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Neural Correlates of the Mother-to-Infant Social Transmission of Fear

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Although clinical and basic studies show that parental trauma, fear, and anxiety may be transmitted to offspring, the neurobiology of this transmission is still not well understood. We recently demonstrated in an animal model that infant rats acquire threat responses to a distinct cue when a mother expresses fear to this cue in their presence. This ability to acquire maternal fear through social learning is present at birth and, as we previously reported, depends on the pup's amygdala. However, the remaining neural mechanisms underlying social fear learning (SFL) in infancy remain elusive. Here, by using [^{14}C]2-deoxyglucose autoradiography, we show that the mother-to-infant transmission of fear in preweaning rats is associated with a significant increase of activity in the subregions of the lateral septum, nucleus accumbens, bed nucleus of stria terminalis, retrosplenial cortex, paraventricular nucleus of the thalamus, mediodorsal and intralaminar thalamic nuclei, medial and the lateral preoptic nuclei of the hypothalamus, and the lateral periaqueductal gray. In contrast to studies of adult SFL demonstrating the role of the anterior cingulate cortex and possibly the insular cortex or research of infant classical fear conditioning showing the role of the posterior piriform cortex, no changes of activation in these areas were observed. Our results indicate that the pup's exposure to maternal fear activates a number of areas involved in processing threat, stress, or pain. This pattern of activation suggests a unique set of neural mechanisms underlying SFL in the developing brain. © 2016 Wiley Periodicals, Inc.

Key words: social fear learning; parental anxiety; intergenerational transmission

Clinical studies indicate that emotional trauma and fear may be transmitted across generations (Yehuda et al., 2005; Murray et al., 2008; Roberts et al., 2012; Eley et al., 2015). Parental history of posttraumatic stress disorder (PTSD) increases the child's risk of developing PTSD (Roberts et al., 2012), whereas parental diagnosis of a specific phobia increases the occurrence of phobia in the offspring (Murray et al., 2008; Lara et al., 2012). One of the features shared by PTSD and specific phobias is the presence of distinct, trauma-related (as in PTSD) or phobia-related cues that trigger threat responses. Intergenerational transmission of stress, fear, and anxiety may be explained by a wide range of mechanisms, including genetic and epigenetic mechanisms, environmental factors, or gene environment interactions (Bowers and Yehuda, 2016). Recent studies have suggested that parental modeling, such as display of anxious behaviors in the child's presence and children's ability to learn these behaviors from parents, are major factors in the intergenerational transmission of fear and anxiety (de Rosnay et al., 2006; Aktar et al., 2013; Eley et al., 2015). Although existing research sheds some light on the biological basis of intergenerational transfer of nonspecific stress and anxiety (Klengel et al., 2016), little is known about the mechanisms controlling intergenerational social transmission of specific threat responses. To study neural mechanisms of the intergenerational social transmission of fear, we used a social fear learning (SFL) paradigm.

SFL, also referred to as *vicarious fear learning*, is a behavioral tool well suited for investigating mechanisms of intergenerational transmission of threat responses triggered by distinct cues, such as in PTSD and phobias (Olsson and Phelps, 2007). In SFL, an animal acquires fear responses to a neutral cue through exposure to a conspecific expressing fear to this cue. SFL is thus a form of associative fear learning in which a classical noxious unconditioned stimulus (Debiec et al., 2010) is replaced with an expression of fear by a conspecific animal.

SIGNIFICANCE:

Children learn adaptive and maladaptive anxious behaviors from their parents. However, neural mechanisms of this parent-to-child fear transmission remain elusive. Here, using a rodent model of mother-to-infant social transmission of fear and autoradiography imaging, we identify a number of threat and stress processing areas in the infant brain activated by an exposure to a frightened mother. Understanding the neurobiology of the parent-to-child fear transmission will contribute to the development of novel methods aimed at prevention and treatment of the intergenerational transmission of maladaptive fear, stress, and trauma.

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Although SFL has been shown by a number of studies across animal species, including humans (Mineka and Cook, 1993; Knapska et al., 2006; Jones et al., 2014; Molapour et al., 2015), the neural mechanisms of SFL remain to be elucidated. Research suggests that SFL and classical fear conditioning share similar neural mechanisms; e.g., studies in adult humans and rodents show that both SFL and classical fear conditioning engage the amygdala, hippocampus, affective pain processing systems (including the midline and intralaminar thalamic nuclei [MITN]), anterior cingulate cortex (ACC), and midbrain structures, such as the periaqueductal gray (PAG) subdivisons, which are directly involved in controlling threat responses (Olsson et al., 2007; Jeon et al., 2010; Molapour et al., 2015). Despite the growing number of studies of SFL in adults, little is known about the mechanisms of social transmission of fear in infancy.

