Intergenerational transmission of emotional trauma through amygdala-dependent mother-to-infant transfer of specific fear

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Emotional trauma is transmitted across generations. For example, children witnessing their parent expressing fear to specific sounds or images begin to express fear to those cues. Within normal range, this is adaptive, although pathological fear, such as occurs in posttraumatic stress disorder or specific phobias, is also socially transmitted to children and is thus of clinical concern. Here, using a rodent model, we report a mother-to-infant transfer of fear to a novel peppermint odor, which is dependent on the mother expressing fear to that smell in pups' presence. Examination of pups' neural activity using c-Fos early gene expression and ^{14}C 2-deoxyglucose autoradiography during mother-to-infant fear transmission revealed lateral and basal amygdala nuclei activity, with a causal role highlighted by pharmacological inactivation of pups' amygdala preventing the fear transmission. Maternal presence was not needed for fear transmission, because an elevation of pups' corticosterone induced by the odor of the frightened mother along with a novel peppermint odor was sufficient to produce pups' subsequent aversion to that odor. Disruption of axonal tracts from the Grueneberg ganglion, a structure implicated in alarm chemosignaling, or blockade of pups' alarm odor-induced corticosterone increase prevented transfer of fear. These memories are acquired at younger ages compared with amygdala-dependent odor-shock conditioning and are more enduring following minimal conditioning. Our results provide clues to understanding transmission of specific fears across generations and its dependence upon maternal induction of pups' stress response paired with the cue to induce amygdala-dependent learning plasticity. Results are discussed within the context of caregiver emotional responses and adaptive vs. pathological fears social transmission.

necklace glomeruli | pheromone | olfaction | PTSD | social referencing

Children, including infants, use their parents' emotions to guide their behavior and learn about safety and danger (1–4). The infant's ability to regulate behavior in novel situations using the caregiver's emotional expression is known as social referencing and occurs in humans and nonhuman primates (1). Although parental physical presence itself or particular cues indicating parental presence, such as voice, touch, or smell typically signal safety for the child, infants are especially responsive to the caregiver's communication during threats (3–5). This social learning is critical for enhancing survival through an adaptation to the environment but also provides transmission of pathological fears, such as occurs in posttraumatic stress disorder (PTSD) or in specific phobias (3–7).

Despite existing evidence that children are sensitive to parental fear and anxiety, the neurobiological mechanisms for the transmission of parental specific fear to the offspring have remained elusive (2–7). Animal studies investigating the impact of parental stress on the offspring focused on the history of parental trauma, quality of maternal care, and resultant overall behavioral alterations in the offspring (7, 8). However, to develop efficient survival strategies, progenies must learn about specific environmental threats triggering parental fear (9).

Most of what we know about fear learning comes from studies using fear conditioning (FC) (10). In FC, a neutral sensory cue [conditioned stimulus (CS)] is paired with a noxious event [unconditioned stimulus (US)]. Animal studies indicate that the amygdala's lateral and basal nuclei (LBA) play an important role in FC (10). However, FC in infant rats is naturally attenuated until postnatal day (PND) 10 due to low levels of the stress hormone corticosterone (CORT) during the stress hyporesponsive period (11–15). This fear suppression continues in older pups (until PND 16) in the mother's presence due to social buffering (attenuation) of the shock-induced CORT increase (15).

To study the intergenerational transmission of fear to specific triggers, we developed a mother-to-infant social fear learning paradigm. In social fear learning, an organism learns fear through an exposure to a conspecific expressing fear to a discrete CS. Social fear learning may thus serve as a model explaining how defense responses to specific triggers are transmitted between individuals. Social fear learning has been demonstrated in primates, including humans and in rodents, and involves the amygdala (16–19).

Results

Social Transmission of Fear from Mothers to Pups. Before pregnancy, adult female rats were olfactory FC, in which a neutral peppermint odor was paired with a mild electric foot shock US (*Materials and Methods*; for a schematic diagram of the experiment, see Fig. 1*A*). After birth, these mothers were presented with the CS odor to evoke fear in the presence of the pups. Specifically, at PND 6 or 7, pups and the mothers that had previously received fear conditioning (MFC) were either exposed

Significance

Despite clinical evidence that specific fear is transmitted across generations, we have little understanding of mechanisms. Here, we model social transmission of mother-to-infant fear in rodents. We show that maternal fear responses to a conditioned fear odor are sufficient to induce robust fear learning throughout infancy, with robust retention. Assessment of mechanism showed that maternal fear expression increases pups' stress hormone corticosterone and amygdala activation to induce this cue-specific fear learning. Suppressing pups' amygdala or preventing pups from mounting a stress response blocked this fear learning. Specific fears may thus be transferred across generations through maternal emotional communication and infant's associative learning mechanisms. Elucidating the mechanisms of this transmission may inform the development of novel therapeutic and preventive approaches.

