimulation of the ascending NE afferents ([24]; Sullivan et al., in prep.), in the absence of any other reinforcer, is sufficient to produce learned responses to that odor.

This well described, special role of NE in early olfactory learning may be indicative of unique features of the developing olfactory system, or alternatively, may represent unique characteristics of developing NE/locus coeruleus–target interactions in general. In support of the latter hypothesis, the physiology of the immature locus coeruleus is markedly different from the mature system. For example, while locus coeruleus responses to sensory stimuli are rapidly terminated by NE α_2 -receptor-mediated autoinhibition in the mature rat, this autoinhibition is not functional until the middle of the second postnatal week [6]. Furthermore, locus coeruleus single-units are activated by a wider range of stimuli in neonates compared to adults [12], and electrical coupling between locus coeruleus cells in neonates can result in a larger population of activated cells for a given input compared to adults [1].

To begin to address this issue, we examined the role of NE in associative learning in a second sensory system that

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is an important mediator of pup behavioral development—the facial vibrissa somatosensory system. The somatosensory system was chosen as another sensory system in which to assess the role of NE in early associative learning because: (1) this system is present and functional in the neonate [3] and is important to pup survival [26], (2) somatosensory associative conditioning has been demonstrated in neonates [7], (3) the locus coeruleus innervates the somatosensory system at a number of different levels along the ascending somatosensory pathway [15] and (4) norepinephrine has been implicated in development, function and plasticity within this system [9,13,22].

The results demonstrate that early associative somatosensory conditioning requires and involves NE in a manner similar to that previously demonstrated for early olfactory learning. These results suggest that the unique characteristics of the developing noradrenergic system may play a critical role in early memory formation in general.

The subjects were male and female rat pups (no more than 1 male and 1 female from a litter were used in an experimental condition) born of Long–Evans rats (Harlan Sprague–Dawley) in the vivarium at the University of Oklahoma. Dams were housed in rectangular polypropylene cages (34 × 29 × 17 cm) lined with wood chips in a temperature and light controlled room. Ad libitum food and water were available at all times. Births were checked twice daily. The day of birth was considered to be PN0.

Associative conditioning. At PN3 or PN4, pups were placed in plastic petri dishes (10 cm diameter) and left unrestrained. A 10 min acclimation period preceded training. The conditioned stimulus (CS) consisted of 30 s whisker stimulation. All whiskers on one side of the snout were stimulated manually for 30 s (about 50 sweeps back and forth across the entire whisker field) using a wooden rod about 1 mm in diameter. Stimulation included repeated flexion of every mystacial vibrissae, without stimulating the intravibrissal hair or skin on the snout. The side of the snout that was stimulated was alternated between litters to control for potential laterality bias of individual pups [21]. At the end of CS stimulation, and co-terminating with the CS, an unconditioned stimuli (US) was delivered consisting of an electrical shock (0.5 mA, approximately 0.5 s duration) to the pup's hind quarter. Training consisted of 8 stimulus presentations with a 3-min inter-trial interval. Pups in the PAIRED experimental group received the CS with a co-terminating US. Additional groups in the two experiments reported here included CS-ONLY, US-ONLY and/or RANDOM CS and US presentations. Following training, the pups were returned to the litter until testing.

A behavioral rating scale [2] was used to quantify behavior during a 10 s pre-CS period, during the CS and during the US. The scale quantified the number of elements moved and sustained for 2 s [2,16]. The scale range was 0–5 (0, no movement, 1: movement of one element (e.g., head or a limb), 2: movements of two elements (e.g., treading), 3: movements of three elements (e.g., pivot), 4:

movements of four elements (e.g., locomotion), 5: movements of 5 elements (e.g., roll over).

Four hours after training, conditioned behavioral responses to 5, 30 sec presentations of whisker CSs were assessed. Pups were placed in plastic petri dishes and allowed to acclimate for 10 min. As during training, behavior was observed before and during each presentation of whisker CS. Behavioral responses to the CS, based on the 5-point scale above, were summed across the five test trials for each animal, and averaged within groups for analysis.

Pharmacological manipulations. Pups were injected 45 min prior to training with either the NE β -receptor antagonist DL-propranolol (0, 1 or 2 mg/kg, i.p.) or β -receptor agonist isoproterenol (0, 1, 2, or 4 mg/kg, s.c.; 19). Pups were injected, given 5 min to recuperate, then returned to the nest for 30 min.

As previously reported [7], association of facial vibrissa stimulation and shock produced conditioned behavioral activation to subsequent vibrissa stimulation alone (Fig. 1). Acquisition of conditioned responding to vibrissa stimulation involved and required NE β -receptor activation. Animals were injected with 0, 1 or 2 mg/kg of the NE β -receptor antagonist DL-propranolol prior to training in either the PAIRED or RANDOM whisker stimulation CS-shock US paradigm ($n = 6$ pups condition⁻¹ dose⁻¹). Acquisition of conditioned behavioral activity was tested 4 h later, at a time when the physiological effects of propranolol are over [23]. Blockade of NE β -receptors (10 or 20 mg/kg propranolol) during PAIRED associative conditioning prevented acquisition of a learned behavioral response compared to saline injected pups (Fig. 1; ANOVA, group × dose interaction, $F(2,30) = 5.97$, $p = 0.05$). Post-hoc Fisher analysis showed PAIRED-SALINE was significantly different from all other groups ($p < 0.05$).