We recently reported that infant rats can acquire fear responses from their mother through SFL (Debiec and Sullivan, 2014). This mother-to-infant fear transmission was mediated by alarm chemosignaling and depended on the pup's amygdala (Debiec and Sullivan, 2014). Specifically, we found that the acquisition of SFL in preweaning rats was accompanied by increased activity in the lateral, basal, central, medial, and cortical nuclei of the amygdala and that a pharmacological inactivation of the lateral nucleus of the amygdala prevented the acquisition of SFL (Debiec and Sullivan, 2014). Although in our previous study we observed that SFL occurs at the end of the first week of life (Debiec and Sullivan, 2014), it has not been determined whether rats are born with the ability to acquire specific threat responses through social learning. We hypothesize that SFL mechanisms in pups are functional at birth. Also, the involvement of neural sites other than the amygdala in early SFL remains to be characterized. Previous fear conditioning studies in rodents have shown the key role of the olfactory processing areas, such as the posterior piriform cortex (PPir; e.g., Raineki et al., 2009); however, the role of the PPir in SFL has not been determined. Also, it is unlikely that the hippocampus or the neocortical structures, such as the ACC, that contribute to the formation of fear memories in adults (Toyoda et al., 2011) are involved in the infant SFL because neither is fully functional until after weaning (Rudy, 1993; Raineki et al., 2010). We hypothesize that SFL in infancy occurs without the involvement of the hippocampus or neocortical structures. This study addresses the two questions raised above, whether SFL is present at birth and which extra-amygdala structures are potentially involved in SFL in infancy. We show that SFL occurs on the day of birth, suggesting that pups are born with the ability to acquire SFL. To assess neural sites activated during infant SFL, we use the [^{14}C]2-deoxyglucose (2-DG) autoradiograms obtained during the previously described experiment (Debiec and Sullivan, 2014). 2-DG autoradiography is a method that allows the assessment of activity by measuring the activity-dependent uptake of the radiographic marker. 2-DG has been especially useful in the whole-brain analysis of neural activity in rodent pups (Sullivan et al., 2000; Raineki et al., 2009; Marquez et al., 2013). Using 2-DG autoradiography, we identify several neural structures activated in the pup's brain during the mother-to-infant transmission of fear through social learning.

MATERIALS AND METHODS

Animals

Animals used in this study were male and female Long-Evans rats born and bred in our colony (originally acquired from Harlan Laboratories, Indianapolis, IN). Mothers or "substitute" mothers were multiparous females. Animals were housed in polypropylene cages (34 × 29 × 17 cm) with an abundant amount of wood shavings for nest building. Rats were kept in a 20°C ± 1°C environment under a 12-hr light/dark cycle. Food and water were provided ad libitum. To prevent litter effects on statistical analysis, no more than one female and one male from a litter were used in each experimental condition. All animal care and experimental procedures were conducted in accordance with the National Institutes of Health guidelines for the care and use of experimental animals and were approved by the University of Michigan institutional animal care and use committee.

Fear Conditioning of Mothers

Maternal olfactory fear conditioning took place in a conditioning chamber constructed of aluminum and Plexiglas walls (Coulbourn Instruments, Allentown, PA) with a metal stainless-steel rod flooring that was attached to a shock generator (model H13-15; Coulbourn Instruments). The chamber was enclosed within an acoustic-isolation ventilated cubicle (Model H10-24A; Coulbourn Instruments). Stimuli presentations were controlled by Freezeframe, and behavior was recorded. Conditioned stimulus (CS) odor (pure peppermint; McCormick, Hunt Valley, MD) was delivered by a flow dilution olfactometer controlled by a ChronTrol (ChronTrol Corporation, San Diego, CA) at a 2-liter/min flow rate and at a concentration of 1:10 peppermint vapor. The unconditioned stimulus (US) was a 0.5-sec (0.6-mA) electric foot shock delivered through a grid floor. This study used a "substitute mother" procedure. Substitute mothers were dams matched with pups' mothers for postpartum period and diet. Previous studies have shown that mothers accept all pups and that pups fail to distinguish between their mother and a substitute mother matched for the same postpartum period and the same diet (Moriceau and Sullivan, 2006; Debiec and Sullivan, 2014). Substitute mothers were conditioned during the lactation period. Animals were placed in the conditioning chamber and given a 10-min acclimation period. Conditioning consisted of six conditioning trials with a 30-sec CS odor that coterminated with a US shock (the intertrial interval [ITI] was randomly generated and was 4 min on average). Unfrightened mothers were dams that did not receive fear conditioning but instead were exposed to the equivalent number of CS odor presentations (Fig. 1) or the equivalent number of unpaired CSs and USs (Fig. 2) and did not express fear during a subsequent re-exposure to the CS in the pups' presence (Debiec and Sullivan, 2014; Table I).