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to the CS (group MFC-CS) or not (group MFC-no CS). A third group included pups from mothers that were preexposed to only the odor CS (no shock) then reexposed to the same CS while with their pups (CS alone-CS). Mothers of MFC-no CS pups expressed some fear to context and served as control fearful mothers with no CS present while interacting with pups (Table 1; cue reexposure and maternal behavior). Seven days later, pups received memory tests to the CS using a Y-maze (Fig. 1*B*). The ANOVA for the Y-maze test revealed a significant effect [$F(2, 15) = 10.5$; $P < 0.002$]. Post hoc test indicated a significant difference in CS-odor choices between the MFC-CS group compared with the other groups ($P < 0.05$; Fig. 1*B*). This pattern of findings shows that a co-occurrence of maternal fear and the olfactory CS cue produces in pups subsequent aversive responses to this CS as maternal fear alone (MFC-no CS) or the CS alone had no effect on pups' behavior. A similar pattern of results was observed during the CS cue exposure test when the MFC-CS pups expressed significantly more freezing than the two control groups (Fig. S1).

However, these results do not rule out the possibility that pups' fear responses to the CS were due to nonlearning mechanisms, such as nonspecific maternal behavior (7, 8). Although FC occurred before breeding, mothers previously fear-conditioned showed less nurturing behaviors and rougher handling of pups during presentation of the CS (Table 1) and potentially throughout rearing. To control for the possibility that these altered maternal behaviors mediated pups fear to the CS, we used "substitute mothers," a manipulation made possible by maternal acceptance of all pups, as well as pups' failure to distinguish between their mother and a substitute mother matched for the same postpartum period and the same diet (12–15). Substitute mothers received olfactory FC (or were exposed to the CS alone). On the following day, pups' biological mother was removed from the nest, and the substitute mother was placed in the nest and permitted an hour to settle down and nurse the pups at PND 13 before the CS-odor exposure. The first two groups included pups with their substitute mothers previously FC; one group was reexposed to the CS (MFC-CS), the other was not (MFC-no CS). A third group included pups with the substitute mother with prior exposure to the CS alone (no shock) and then reexposed to this CS (CS alone-CS) (Table S1; maternal behavior during CS reexposure). Statistical analysis of the Y-maze test on the following day revealed a significant effect [$F(2, 15) = 7.636$; $P < 0.006$]; post hoc test indicated a significant difference in CS-odor choices between the MFC-CS group compared with other groups ($P < 0.05$; Fig. 1*C*). This socially transmitted CS-odor aversion persisted at least until adolescence (PND 43) and did not generalize to a novel odor (Fig. S2). Older weaning-aged pups (PND 18–19) also learned maternal fear through social transmission (Fig. S3).

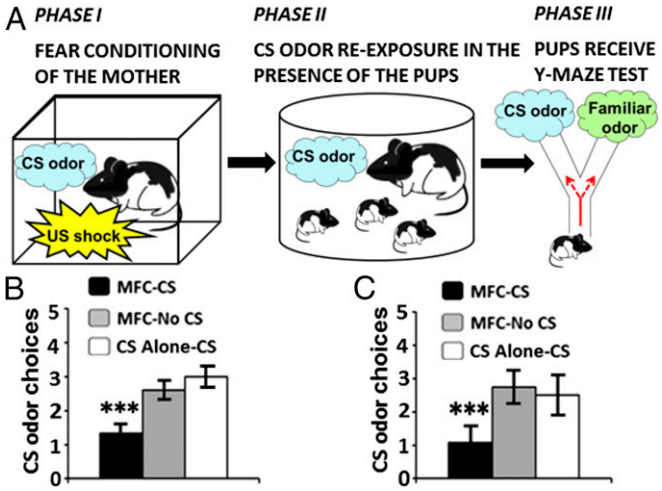

Fig. 1. Social transmission of fear from mothers to pups. (*A*) Schematic diagram illustrating experiments reported in *B* and *C*. The mothers were odor fear conditioned (MFC). Next, the odor CS was presented to the mother in the presence of her pups (MFC–CS). Control groups for mother–infant exposure include pups of MFC not presented with the CS (MFC-no CS) and pups with the mother exposed the CS without the US shock (CS alone) and later presented with this odor while with her pups (CS alone-CS; $n = 6$ per group). Pups were then tested in a Y-maze to assess whether they showed aversion to the CS. (*B*) PND 6–7 pups exposed to mother previously fear conditioned and reexposed to the CS in pups presence (MFC-CS) avoid this CS during the Y-maze test 7 d later (*C*). PND 13 pups were exposed to a substitute mother frightened with a previously trained olfactory CS (MFC-CS) or controls (MFC-CS and CS alone-CS) ($n = 6$ per group). At testing, 24 h later MFC-CS pups expressed aversion to this CS but not controls. All bars indicate mean ± SEM. ***$P < 0.006$, ANOVAs.