Conversely, association of whisker stimulation CS with NE β -receptor activation was sufficient to produce condi-

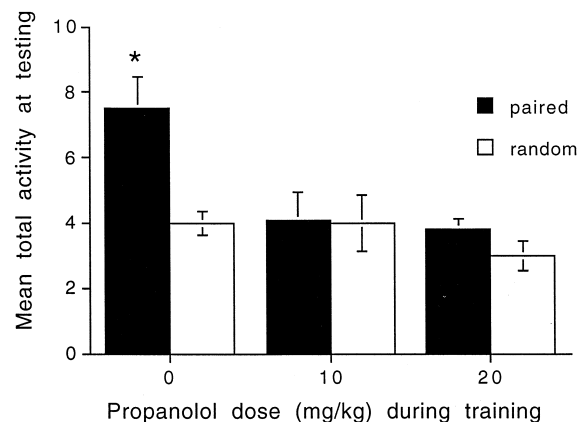


Fig. 1. The NE β -receptor antagonist propranolol blocked associative somatosensory learning in neonates in a dose-dependent manner. Asterisk represents significant difference from all other groups ($p < 0.05$).

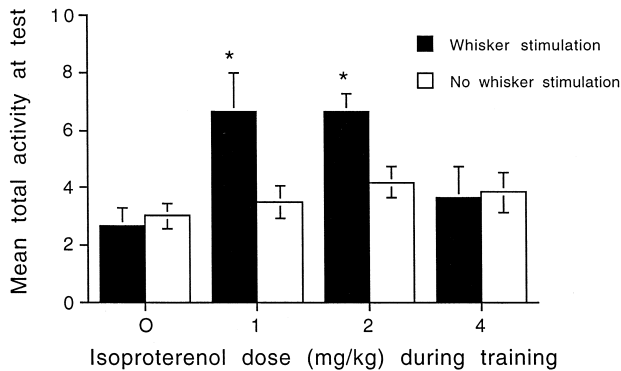


Fig. 2. Association of the NE β -receptor agonist isoproterenol with vibrissa stimulation was sufficient to produced subsequent conditioned behavioral activation to whisker stimulation alone, in a dose-dependent manner. Asterisk represents significant difference from all other groups ($p < 0.05$).

tioned responding in a dose-dependent manner (Fig. 2). Animals were injected with 0, 1, 2 or 4 mg/kg of the NE β -receptor agonist isoproterenol prior to training in either the whisker stimulation CS-ONLY or no stimulation (placed in training dish and left alone) paradigm ($n = 6$ pups per condition/dose). As with propranolol, acquisition of conditioned behavioral responses was tested 4 h later [23]. Association of whisker stimulation with a moderate dose of isoproterenol was sufficient to induce subsequent enhanced behavioral activity in response to the whisker CS alone (ANOVA, main effect for condition, $F(3,40) = 5.39$, $p < 0.01$). The ability of isoproterenol to support conditioning showed an inverted-U dose–response curve similar to that observed with early olfactory conditioning [16,19], with low (0 mg/kg) and high (4 mg/kg) dose groups no different from the naive group. Post-hoc Fisher tests revealed that pups receiving CS stimulation paired with 1 or 2 mg/kg isoproterenol were significantly different from all other groups ($p < 0.05$).

The present results demonstrate that early somatosensory associative conditioning involves and requires NE β -receptor activation. These results are similar to those reported for the behavioral and neural effects of early olfactory conditioning [16], and suggest that NE has a generalized role in associative memory during early development. These findings parallel the role of NE in developmental sensitive periods found using sensory deprivation paradigms [4], but suggest a more specialized function for NE in shaping ontogeny of sensory system circuits. It has been hypothesized that, due to the characteristics of the immature locus coeruleus [10], biologically relevant, behaviorally arousing stimuli cause an intense, prolonged release of NE that allows modification of those sensory systems that are co-active, forming a memory of, or experience-dependent change in response to, that stimulus [17].

Recent work in our laboratory [7] suggests that early somatosensory associative learning may be subcortically

mediated. Although cortical somatosensory barrels are anatomically present during the first postnatal week [5,14], vibrissa stimulus-evoked activity cannot be detected with functional imaging techniques (e.g., C^{14} 2-deoxyglucose uptake) until PN7–PN8 [7,11,25]. If early somatosensory learning is subcortical in origin, this may represent another parallel with early olfactory learning. Early olfactory learning is correlated with functional changes in the subcortical olfactory bulb [8,17]. Thus, during development NE may act to shape subcortical response patterns to biologically significant sensory input. This early, associative shaping of subcortical response patterns could, in turn, have a significant impact on the development of subsequent cortical responsiveness to specific stimuli.

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