Mother-to-Infant Social Transmission of Fear

Exposure of pups and mothers to the CS took place in the home cage on postnatal day (PN) 0 (Fig. 1) or PN13 and PN14

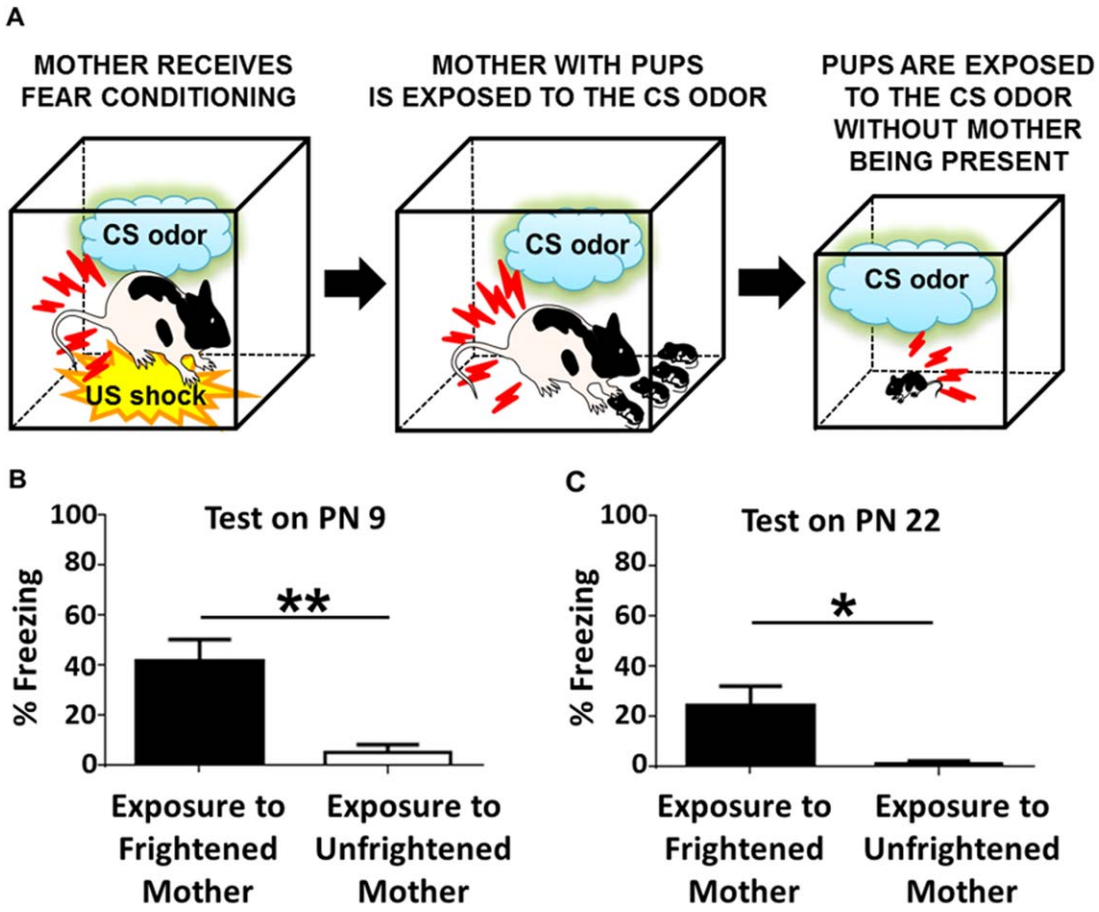

Fig. 1. Mother-to-infant social transfer of fear on the day of birth. **A:** Diagram illustrating behavioral procedures used in the mother-to-infant transmission of fear experiment. PN0 pups were exposed to a mother previously fear conditioned and re-exposed to the CS in pups' presence (exposure to frightened mother). Another group of PN0 pups was exposed to a mother with prior exposure to the CS alone (without the US) and then re-exposed to this CS while with the pups (exposure to unfrightened mother). One group of animals from each experimental group was re-exposed to the odor that served as the CS in maternal fear conditioning on PN9, whereas the other group was re-exposed to the same CS on PN22. **B:** PN0 pups from the exposure-to-frightened-mother group show significantly higher levels of freezing behavior on CS presentation than the exposure-to-unfrightened-mother pups on PN9 (n = 6 for each group). **C:** PN0 pups from the exposure-to-frightened-mother group show significantly higher levels of freezing behavior on CS presentation than the exposure-to-unfrightened-mother pups on PN22 (n = 9 for each group). All bars indicate mean ± SEM. $^{\star}P < 0.05$, $^{\star\star}P < 0.01$.

(Fig. 2). For the duration of the experiment, a biological mother was replaced in the home cage with a substitute mother. The biological mother then remained in the colony in the new home cage, whereas pups in the cage with the substitute mother were moved to the experimental room. Animals were given a 1-hr adaptation period, after which all mothers were settled and were nursing the litter. The four 30-sec CS odor presentations were delivered by an olfactometer (concentration of 1:10 peppermint vapor, 2-liter/min flow rate) to mother and pups with a 10-min ITI. Immediately afterward, pups were removed from the cage with a substitute mother, returned to the colony room, and placed in the home cage with their mother.