Table 1. Maternal behavior during fear-inducing odor CS

	Percent of observation periods in which behaviors occurred		
	MFC-CS	MFC-no CS	CS alone-CS
Maternal behaviors			
Fearful/defensive	78.0	30.22	0.0
Freezing	80.5	95.6	0.0
Rough/abusive	6.14	0.0	0.0
Nurturing	33.0	37.0	68.8
Neutral	18.4	74.05	39.77
Mother and pup in nest	56.66	62.5	75.0

Maternal behavior (%) during reexposure to MFC-CS and controls including a MFC not presented with the CS (MFC-no CS) or a control mother exposed to the odor CS without the US shock (CS alone) and later presented with this odor while with her pups (CS alone-CS). Fearful/defensive behaviors include freezing (the percent value of fearful/defensive behaviors accounted for by freezing is displayed in brackets), startle, escaping, covering the source of odor, and covering pups with bedding. Rough/abusive behaviors include stepping/jumping on pup and throwing/dropping/dragging/pushing away/rough handling pup. Nurturing behaviors include nursing, grooming/licking/retrieving pup. Neutral behaviors include sleeping, resting, self-grooming, eating, and drinking (for details, see *SI Materials and Methods*). Supplemental analysis showed that maternal fear expression (CS cue-induced freezing) strongly correlated with pups' subsequent CS-induced fear and avoidance behavior (*SI Supplemental Analysis*).

Odor of Frightened Mother Triggers Pup Stress Response and Reinforces Aversive Learning. We have shown above that fear responses to a specific odor are acquired through coexposure to fearful mothers and the CS. Earlier studies demonstrate that social transmission of fear may occur through observational learning in older animals (16–19), although infant rats lack functional visual and auditory sensory systems until they enter the third week of life (12). However, at birth, pups have a well-developed olfactory system, which supports olfactory learning and infant–mother communication (12–15). Olfaction remains important throughout life in rodents, including communicating fear using an alarm odor to support social transmission of fear (20–22). Thus, we explored whether the frightened mother's odor and its ability to increase pups' CORT was important for pups' learning of socially transmitted fear. As illustrated in Fig. 2, pups were physically isolated from their mother but received the odor of a frightened mother (by exposure to the previously trained CS cue) via an olfactometer (OFM-CS; Fig. 2*A*). Controls included pups exposed to the odor of a mother that was not frightened (OM-CS) or a neutral odor alone (CS only). The next day, pups were tested in a Y-maze and results showed a significant effect [$F(2, 15) = 7.308$; $P < 0.007$]. Post hoc means comparisons test indicated significant difference in CS-odor choices between the OFM-CS group compared with the two other groups ($P < 0.05$; Fig. 2*B*). This pattern of findings demonstrates that

pups may acquire an aversion to the CS through association with the odor of the frightened mother and the potential release of a maternal alarm odor (20–22).

Because the stress hormone CORT is increased by the alarm odor (21) and is critical for pups' fear learning (12–14), we next explored the possibility that the alarm odor induced an increase in pups' CORT and was critical for pups socially transmitted FC. It should be noted, however, that maternal presence typically socially buffers pups to suppress the US-induced CORT increase and blocks FC until PND 16, although these mothers did not express fear (15). We hypothesized that maternal fear and associated release of the alarm odor was capable of overriding social buffering and increased pups' CORT levels to permit fear learning. To this end, one group of pups at PND 11–12 underwent mother–pups transmission using OFM-CS, whereas the control group was exposed to the odor of the (substitute) mother that was not frightened paired with the CS cue (OM-CS) and pups' blood was collected for CORT RIA (*SI Materials and Methods*). The *t* test with Welch's correction for unequal variance revealed a significant effect of exposure [$t(16, 18) = 2.133$, $P < 0.05$, with the OFM-CS group displaying significantly higher CORT] (Fig. 2*C*). We then asked whether lowering pups' CORT levels would affect mother–infant transmission of fear. Prior (90 min) to the procedure for social transmission of fear using the odor of the frightened (substitute) mother, pups at PND 11–12 were injected either with a CORT synthesis inhibitor metyrapone (50 mg/kg, i.p.) or saline and returned to the dam until training. All pups received pairings of the odor of the frightened mother paired with the olfactory CS (OFM-CS). The next day, pups were tested with a Y-maze and showed that the CORT block group failed to learn the odor aversion [$t(14) = 5.465$, $P < 0.002$] (Fig. 2*D*). This pattern of findings demonstrates that activation of the infant's HPA axis, which is induced by the odor of the frightened mother, plays an important role in the acquisition of socially transmitted maternal fear responses.

