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Developmental Changes in Olfactory Behavior and Limbic Circuitry

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Introduction

The olfactory system of infant rats is not an immature version of the adult system but is organized to ensure infants form the attachment to the caregiver necessary for survival. We study a sensitive period during the first nine days of life when rat pups have unique learning abilities that ensure pups quickly and reliably learn a preference for the maternal odor that underlies pup orientation to the mother and nursing. There are two unique aspects of this early learning. First, neonatal rat pups have an increased ability to acquire odor preferences. It produces corresponding metabolic and anatomical changes in the olfactory bulb that is supported by norepinephrine from the hyperfunctioning neonatal locus coeruleus (Sullivan, 2003; Moriceau and Sullivan, 2004a). The neonatal learned odor preference and the associated olfactory bulb changes are maintained into adulthood. Secondly, which is the focus of this paper, neonatal pups have a decreased ability to acquire learned odor aversions (Sullivan *et al.*, 1986, 2000; Camp and Rudy, 1988), presumably due to lack of amygdala participation in aversive conditioning (assessed by 2-DG; Sullivan *et al.*, 2000). Specifically, during the sensitive period, a novel odor paired with painful 0.5mA shock produces a subsequent odor preference in pups. [Neonatal (<PN9) pups could learn an odor aversion if that odor is paired with malaise (LiCl or strong shock >1.2mA; Spear and Rudy, 1991).] It should be noted that pups of this age feel pain (Haroutunian and Campbell, 1979; Barr, 1995) and do not show a preference for the shock, only the odor. We also find that the amygdala, a brain area necessary for adult odor–shock fear conditioning (Fanselow and Gale, 2003; Maren, 2003), does not participate in this shock-induced odor preference (Sullivan *et al.*, 2000). We suggest that the failure of the amygdala to participate in the neonatal sensitive period odor shock conditioning results in the odor preference. On postnatal day (PN) 10, when walking emerges (Bolles and Woods, 1964), naïve pups easily learn to avoid odors paired with shock and the amygdala participates in this conditioning (Sullivan *et al.*, 2000). We suggest that the functional emergence of the amygdala in odor shock conditioning at PN10 permits pups to learn a shock-induced odor aversion using a fear conditioning paradigm. Thus, during the sensitive period the neonate appears to use a unique neural circuitry for learning. Considering structures that support adult learning are probably not functional in the neonate (amygdala, hippocampus, cerebellum, frontal cortex) and connections between these areas are still maturing, it is not surprising that neonatal pups are using a different circuit (Verwer *et al.*, 1996; Nair and Gonzalez-Lima, 1999; Stanton, 2000; reviewed in Sullivan, 2003).

Altering the day at which the sensitive period ends

We have been able to modify the age that pups begin to learn an odor aversion from odor–shock conditioning and participation of the amygdala simply by modifying corticosterone (CORT) levels (Moriceau and Sullivan, 2004b). The neonate's sensitive-period is coincident with the 'stress hyporesponsive period' (SHP). The SHP is characterized by low basal CORT levels and the inability of most stressful stimuli to increase CORT, such as shock (Levine, 1962), unless pups experience prolonged separation from the mother (Stanton *et al.*, 1988). In our experiments, pups are away from the mother for ~1 h, which does not alter CORT levels and is within the range mothers would normally be absent from the nest. We assessed the effects of manipulating CORT levels by increasing and removing CORT, respectively, during (PN8) and after (PN12) the sensitive period. We found that CORT (3 mg/kg, i.p.) prior to conditioning enabled PN8 PAIRED pups (sensitive period) precociously to learn an odor aversion, prevented the acquisition of the olfactory bulb learning-induced neural changes and permitted the amygdala (basolateral/lateral complex) to participate in the learning. Moreover, PN12 CORT depleted (adrenalectomy, ADX) pups continued shock-induced odor preference learning, acquired the olfactory bulb neural changes and the amygdala did not participate in the learning. CORT replacement in ADX PN12 pups enabled pups to learn a shock-induced odor aversion, prevented the olfactory bulb learning-induced changes and the amygdala participated in odor–shock conditioning. We propose that it is the activation of the amygdala by CORT, either directly or indirectly, that permits the odor aversion learning and determines the end of the pups sensitive period for learning.

Fear to predator odor and amygdala participation also emerges at PN10

Under most circumstances, adult male rats eat pups and their odor is therefore classified as a predator odor to pups (Mennella and Moltz, 1988). Fear to predator odors, as measured by freezing to adult male odor, emerges at PN10 in rat pups (Takahashi and Rubin, 1993) and is coincident with the amygdala participating in the response to predator odor (Wiedenmayer and Barr, 2001). This suggests that the amygdala is important in pups' responsiveness to naturally fearful odors.

Ontogeny of fear to predator odors can be controlled by CORT

In striking similarity to learned fear, Takahashi and Rubin (1993) have shown that the emergence of fear to predator odor is strongly influenced by increases in CORT levels associated with the end of the stress hyporesponsive period. Specifically, they were able to precociously induce freezing in neonatal pups by injecting CORT. In strong support of this work, prolonged exposure to predator odor (30 min) in neonatal pups can elicit freezing (Gould and Cameron, 1997), although this duration of exposure increases endogenous CORT levels (Moriceau and Sullivan, 2004b). In our recent work, we replicated the behavioral work of Takahashi and Rubin (1993) and assessed whether amygdala activation was coincident with the behavioral manipulations induced by CORT manipulation. Our results indicate that we could prematurely activate the expression of freezing and the basolateral/lateral complex of the amygdala (cfos) in neonates through exogenous CORT. Additionally, we were also able to retard the developmental expression of fear to predator odor and

amygdala activation through adrenalectomy (ADX). These results replicate Wiedenmayer and Barr (2001) and Takahashi and Rubin (1993), but extend their results to suggest CORT is implicated in amygdala (basolateral complex) activation, either directly or indirectly, to permit the expression of infant fear. This is in contrast to the adult literature, which suggests predator odor activates the central nucleus, basolateral complex, and medial amygdala (Kemble *et al.*, 1990; Misslin, 2003; Li *et al.*, 2004). However, the central and the medial nuclei of the amygdala are not activated, at least by naturally fearful odors until after weaning (Wiedenmayer and Barr, 2001) suggesting the role of specific amygdala nuclei may change during development depending on the task.

These results indicate that CORT has the ability to alter the ontogenetic emergence of both learned and natural fear and suggesting the attachment system could be modified by environmental factors. First, the sensory stimulation and milk pups receive during maternal interactions maintains pups' low CORT levels (Kent *et al.*, 1997). Secondly, a stressed mother may raise pups' CORT level two ways: by decreasing her maternal care and transmitting her high CORT levels to pups through her milk (Yeh, 1984).

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