Fear Memory Test

On PN9 or PN22, pups were individually placed in a 600-ml clear plastic beaker and given a 2-min adaptation period. Subsequently, pups received three presentations of a peppermint CS odor (30 sec) delivered by an olfactometer (concentration of 1:10 peppermint vapor, 2-liter/min flow rate) with a 4-min ITI. After the completion of the experiment, pups were returned to the dam. The freezing behavior was videotaped and scored by a blind observer. An average of the three scores for each CS for each pup was used for statistical analysis. Behavior was displayed as percentage of freezing during the CS exposure.

Assessment of Neural Correlates With Autoradiography

For the assessment of neural activity, we used autoradiograms obtained from a previously reported experiment (Debiec and Sullivan, 2014). PN13 and PN14 pups were injected with [^{14}C]2-deoxyglucose (2-DG; 20 μCi/100 g, s.c.) 5 min before the mother-to-infant social transmission of fear procedure (see above). Immediately after the social transmission of fear procedure, pups

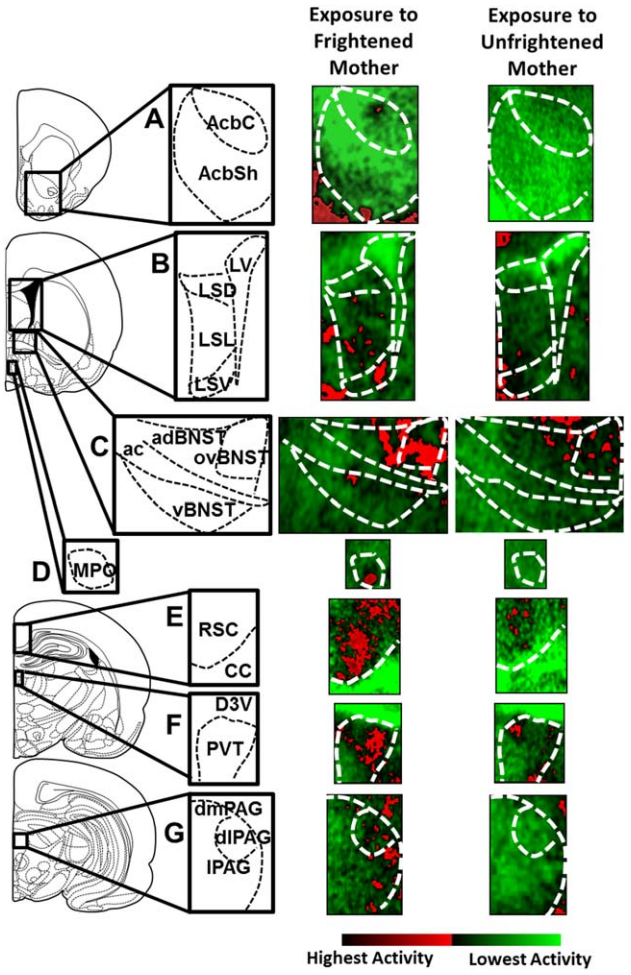

Fig. 2. Autoradiography of mother-to-infant transfer of fear. [^{14}C]2-deoxyglucose (2-DG) autoradiography images were taken from PN13 and PN14 pups either exposed to a mother previously fear conditioned and re-exposed to the CS in pups' presence (exposure to frightened mother) or staying with mother not expressing fear while with the pups (exposure to unfrightened mother). Left lane shows the locations of the selected examined brain areas. Middle and right lanes show representative 2-DG autoradiographic images of the selected brain areas. **A:** AcbC and AcbSh. **B:** LSD, LSL, LSV, and lateral ventricle (LV). **C:** adBNST, vBNST, and ovBNST, and anterior commissure (ac). **D:** MPO. **E:** RSC and CC. **F:** PVT and dorsal third ventricle (D3V). **G:** dmPAG, dlPAG, and lPAG. Color gradation shows neural activity (pseudocolor images displayed in ImageJ Red/Green Lookup Table) from light green (no activity) to dark red (highest activity).

were euthanized, and their brains were quickly removed, frozen in 2-methylbutane (−45°C), and stored in a −70°C freezer. Subsequently, brains were sectioned (20 μm) in a −20°C cryostat, and every other section was placed on a coverslip and exposed for 5 days along with standards (^{14}C standards 10 × 0.02 mCi; American Radiolabeled Chemicals, St. Louis, MO) to X-ray film. 2-DG uptake was assessed in ImageJ (NIH). The uptake of 2-DG was expressed relative to uptake in the corpus callosum (CC). 2-DG uptake in the CC did not vary across conditions. 2-DG uptake was measured by an observer blind to the experimental condition.