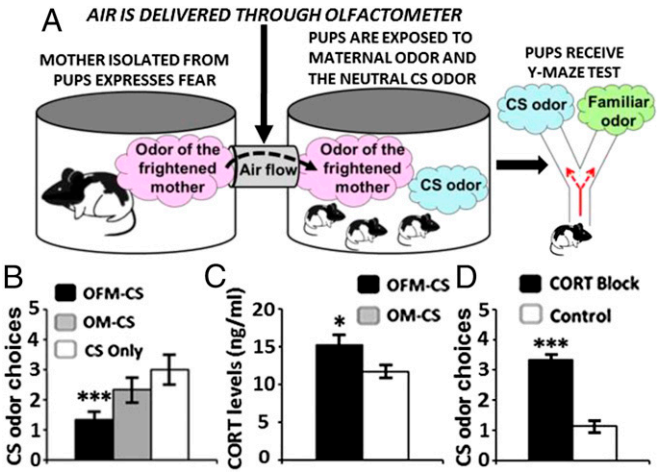

Fig. 2. Odor of frightened mother triggers pup stress response and reinforces aversive learning. (*A*) Schematic diagram of experiment reported in Fig. 2 *B* and *C* on PND 11–13 pups. A mother was placed within an olfactometer and presented with the fear inducing odor CS. The odor of the frightened mother (OFM) was then presented to pups along the odor CS, which was still neutral to pups (OFM-CS). Controls included pups exposed to the odor of the mother (OM) that was not frightened paired with the neutral CS (OM-CS) or pups exposed to the neutral odor only (CS only); $n = 6$ per group. The next day all pups were given a Y-maze test. (*B*). OFM-CS pups compared with the two control groups (OM-CS and CS only) express subsequent aversion to this CS. (*C*) Exposure to the odor of the frightened mother (OFM-CS pups; $n = 12$) elevates CORT levels compared with the controls exposed to the odor of the calm mother (OM-CS; $n = 8$). (*D*) Pharmacological blockade of CORT synthesis (CORT block; $n = 9$) by metyrapone impairs the mother-to-pups transmission of fear in OFM-CS pups compared with the OFM-CS saline controls (control; $n = 7$). All bars indicate mean ± SEM. *$P < 0.05$, *t* tests; ***$P < 0.007$, ANOVAs.

Amygdala and Olfactory Autoradiography of Mother-to-Infant Transfer of Fear. To assess neural activity during the transmission of fear, pups were injected with ^{14}C 2-deoxyglucose (2-DG) (13) 5 min before the mother-to-pups social transmission of fear. Groups included pups with their 'substitute mother' previously fear conditioned and then CS reexposed while with pups (MFC-CS) or not (MFC-no CS). A third group included pups with a substitute mother that had not received prior FC and instead had been exposed to unpaired presentations of the CS and the US, and then reexposed to the CS with pups (no MFC-CS; *SI Materials and Methods*). Pups' brains were removed and processed for autoradiography. Areas of interest were the amygdala, a key structure for FC (10), and olfactory structures (20). Significant differences were found throughout amygdala nuclei: lateral (LA) [$F(2, 14) = 7.545$; $P < 0.008$]; basal (BA) [$F(2, 14) = 5.564$; $P < 0.03$]; central (CeA) [$F(2, 14) = 13.68$; $P < 0.0006$]; cortical (CoA) [$F(2, 14) = 5.032$; $P < 0.03$]; medial (MeA) [$F(2, 14) = 5.988$; $P < 0.02$] (Fig. 3 *A* and *B*), and post hoc tests ($P < 0.05$) revealed increased uptake in all MFC-CS amygdala nuclei compared with the two other groups. This pattern of findings indicates that the infant's amygdala plays a role in the mother-to-infant transfer of fear. Analysis of the main olfactory bulb showed no significant differences in the rostral portion (r-MOB; $P = 0.24$; Fig. 3 *C* and *D*), although analysis of the caudal portion of the MOB (c-MOB) showed a significant difference [$F(2, 14) = 8.438$; $P < 0.004$] (Fig. 3 *C* and *D*), with post hoc tests revealing that the MFC-CS group was significantly different from both controls. The MFC-CS group showed increased 2-DG uptake located in the isolated areas of the glomerular layer (c-MOB-iGl) forming a characteristic ring encircling the c-MOB. This pattern of neural activity resembles the location of the necklace glomeruli (NG), which more rostrally form a ring around the anterior portion of the accessory olfactory bulb (AOB) and moving posteriorly, encircle the entire trunk of the c-MOB (23). Interestingly, we observed the highest activity in the ventral aspect of the c-MOB (Fig. 3*D*), which has been shown to contain the largest NG (23). The robust activity in the c-MOB-iGl suggests that this area is involved in the transfer of fear, possibly through the Grueneberg ganglion (GG)-NG olfactory subsystem, which was recently shown to be involved in alarm odor processing (21) and which was further explored below. Within the vomeronasal organ (VNO)–AOB subsystem (20), significant differences were found for the AOB [$F(2, 14) = 6.090$; $P < 0.02$]; post hoc tests showed increased AOB activity in both groups with mothers expressing fear (Table 1 and Table S1) compared with the no MFC-CS group ($P < 0.05$; Fig. 3 *C* and *D*) suggesting that the infant's AOB is responsive to maternal threat communication.

Amygdala and Olfactory Expression of the Immediate Early Gene c-Fos Following the Mother-to-Infant Transfer of Fear. To verify our autoradiography data at a cellular resolution level, we measured neural activity using expression of the immediate early c-Fos gene. In this experiment, pups underwent the procedure for mother–pups social transmission of fear using the odor of the frightened mother (Fig. 2*A* and *SI Materials and Methods*). One group included pups exposed to the odor of the frightened mother paired with the CS (OFM-CS), whereas another group included pups exposed to the odor of the (unfrightened) mother paired with the CS (OM-CS). Amygdala nuclei demonstrating high 2-DG uptake during the mother-to-infant transmission of fear also showed significant higher levels of c-Fos: [*t* test: LA $t(8) = 3.26$, $P < 0.02$; BA $t(8) = 2.33$, $P < 0.05$; CeA $t(8) = 3.72$, $P < 0.006$; CoA $t(8) = 2.39$, $P < 0.05$; MeA $t(8) = 2.712$, $P < 0.03$] (Fig. 4 *A* and *B*). For the olfactory system, MOB analysis included the granule cell layer (MOB-Gr), associated with olfactory FC c-Fos changes in pups (24) as well as the AOB granule (AOB-Gr) and mitral (AOB-Mi) cell layers because these areas are involved in alarm odor signaling (20). Finally, we assessed

