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Research Report

Developmental emergence of fear learning corresponds with changes in amygdala synaptic plasticity

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ABSTRACT

Mother–infant attachment is facilitated in altricial rodents through unique neural mechanisms that include impaired neonatal fear conditioning until the time that pups first begin to leave the nest (sensitive period). Here, we confirmed the developmental emergence of odor fear conditioning in neonatal rat pups, and examined synaptic plasticity of inputs to the basolateral amygdala *in vitro*. Coronal slices through the amygdala were obtained from sensitive (<10 days) and post-sensitive (>10, <19 days) period pups. Field potentials were recorded in the basolateral amygdala in response to stimulation of either the external capsule (neocortical inputs) or fibers from the cortical nucleus of the amygdala (olfactory inputs). The effects of tetanic stimulation were examined in each pathway. In both pathways, tetanic stimulation induce significant long-term synaptic plasticity in post-sensitive period pups, but no significant plasticity in sensitive period pups incapable of learning odor aversions. $GABA_A$ receptor blockade in post-sensitive period slices reverts synaptic plasticity to sensitive period characteristics. The results suggest that sensitive period deficits in fear conditioning may be related to impaired amygdala synaptic plasticity and the immature state of GABAergic inhibition and/or its modulation in the neonatal amygdala.

1. Introduction

Forming social attachments is fundamentally important for survival in many altricial species. This is highlighted by the presence of specialized learning circuits during 'sensitive periods' of social attachment formation where some forms of learning are facilitated, while others are attenuated. For example, altricial rat pups are dependent on maternal care for survival and exhibit facilitated sensitive period odor preference learning to the maternal odor, which is then used for approach to the caregiver and nipple attachment. Sensitive period pups also show attenuated aversion learning, presumably to prevent pups from learning to avoid the maternal odor. For rat pups, the temporal association of maternal odor with a variety of other maternally generated stimuli, such as grooming, warmth, or milk results in learned approach, nipple attachment and behavioral activation responses by the neonate on subsequent presentation of that odor (Galef and Sherry, 1973; Johanson and Hall, 1979; Johanson and Teicher, 1980; Brake, 1981; Pedersen et al., 1982; Alberts and May, 1984; Sullivan et al., 1986a,b; Wilson and Sullivan, 1994). Importantly, the range of interactions with the mother includes painful stimuli, such as biting and being stepped upon, yet neonates fail to learn an aversion to odors paired with such painful stimulation and instead learn to prefer the odor (Haroutunian and Campbell, 1979; Sullivan et al., 1986a,b, 2000; Camp and Rudy, 1988; Moriceau and Sullivan, 2004a; Roth and Sullivan, 2005). As pups mature and begin to explore the extra-nest environment around postnatal day (PN) 10 (Bolles

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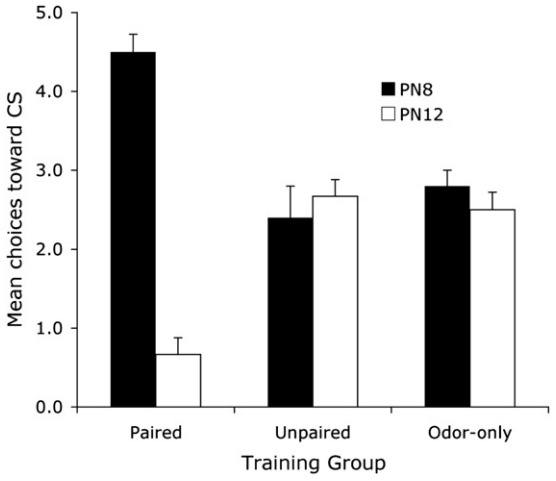

Fig. 1 – Mean (±sem) number of choices toward the conditioned stimulus (CS) odor during the Y-maze test (total of 5 trials) for PN8 and PN12 pups.

and Woods, 1964), more 'adult-like' fear and inhibitory learning emerges (Haroutunian and Campbell, 1979; Blozovski and Dumery, 1987; Camp and Rudy, 1988; Sullivan et al., 2000; Moriceau and Sullivan, 2004a; Roth and Sullivan, 2005).

Here we explore the neural correlates of attenuated aversion learning and the emergence of fear conditioning in sensitive period and post-sensitive period pups. In adult rats, the amygdala plays a critical role in fear conditioning (Sananes and Campbell, 1989; Rosenkranz and Grace, 2002; Davis et al., 2003; Fanselow and Gale, 2003; LeDoux, 2003; Debiec and Ledoux, 2004; Sevelinges et al., 2004; Schroeder and Shinnick-Gallagher, 2005). Association of a conditioned stimulus and, for example, footshock in juvenile or adult rats causes activation of the amygdala, and induces a modification of conditioned stimulus-evoked responses of amygdala neurons (Rosenkranz and Grace, 2002). Lesions of the amygdala prevent or retard fear learning and memory (LaBar and LeDoux, 1996; Setlow et al., 2000; Gale et al., 2004). Furthermore, synaptic plasticity of cortical and thalamic inputs to the basolateral nucleus of the amygdala appears necessary for normal fear conditioning (Blair et al., 2001; Maren, 2005), such that manipulations that impair or enhance such plasticity also impair or enhance acquisition of behaviorally expressed learned fear (e.g., (Campeau et al., 1992; Davis et al., 1994; Szinyei et al., 2007).