TABLE I. Maternal Behavior During Fear-Inducing CS Odor Exposure (Observation Periods in Which Behaviors Occurred [%])*

Maternal behaviors	Frightened mother	Unfrightened mother
Fearful/defensive	53	0
Rough/abusive	0	0
Nurturing	0	52
Neutral	47	48
Mother and pup in the nest	28	36

*Maternal behavior in the presence of the pups during re-exposure to the conditioned (frightened mother) or neutral (unfrightened mother) cue. Fearful/defensive behaviors include freezing, startle, escaping, covering the source of odor, covering pups with bedding; rough/abusive behaviors include stepping/jumping on pup, throwing/dropping/dragging/pushing away/rough handling of pup; nurturing behaviors include nursing, grooming/licking/retrieving pup; neutral behaviors include sleeping, resting, self-grooming, eating, and drinking (see Materials and Methods).

For image analysis, a default gray scale was used. For a better display, the images shown in Figure 2 were converted to pseudocolor in the ImageJ Green/Red Lookup Table (see Fig. 2 legend).

Statistical Analysis

Data were analyzed in Prism 5 (GraphPad Software, La Jolla, CA) by *t*-test. $P < 0.05$ was considered significant.

RESULTS

Mother-to-Infant Social Transfer of Fear Occurs on the Day of Birth

PN0 pups exposed to a mother expressing fear to the previously trained CS odor (exposure to frightened mother, n = 6) as well as pups exposed to a mother that was not frightened (exposure to unfrightened mother, n = 6) showed negligible levels of freezing behavior prior to the presentation of the same CS odor on PN9 (percentage of freezing behavior during a 1-min time interval prior to the presentation of the first CS for the exposure-to-frightened-mother group was 3.89 ± 2.36; for the exposure-to-unfrightened-mother group, it was 2.59 ± 0.88; $P > 0.05$). However, during subsequent presentations of the CS odor, exposure-to-frightened-mother pups displayed significantly higher levels of freezing behavior compared with the exposure-to-unfrightened-mother controls (Student's *t*-test, $P < 0.01$; Fig. 1B, see Table I for maternal behavior during the CS odor re-exposure). Testing on PN22 of another group of pups that had been exposed on PN0 to a mother frightened by a presentation of the previously conditioned CS odor (exposure to frightened mother; n = 9) compared with pups exposed to a mother that had not been frightened (exposure to unfrightened mother; n = 9) revealed that pups with a history of exposure to a frightened mother on PN0 expressed significantly higher levels of freezing behavior when presented with the same CS odor (Student's *t*-test, $P < 0.05$; Fig. 1C).

Brain Autoradiography of Mother-to-Infant Social Transfer of Fear

Analysis of the 2-DG autoradiogram results by *t*-test showed that PN13 and PN14 pups exposed to a frightened mother (n = 7) compared with pups exposed to an unfrightened mother (n = 6) displayed a significant increase in neural activity in several sites, including the nucleus accumbens (Acb) core (AcbC; $P < 0.05$), Acb shell (AcbSh; $P < 0.05$), lateral septum (LS) portion dorsal (LSD; $P < 0.001$), LS portion lateral (LSL; $P < 0.001$), LS portion ventral (LSV; $P < 0.05$), anterodorsal bed nucleus of stria terminalis (adBNST; $P < 0.05$) ventral BNST (vBNST; $P < 0.05$), oval nucleus BNST (ovBNST; $P < 0.05$), retrosplenial cortex (RSC; $P < 0.01$), hypothalamic nucleus medial preoptic (MPO; $P < 0.01$), hypothalamic nucleus lateral preoptic (LPO; $P < 0.01$), hypothalamic nucleus paraventricular (PVN; $P < 0.05$), thalamic nucleus paraventricular (PVT; $P < 0.05$), midline and intralaminar thalamic nuclei (MITN; $P < 0.05$), and lateral portion of the PAG (lPAG; $P < 0.05$). No significant differences among the experimental groups ($P > 0.05$) were observed in the prelimbic cortex (PL), ACC, insular cortex (IC), anterior piriform cortex (APir), PPir, dorsal hippocampus, ventral hippocampus, primary auditory cortex (Au1), ventral posterolateral nucleus of the thalamus (VPN), ventral posteromedial nucleus of the thalamus (VPM), medial geniculate nucleus of the thalamus (MG), ventrolateral PAG (vlPAG), or dorsomedial PAG (dmPAG). The complete list of examined structures with corresponding results is displayed in Table II. Figure 2 includes representative autoradiography images from selected areas showing significant increase of neural activity during the exposure to a frightened mother.