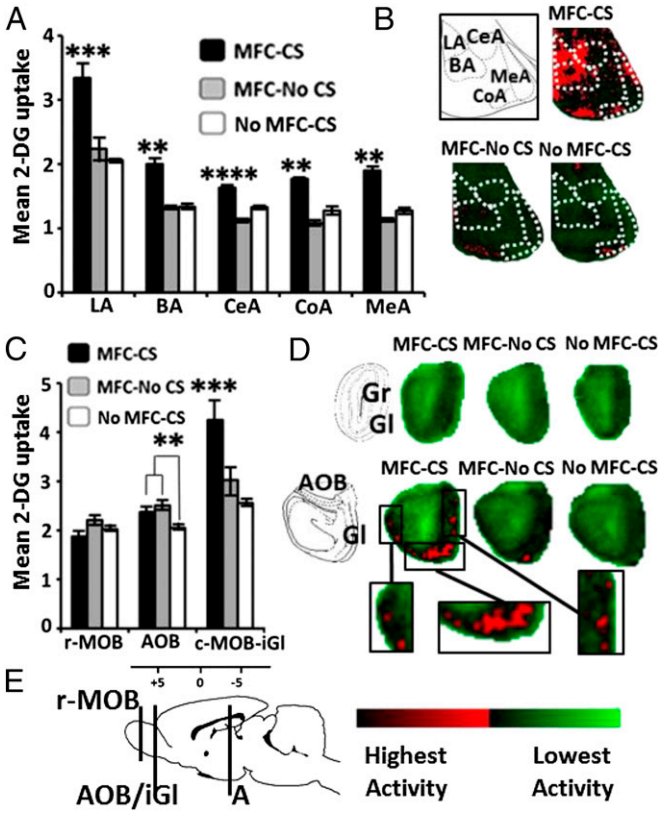

Fig. 3. Amygdala and olfactory autoradiography of mother-to-infant transfer of fear. PND 13–14 pups received injections of 2-DG radiographic marker before exposure to the mother previously fear conditioned (MFC) expressing fear in response to the CS odor (MFC-CS; $n = 7$). Controls included pups exposed to MFC that did not receive CS exposure (MFC-no CS; $n = 4$) and pups with mothers that instead of fear conditioning (no MFC) had been subjected to unpaired presentations of the CS and a foot shock US and then with pups were reexposed to the CS odor (no MFC-CS; $n = 6$). Following exposure, 2-DG uptake was assessed in brains from all groups. (*A*) MFC-CS pups display increased 2-DG uptake in the amygdala nuclei: LA, BA, CeA, CoA, and MeA. (*B*, *Upper Left*) Location of the examined amygdala nuclei followed by representative images (with outlined amygdala nuclei) from each experiment. (*C*) All experimental groups display similar 2-DG uptake in the r-MOB; MFC-CS and MFC-no CS pups display increased 2-DG uptake in the AOB compared with the no MFC-CS group, and only the MFC-CS group (compared with the two control groups) show increased uptake in the c-MOB-iGl. (*D*) Representative images from each experimental group showing 2-DG uptake in olfactory areas. (*Top*) From left: schematic diagram of the coronal section of the r-MOB followed by the representative images of the r-MOB. (*Middle*) From left: schematic diagram of the coronal section of the c-MOB and AOB followed by the representative images (Gr, granule cell layer; Gl, glomerular layer; ON, olfactory nucleus). (*Bottom*) From left: magnified images from the representative MFC-CS brain displayed in *D* showing augmented 2-DG uptake in the c-MOB-iGl. (*E*, *Left*) Sagittal section of the rat brain showing distances from Bregma to coronal sections where neural activity of the examined structures was measured. (*Right*) Color gradation showing neural activity (pseudocolor images displayed using ImageJ Red/Green Lookup Table: from light green (no activity) through dark green to dark red (highest activity). All bars indicate mean ± SEM. $^{**}P < 0.03$; $^{***}P < 0.008$; $^{****}P < 0.0006$, ANOVAs.