The failure of odor–pain association to induce learned fear in neonates may in part be due, therefore, to the immature state of amygdala circuitry during the early postnatal period. In the adult, neocortical and thalamic inputs to basolateral nucleus neurons demonstrate long-term synaptic plasticity following tetanic stimulation, and this plasticity may either be expressed as potentiation or depression depending on the conditions and presence or absence of $GABA_A$ receptor antagonists (Rogan et al., 1997; Heinbockel and Pape, 2000; Rammes et al., 2001; Kaschel et al., 2004). Furthermore, plasticity is expressed at both excitatory and inhibitory synapses (Rogan et al., 1997; Bauer and LeDoux, 2004; Szinyei et al., 2007). Both amygdala synaptic plasticity and learned fear are modulated by a number of factors, including neuromodulators (Rosenkranz and Grace, 2002; Azad et al., 2004), steroid hormones (Setlow et al., 2000) and level of GABAergic inhibition (Watanabe et al., 1995; Rammes et al., 2000). Importantly however, while GABA synthetic enzymes (e.g., GAD (Stork et al., 2000)) and receptor subunits (Zhang et al., 1991) are present at birth in the amygdala, they do not attain adult levels there until several weeks later, suggesting a potential late emergence for the mature expression of amygdala synaptic plasticity (Gilbert and Cain, 1981). In fact, odor–foot shock association that induces amygdala activation (e.g., c-fos labeling) and learned fear in PN12 rat pups, induces neither amygdala activation nor fear in PN10 pups (Sullivan et al., 2000; Moriceau and Sullivan, 2004b, Roth and Sullivan, 2005). It should be noted that pain threshold to footshock is very similar across this age range of pups (Emerich et al., 1985; Barr, 1995; Sullivan et al., 2000; Fitzgerald and Beggs, 2001).

The present report was an examination of synaptic plasticity in two afferent pathways to the basolateral nucleus of the amygdala *in vitro*, before and after the age at which fear conditioning emerges in the rat. Given that neonatal maternal recognition is primarily olfactory mediated, we examined the putative input from the cortical nucleus of the amygdala to the basolateral nucleus. The cortical nucleus of the amygdala receives direct input from the olfactory bulb (Shipley and Ennis, 1996), and olfactory evoked responses within the amygdala are known to be modified by fear conditioning (Rosenkranz and Grace, 2002). To allow our results to be compared to the extant literature on amygdala synaptic plasticity, we also examined the neocortical input to basolateral nucleus. We hypothesized that the during the sensitive period for learned odor-guided attachment to the mother, plasticity within circuits mediating fear conditioning

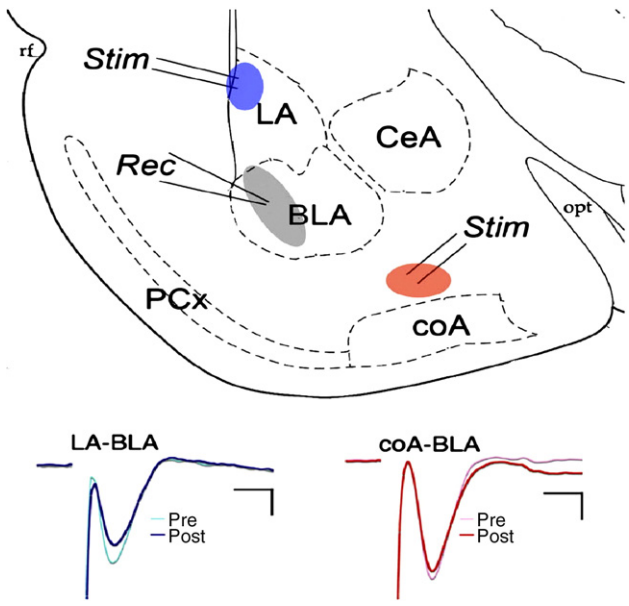

Fig. 2 – (Top) Schematic representation of coronal amygdala slice showing approximate stimulation (Stim) and recording (Rec) electrode placements. Only one pathway was tested in each slice. (Bottom) Examples of evoked potentials before and after tetanic stimulation of the two pathways. LA = lateral nucleus, BLA = basolateral nucleus, ceA = central nucleus, coA = cortical nucleus, PCx = piriform cortex. Calibration is 2 ms and 2 mV for LA–BLA pathway and 2 ms and 5 mV for coA–BLA pathway.