TABLE II. Structures Examined★

Region	Paired-CS	CS only
Forebrain and Cortex		
Prelimbic cortex (PL)	1.959 ± 0.270	1.764 ± 0.191
Anterior cingulate cortex (ACC)	2.524 ± 0.224	1.987 ± 0.081
Insular cortex	1.829 ± 0.058	1.701 ± 0.033
Anterior piriform (APir)	2.791 ± 0.193	2.886 ± 0.159
Posterior piriform (PPir)	2.311 ± 0.207	2.123 ± 0.151
Nuc. Accumbens core (AcbC)	2.268 ± 0.175†	1.791 ± 0.029
Nuc. Accumbens shell (AcbSh)	2.276 ± 0.166†	1.793 ± 0.046
Lateral septum dorsal (LSD)	1.708 ± 0.032§	1.312 ± 0.061
Lateral septum lateral (LSL)	2.311 ± 0.119§	1.610 ± 0.093
Lateral septum ventral (LSV)	1.723 ± 0.087†	1.380 ± 0.068
Anterodorsal BNST (adBNST)	3.024 ± 0.405†	1.892 ± 0.149
Oval nucleus BNST (ovBNST)	3.929 ± 0.565†	1.879 ± 0.128
Ventral BNST (vBNST)	1.690 ± 0.072†	1.479 ± 0.060
Retrosplenial cortex (RSC)	2.498 ± 0.132‡	2.009 ± 0.060
Dorsal hippocampus	1.825 ± 0.094	1.712 ± 0.038
Ventral hippocampus	3.237 ± 0.384	2.277 ± 0.131
Primary aud. Cortex (Aul)	2.800 ± 0.184	2.279 ± 0.151
Hypothalmus		
Medial preoptic nucleus (mPO)	2.995 ± 0.262‡	1.650 ± 0.150
Lateral preoptic nucleus (lPO)	2.305 ± 0.121‡	1.790 ± 0.098
Paraventricular nucleus (PVN)	2.469 ± 0.217†	1.690 ± 0.061
Thalamus		
Paraventricular nucleus thalamus (PVT)	3.773 ± 0.330†	2.691 ± 0.223
Mediodorsal and intralaminar thalamic nuclei (MITN)	2.770 ± 0.255†	1.984 ± 0.087
Ventral posterolateral nucleus (VPN), Ventral posteromedial nucleus (VPM)	3.213 ± 0.177	3.068 ± 0.230
Medial geniculate nucleus (MG)	4.215 ± 0.469	4.280 ± 0.445
Periaqueductal gray		
Dorsomedial PAG (dmPAG)	2.044 ± 0.187	1.807 ± 0.088
Lateral PAG (lPAG)	2.610 ± 0.185†	2.017 ± 0.126
Ventrolateral PAG(vlPAG)	2.142 ± 0.108	1.949 ± 0.214

★Group means were tested using the t-test with Welch's correction for unequal variance. Data are presented as means ± SEM.
†$P < 0.05$ compared with exposure to unfrightened mother.
‡$P < 0.01$ compared with exposure to unfrightened mother.
§$P < 0.001$ compared with exposure to unfrightened mother.

DISCUSSION

The present study shows that PN0 pups may acquire maternal CS-specific threat responses through SFL (Fig. 1). Maternal expression of fear to the CS in the newborns' presence (Table I) was sufficient to produce CS-controlled freezing responses that persisted at least until after weaning on PN22 (Fig. 1). These results are consistent with our previous findings showing that, during the first postnatal week, pups may acquire from their mother long-lasting CS-specific threat responses (Debiec and Sullivan, 2014). The current study extends our earlier findings and suggests that pups are born with an ability to learn about threats through social learning. This is in stark contrast to studies on classical fear conditioning demonstrating that, until PN10, electric shock-reinforced aversive learning is physiologically attenuated (Sullivan et al., 2000). The present study, together with our previous work (Debiec and Sullivan, 2014), indicates a unique role of maternally transmitted emotional learning that allows infants to develop defense responses against possible environmental threats when they are still with the mother in the nest and before they are able to learn through their own harmful experiences, such as those occurring in fear conditioning. However, given the small sample, the interpretation of these data is limited, and future research with a larger sample is required to substantiate our current findings.

Most of what we know about the neural mechanisms of SFL comes from studies of adult organisms. We previously showed that transfer of maternal fear responses in infancy through SFL is mediated by alarm chemosignaling and depends on the pup's amygdala (Debiec and Sullivan, 2014). A surgical disruption of alarm pheromone processing pathways or pharmacological inactivation of the amygdala in preweaning pups prevented social transmission of fear with alarm pheromone (an odor of the frightened mother). However, both alarm chemosignaling and amygdala-dependent fear learning, including SFL, engage several neural sites and systems (Kiyokawa et al., 2005; Herry and Johansen, 2014). Our experiment with 2-DG autoradiography allowed identification of several

extra-amygdala sites in the pup's brain that were activated by the maternal expression of fear (Fig. 2, Table II).