the glomerular layer of the c-MOB because our 2-DG data showed high neural activity in this area (Fig. 3 *C* and *D*). We did not observe any difference in c-Fos immunoreactivity in the MOB-Gr ($P < 0.05$), AOB-Gr ($P < 0.05$), and AOB-Mi ($P < 0.05$; Fig. 4 *C* and *D*). However, we found increased c-Fos uptake in the isolated glomeruli encircling the c-MOB (c-MOB-iGl), a pattern similar to our 2-DG findings and consistent with the NG location. For analysis of c-Fos expression, we used six isolated glomeruli (three from the left and three from the right side) from each brain. The average score of c-Fos expression in one isolated glomerulus for each brain was calculated. Data in Fig. 4*C* were displayed as a sum of all average scores per glomerulus per brain (all iGls) and as an average score per glomerulus per brain (single iGl). The *t* test showed increased c-Fos expression in the OFM-CS group [all iGls; *t* test: $t(8) = 13.97$, $P < 0.0002$]. Overall, our analysis of c-Fos expression provides further evidence that the MOB is not activated during the social transmission of fear. Although we observed a trend of increased c-Fos reactivity in the AOB-Mi, our data do not support the role of the AOB in the mother-to-infant transmission of fear. Robust c-Fos immunoreactivity in the amygdala and the isolated glomeruli encircling the c-MOB suggests the role of these structures in the intergenerational transmission of fear in our paradigm. Our behavioral results show that the odor of the frightened mother triggers pup stress response and supports infant social fear learning (Fig. 2). In addition, our 2-DG and c-Fos data show increased neural activity in the isolated glomeruli encircling the c-MOB, the location pattern similar to the organization of the NG. Together, our behavioral and imaging data strongly suggest the involvement of the maternal alarm odor and the GG-NG pathway in the mother-to-infant transmission of fear.

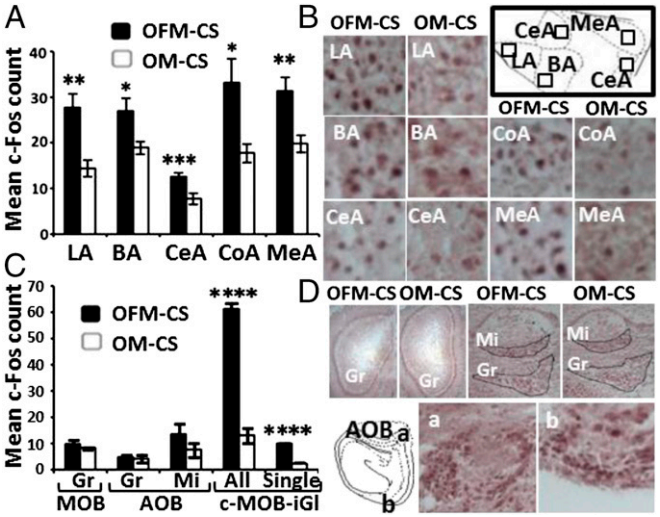

Fig. 4. Amygdala and olfactory expression of the immediate early gene c-Fos following the mother-to-infant transfer of fear. PND 13–14 pups were subjected to the mother–pups fear transfer training through pairing of OFM with group OFM-CS. Control group included pups exposed to the OM that was calm paired with the CS (group OM-CS); $n = 5$ per group. (*A*) Increased c-Fos expression in the OFM-CS group (compared with the OM-CS pups) in the amygdala nuclei: LA, BA, CeA, CoA, and MeA. (*B*) Representative images from each experimental group showing c-Fos expression (dark dots) in the amygdala nuclei. (*Top Right*) Schematic diagram of the examined amygdala nuclei (squares in each nucleus indicate the approximate position of the representative microphotographs). (*C*) Similar levels of c-Fos expression in both groups in the MOB-Gr, AOB-Gr, and AOB-Mi; increased c-Fos immunoreactivity in the MFC-CS group (compared with the OM-CS group) in the c-MOB-iGl. All, c-Fos expression in all assessed c-MOB-iGl: six per brain; single, comparison of c-Fos immunoreactivity in the single isolated glomerulus from the c-MOB-Gl area in the OFM-CS group with a corresponding glomerulus in the OM-CS group. (*D*) Representative images showing c-Fos immunoreactivity (dark dots) in the examined areas. (*Upper*) From left: c-Fos expression in the MOB-Gr and the AOB (Gr and Mi); (*Lower*) Representative images showing high c-Fos immunoreactivity in the isolated glomeruli encircling the c-MOB in the OFM-CS group; From left: schematic drawing of the coronal section of the c-MOB and the AOB (*a* and *b* show positions where isolated glomeruli displayed on the right were located). All bars indicate mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.03$, $^{***}P < 0.006$, $^{****}P < 0.0002$, unpaired Student *t* test; n.s., nonsignificant.

Grueneberg Ganglion Axotomy Prevents Infant Fear Learning Using Maternal Alarm Odor. To examine the role of the GG-NG pathway in the social transmission of fear, PND 2–3 pups underwent either GG axotomy or sham surgery (*SI Materials and Methods*). Previous work shows that disruption of GG axonal tracts abolishes fear responses to the alarm odor in adults (21). Twelve days following the GG axotomy/sham procedure, pups were exposed to the odor of the frightened mother paired with a neutral lemon odor CS. The next day, pups were tested in a Y-maze (lemon odor). To ensure that our surgical procedure left the non–GG-NG fear learning intact (21), pups were olfactory FC to a novel CS odor (peppermint) using UC shock and assessed by the Y-maze test. After the completion of the experiment, GG axotomy lesions were microscopically verified by an experimenter blind to the treatment condition. Only data from subjects with bilateral lesions (GG axotomy) or no disruption of the GG axonal tracts (sham) were used for analysis. The *t* test revealed a significant effect of the surgical procedure, $t(10) = 2.401$, $P < 0.04$, with the GG axotomy group failing to show mother-to-infant transfer of fear, although shock FC learning was left intact ($P < 0.05$; Fig. 5*A*). Thus, the intact GG-NG pathway is critical for the social transmission of fear using the odor of the frightened mother.

Amygdala Inactivation Prevents Mother-to-Infant Transfer of Fear. Our data show robust infant amygdala activation during the social transmission of fear (2-DG, c-Fos) consistent with adult social fear learning data (18, 25). To determine if the amygdala is causally involved in social transmission of fear in pups, PND 11–13 pups were bilaterally implanted with intra-LBA amygdala cannulae and 2–3 d later (14) had the amygdala suppressed (using $GABA_A$ receptor agonist muscimol) or received vehicle (control) infusion followed by the social transmission of fear procedure. The next day, pups were tested in the Y-maze, and then brains were removed to verify cannulae placement (Fig. S4). Only data from animals with injector cannula tip bilaterally located in the LBA were used for analysis. The *t* test revealed a significant effect of treatment, $t(8) = 4.714$, $P < 0.02$, with the muscimol group failing to demonstrate the decreased number of the CS-odor choices indicative of learning, as observed in controls (Fig. 5*B*). Thus, LBA inactivation prevented mother-to-pup fear transmission.

Discussion

In this study we showed that maternal CS-specific fear responses can be transmitted to the offspring in rats using a social fear learning paradigm within the nest (Fig. 1). A possible history of maternal rough handling alone was insufficient to produce specific fear responses in pups: the mothers that expressed contextual fear (Table 1 and Table S1: group MFC-no CS) during interaction with pups (no CS presented) did not produce specific fear to the CS in pups. Thus, pups only acquire the CS-specific fear responses from their mother if she expresses CS-specific fear in the presence of pups.

During the stress hyporesponsive period of pups (until PND 10), FC is physiologically suppressed and continues to be suppressed if mothers socially buffer the pups during FC until PND 16 (11–15). However, we show that pups as young as PND 6 are capable of FC if this learning is reinforced by maternal fear communication in the presence of the CS odor.

Furthermore, similarly to attachment-related odor learning, this fear-related early learning is robustly retained. Research shows that brief FC in infancy is usually short-lived, a phenomenon known as infantile amnesia or infantile forgetting, although repeated sessions induce robust retention (26). In contrast, our data demonstrate that one session of maternally transmitted fear learning at PND 13 lasts through early adolescence, indicating retention for at least 30 d (Fig. S2*A*). The ability to acquire maternally transmitted fear during early infancy and before the development of infant amygdala-dependent odor-shock conditioning (12), combined with the lasting character of these socially transferred memories, suggests a unique characteristic of

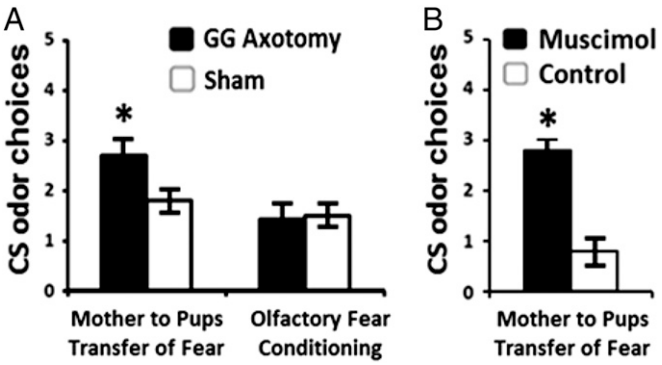

Fig. 5. GG- NG pathway and the amygdala are involved in mother-to-infant transmission of fear. (*A*) GG axotomy prevents infant fear learning using maternal alarm odor. PND 2–3 pups underwent GG axotomy ($n = 7$) or sham procedure ($n = 5$). Twelve days later, pups received the mother–pups transfer of fear using the OFM paired with the odor CS followed 24 h later by the Y-maze test. On the following day, pups received olfactory fear conditioning to a novel odor, and 24 h later, Y-maze test to this odor. GG axotomy pups did not acquire aversive responses to the CS odor paired with the OFM (*Left*); both groups acquired aversion to a distinct odor cue trained during fear conditioning (*Right*). (*B*) Amygdala inactivation prevents mother-to-infant transfer of fear. PND 13–15 pups with implanted cannulae received bilateral intra-LBA infusions of $GABA_A$ receptor agonist muscimol or equivalent volume of saline (control) before the mother–pups transmission of fear using the odor of the frightened mother paired with the odor CS ($n = 5$ per group). Muscimol group did not display CS odor aversion during the Y-maze test 24 h later. All bars indicate mean ± SEM. *$P < 0.04$.

maternally transmitted emotional learning. Infants can learn from their mothers about potential environmental threats before their sensory and motor development allows them a comprehensive exploration of the surrounding environment.