Neural Activity in the Cortex and the Hippocampus

Previous research has identified several neural sites in the forebrain subserving fear learning. A recent study demonstrated that pharmacological inactivation of the ACC in adult mice prevented the acquisition of SFL, suggesting that the ACC plays a critical role in SFL (Jeon et al., 2010). In accordance with this study, a functional imaging study in adult human subjects showed activation in the ACC during an SFL task (Olsson et al., 2007). The same article reported increased activity in the IC during SFL. Indeed, evidence from human and rodent research suggests that the ACC and the IC play roles in assessing potential threats (Fiddick, 2010). Another neocortical structure known to be involved in the acquisition and expression of classical fear conditioning in adults is the PL (Sotres-Bayon et al., 2012; Sharpe and Killcross, 2015). However, in our study, we did not observe any changes of activity in the pup's ACC, IC, or PL during the mother-to-infant social transmission of fear (Table II). It is likely that SFL in preweaning pups occurs independently of the neocortical structures ACC, IC, or PL because mature cerebral metabolism emerges in rats at about PN20, and maturation of the neocortex is completed at about PN90 (Watson et al., 2006). Similarly, an acquisition of maternal fear responses was not accompanied by an increased 2-DG uptake in the pup's Au1 or hippocampus (Table II). Although the hippocampus and the auditory cortex play important roles in adult fear conditioning (context fear conditioning and auditory fear conditioning, respectively), the auditory modality is not functional until postnatal week 3, and the hippocampus-dependent aversive learning emerges about or after weaning at PN21 (Rudy, 1993; Raineki et al., 2010). However, we did observe increased 2-DG uptake in the RSC (Fig. 2, Table II), which was shown to be involved in context fear conditioning in adults (Robinson et al., 2012; Kwapis et al., 2015); however, its roles in other forms of fear learning are not so well understood (Fig. 2E, Table II).

Research on olfactory learning in preweaning pups indicates the involvement of the piriform cortex (part of the olfactory cortex), with the APir being implicated in odor preference learning (Roth and Sullivan, 2005; Raineki et al., 2009; Morrison et al., 2013) and the PPir in odor aversive learning (Raineki et al., 2009). Our published study indicates that a Grueneberg ganglion olfactory subsystem that processes alarm chemosignaling is involved in pups in the mother-to-infant social transmission of fear (Debiec and Sullivan, 2014). However, in the current study, an exposure to a frightened mother did not produce any changes in the activity of the APir or the PPir (Table II). Although earlier research has suggested that classical fear conditioning and SFL share similar neural mechanisms (Olsson and Phelps, 2007), the observed lack of activation in the PPir suggests that olfaction-mediated infant SFL is controlled by neural systems, at least in part, distinct from the classical olfactory aversive learning mechanisms. The lack of activation in the hippocampus, PL, IC, or ACC, which are all involved in aversive learning in adults, also indicates the distinct neural mechanisms supporting SFL in infancy.

Neural Activity in the Lateral Septum

We found that exposure to a frightened mother is accompanied by increased 2-DG uptake in the LSD, LSL, and LSV (Fig. 2B, Table II). LS nuclei are reciprocally connected to the main olfactory bulb, cingulate cortex, amygdala, hippocampus, hypothalamus, thalamus, and midbrain areas and have been shown by previous research to be involved in affect regulation (Sheehan et al., 2004). Specifically, studies in rodents show that the LS plays an important role in the social modulation of fear (Guzman et al., 2014; Zoicas et al., 2014). Our findings show the activation of the LS during SFL; however, the possible role of the LS in the mother-to-infant transmission of fear awaits determination by further research.

Neural Activity in the Acb and the BNST

We previously demonstrated that early SFL is associated with activation of several amygdala nuclei (Debiec and Sullivan, 2014). Here, we assessed neural activation in the extended amygdala areas, including the Acb shell and stria terminalis subregions. It is well known that the BNST plays an important role in stress, fear, and anxiety (Daniel and Rainnie, 2015). We found that mother-to-infant social transmission of fear was associated with increased 2-DG uptake in the BNST subregions adBNST, ovBNST, and vBNST (Fig. 2C, Table II). This is consistent with a recent study showing that an exposure to alarm pheromone induces expression of the early expression gene *c-Fos* in the BNST in adult rats (Kiyokawa et al., 2005). Our data show that early SFL is accompanied by increased activity in the AcbC and the AcbSh (Fig. 2A, Table II). Although the role of the Acb in fear learning is not very well understood, recent studies in adult rats have shown that inactivation of the Acb disrupts the acquisition of fear-potentiated startle (Schwienbacher et al., 2004) and that active avoidance of threat is associated with increased neural activity in the Acb, especially in the AcbSh (Ramirez et al., 2015). We found that infant SFL is associated with increased activity in the BNST and the Acb subregions. Recent studies have highlighted the role of the BNST and the Acb systems in controlling fear and anxiety states (Daniel and Rainnie, 2015; Ramirez et al., 2015). Our findings suggest that the BNST and the Acb systems may be involved in social learning in infancy.

Neural Activity in the Hypothalamus

It has been well established that hypothalamic structures are involved in processing fear, stress, and pain (Gross and Canteras, 2012). We found that early SFL increased 2-DG uptake in the hypothalamic nuclei MPO,

LPO, and PVN (Fig. 2, Table II). Although, the role of the hypothalamus in SFL is unknown, several hypothalamic structures are critical parts of a defensive circuitry and control social or predator fear (Gross and Canteras, 2012). A recent study showed that a social defeat stress task in adult rats increased c-Fos immunoreactivity in the MPO, LPO, and PVN (Lkhagvasuren et al., 2014). In addition, increased c-Fos expression was observed in the LPO and the PVN during predator exposure in adult mice (Martinez et al., 2008). Increased activity in the MPO, LPO, and PVN during an exposure to a frightened mother suggests that these hypothalamic nuclei may be a part of neural networks supporting SFL in infancy.

Neural Activity in the Thalamus

Previous research has shown that the MITN, which are a thalamic part of the affective pain processing system, are involved in SFL in adult mice (Jeon et al., 2009). Consistent with this finding is our observation that mother-to-infant social transmission of fear was accompanied by increased 2-DG uptake in the MITN (Table II). However, we did not find any augmentation of neural activity during SFL in other hypothalamic pain systems, VPN, or VPM (Gauriau and Bernard, 2002). Our 2-DG analysis showed that infant SFL was accompanied by PVT activation (Fig. 2F, Table II). The PVT is vastly connected with the ACC, BNST, and amygdala, and there is increasing evidence from adult studies showing the role of the PVT in regulating fear and anxiety (Kirouac, 2015); however, the role of the PVT in infant fear has not yet been determined. Although the auditory thalamus plays a key role in adult auditory fear conditioning (Apergis-Schoute et al., 2005), consistent with previous studies showing that auditory modality is not functional until postnatal week 3 (Rudy, 1993), we did not observe any activation in the auditory thalamus (MG) during infant SFL (Table II). In agreement with adult SFL findings, our data suggest that the thalamus is part of the social transmission of fear circuitry in infancy.

Neural Activity in the PAG

It is well known that the PAG is a part of pain and fear processing systems, and, through its connections with the amygdala, it is an important part of classical fear conditioning circuitry (Herry and Johansen, 2014). However, the role of the PAG in SFL has not yet been studied. We found that mother-to-infant social transmission of fear was associated with increased neural activity in the lPAG but not in the dmPAG or in the vlPAG (Fig. 2G, Table II), although all these parts of the PAG are involved in classical fear conditioning in adults. Our findings suggest that the PAG nociceptive and fear processing circuits play a role in infant SFL, just as they do in classical fear conditioning in adults. The fact that SFL, compared with fear conditioning, is associated with the more selective activation of the PAG (activation of the lPAG but not activation of the dmPAG or the vlPAG) suggests that the plausible involvement of the PAG in controlling socially transmitted fear is distinct from its role in fear conditioning.

CONCLUSIONS

Children are very vulnerable to parental stress, fear, and trauma. The impact of maternal stress on the child's behavior and emotional regulation can been observed as early as in infancy (Bosquet Enlow et al., 2011). The effects of parental stress and fear on the offspring are mediated by hereditary, genetic or nongenetic, as well as nonhereditary mechanisms, such as social learning. Although the ability to acquire SFL is present across the life span, the young child's dependence on the caregiver and distinctive sensitivity to the caregiver's emotions determine a special role of SFL in infancy and early childhood. This unique character of SFL in childhood allows the offspring to learn early from parents about possible threats in the surrounding world (Debiec and Sullivan, 2014). Our data indicate the unique nature of SFL, which is present at birth, in contrast to classical fear conditioning, which emerges in pups during the second week of life. Infant rats can thus learn from the mother about environmental threats before their sensory and motor development allows them to acquire aversive learning through their own interactions with the environment outside their nest. However, the same ability to develop adaptive threat responses in conjunction with exposures to parental maladaptive fear may mediate the parent-to-child transmission of disordered fear and anxiety. Indeed, a recently published study of children of twins has shown that children may acquire maladaptive anxiety from their parents through SFL, independently of hereditary mechanisms (Eley et al., 2015). Characterization of the mechanisms of SFL in infancy and early childhood is thus critical for the understanding of the social transmission of adaptive and maladaptive fears.

We have previously demonstrated that the amygdala plays a critical role in social transmission of fear in infancy (Debiec and Sullivan, 2014). Here we show that several neural sites that are involved in processing fear, stress, and pain and that are, in major part, either directly or indirectly connected with the amygdala are activated in the pup's brain during the acquisition of maternal threat responses. The current study provides candidate regions for further research of neural mechanisms of SFL in humans. Identification of the behavioral, neural, and molecular mechanisms of SFL in infancy and early childhood may help in developing early preventive and treatment interventions aimed at disrupting the transfer of parental maladaptive fears to children.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

ROLE OF AUTHORS

D-JC and JD conceived and designed the study. D-JC acquired the data and conducted the statistical analysis. D-JC and JD analyzed and interpreted the data. D-JC prepared the figures. JD wrote the article. JD obtained funding and supervised the study.

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