Similarly to odor-shock FC in pups, the role of CORT is important. Pups precociously learn FC if reared by a maltreating mother, a procedure that increases CORT levels and permits amygdala learning plasticity (12). Here we show that, without early life stress, the mother can quickly raise pups' CORT levels by expressing fear, and this increase in CORT supports amygdala plasticity (see *SI Materials and Methods* for an expanded discussion on CORT).

The critical role of the amygdala in this socially transmitted fear was demonstrated through the inactivation of the LBA by muscimol, which prevented mother-to-infant transmission of fear; this suggests that for the social transmission of fear, pups use a network similar to that required by shock supported FC and social transmission of fear in adults. Notably, however, this social transmission of fear functionally emerges at a younger age compared with shock-supported FC. We have shown that in our model, maternal physical presence is not necessary for fear learning to occur. Pairing of just an odor of the frightened mother with the neutral (to pups) smell of the CS is sufficient to produce subsequent aversive responses in pups that are odor specific (Fig. 2*B*). Additionally, our GG-related increases of neural activity and GG axotomy data suggest the GG is important in fear communication via maternal alarm odor (similar to odor fear communication in adults) and mother–pups transfer of fear. However, this observation does not rule out the possibility that other sensory modalities are involved in the intergenerational transfer of fear at later stages of development (18, 19). Consistent with existing alarm odor signaling literature, our data suggest that through excitation of pups' GG-NG (and possibly VNO-AOB) pathway, maternal olfactory threat information reaches the MeA and CoA and activates the LA, BA, and CeA known to support fear learning and expression (20–22). However, the distinct neural and molecular mechanisms through which maternal alarm odor instructs fear learning in the pups'

amygdala remain to be determined (see *SI Materials and Methods* for an expanded discussion).

A recent study shows that parental traumatic experience may induce neuroanatomical adaptations and related cue-specific behavioral predispositions in offspring (9). Our results, however, demonstrate that parental specific fear behaviors may be transferred to infants through emotional communication and associative learning mechanisms producing lasting memories. These findings provide a model characterizing how parental adaptive and pathological fear may be transmitted to their offspring, such as in PTSD (6, 27, 28) and specific phobias (2). Understanding of the neural and molecular mechanisms controlling intergenerational transmission of fear will help to develop better preventive and therapeutic methods.

Materials and Methods

A detailed description of materials and methods is provided in *SI Materials and Methods*.

Subjects. Male and female Long–Evans rats were born and bred in our colony. All animal care and experimental procedures were conducted in accordance with National Institutes of Health guidelines and were approved by the Nathan Kline Institute's Institutional Animal Care and Use Committee.

Odor Cue Delivery. CS odor (pure peppermint or lemon; McCormick) was delivered by a flow dilution olfactometer controlled by FreezeFrame software (2 L/min flow rate, 1:10 CS odor:air).

Fear Conditioning of Mothers. Mothers were conditioned before breeding or during lactation. Standard Coulborn conditioning boxes were used. Six pairings of a 30-s olfactory CS and electric foot shock US (0.5 s; 0.6 mA) were controlled by FreezeFrame.

Fear Conditioning of Pups. Pups were placed individually in 600-mL clear plastic beakers and were given five pairings of the 30-s olfactory CS and electric tail shock US (1 s; 0.5 mA; Lafayette shock generator) controlled and recorded using EthoVision.

Mother–Pups Social Transmission of Fear. Socially transmitted fear was done in the pup's home cage with four CS presentations controlled by FreezeFrame and videotaped (Figs. 1 and 3 and Figs. S1–S3).

Mother–Pups Transmission of Fear Using the Odor of the Frightened Mother. Mother was placed in a visually and sound-isolated container connected through an olfactometer (allowing continuous flow of air). The odor was delivered to the plastic beakers that contained individual pups. Maternal fear was elicited by four CS presentations that was accompanied by simultaneous CS presentations to each pup (Figs. 2, 4, and 5).

Y-Maze Testing of Pups' Aversion Learning. Pups were given five trials to choose between two arms, one containing the CS odor and the other containing a familiar odor (clean wood bedding).

RIA. Immediately following the socially transmitted FC, trunk blood samples of 300–400 μL were collected and analyzed (Kit brand).

CORT Blockade. Metyrapone (50 mg/kg) or vehicle was injected i.p. 90 min before training.

Cannulae Implantation/Drug Infusions. Pups were implanted and recovered for 2–3 d. LBA pharmacological inactivation occurred with bilateral muscimol (2 mM) or vehicle at 0.1 μL/min for 5 min (total infusion volume 0.5 μL).

GG Axotomy and Verification of Axonal Tract Lesions. GG axotomy was conducted as in previous work (21).

2-DG Autoradiography. Pups were injected with ^{14}C 2-DG (20 μCi/100 g, s.c.) 5 min before the mother–pups transmission FC, the brain removed 45 min later, processed for autoradiography, and analyzed (ImageJ software; National Institutes of Health).

Immediate Early Gene c-Fos Expression. Pup brains were removed 90 min after socially transmitted FC and processed using standard procedures (*SI Materials and Methods*).

Statistical Analysis. Statistical analysis was performed using Student *t* test or ANOVA followed by post hoc Newman–Keuls test. Differences were considered significant when $P < 0.05$.

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