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The role of the medial prefrontal cortex in innate fear regulation in infants, juveniles, and adolescents

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Abstract

In adult animals, the medial prefrontal cortex (mPFC) plays a significant role in regulating emotions and projects to the amygdala and periaqueductal gray (PAG) to modulate emotional responses. However, little is known about the development of this neural circuit and its relevance to unlearned fear in pre-adulthood. To address these issues, we examined the mPFC of 14 (infants), 26 (juveniles), and 38–42 (adolescents) day old rats, to represent different developmental and social milestones. The expression patterns of the neuronal marker FOS were used to assess neurological activity. Muscimol, a GABA agonist, was used to inactivate the prelimbic and infralimbic mPFC subdivisions (400 ng in 200 nl). Animals were exposed to either a threatening or non-threatening stimulus that was ecologically relevant and age-specific. Freezing was measured as an indicator of innate fear behavior. The data indicated that the mPFC is neither active nor responsive to innate fear in infant rats. In juveniles, the prelimbic mPFC became responsive in processing aversive sensory stimulation, but did not regulate freezing behavior. Finally, during adolescence, inactivation of the prelimbic mPFC significantly attenuated freezing, and decreased FOS expression in the ventral PAG. Surprisingly, across all ages, there were no significant differences in FOS levels in the medial and basolateral/lateral amygdala when either mPFC subdivision was inactivated. Taken together, unlearned fear has a unique developmental course with different brain areas involved in unlearned fear in the immature animal than the adult. In particular, the mPFC neural circuitry is different in young animals and progressively develops more capacities as the animal matures.

Introduction

Animals continually monitor their environment and must respond appropriately in order to avoid being harmed, injured or killed. They respond with defensive behaviors and fear if they perceive a stimulus to be dangerous. Animals possess a specialized neural circuit that allows them to assess the valence of sensory stimuli and to generate defensive responses if the stimulus poses a threat. This fear circuit consists of interconnected brain areas, that include the medial prefrontal cortex (mPFC), the amygdala and the periaqueductal gray (PAG), that participate in stimulus processing and the generation of defensive behavior (LeDoux, 2000; Öhman and Mineka, 2001; Rosen, 2004).

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It has been proposed that the mPFC regulates fear behavior by modulating the activity of the amygdala and PAG (Paré et al., 2004; Price, 2005; Peters et al., 2009; Ulrich-Lai and Herman, 2009). According to this model, two subdivisions of the mPFC play opposing roles in fear behavior regulation. The prelimbic subdivision promotes the expression of fear by increasing amygdala output. The infralimbic subdivision, on the other hand, exerts inhibitory control on fear expression by decreasing amygdala output. The infralimbic and the prelimbic mPFC also influence fear behavior through direct projections to columns of the PAG that generate defensive behaviors (An et al., 1998; Floyd et al., 2000; Keay and Bandler, 2001; Gabbott et al., 2005). Findings in adult rodents support this model: inactivation of the prelimbic mPFC decreased conditioned freezing and stimulation increased it; whereas stimulation of the infralimbic mPFC decreased freezing (Vidal-Gonzalez et al., 2006; Corcoran and Quirk, 2007).

Even though animals are able to respond to threats throughout life, little is known about the role of the mPFC in fear regulation in young animals. Fear behavior changes dynamically during early development, with young animals exhibiting different fear reactivity than adult animals (Bronson, 1968; Curio, 1993; Wiedenmayer, 2009). Stimulus assessment is particularly demanding for animals in early development because a stimulus may pose a threat at one age but not at a later age. For example, an unfamiliar male conspecific poses a threat to infant but not juvenile rats (Wiedenmayer et al., 2005). Furthermore, the mPFC may not have the same regulatory function in early life as in adulthood because it continues to develop postnatally (van Eden et al., 1990; Benes et al., 2000). For example, the mPFC does not support fear memory extinction in infant rats but becomes functional only in juvenile rats (Kim et al., 2009).

The present study investigates whether the mPFC contributes to unlearned fear behavior in young rats and whether its function changes during development. In particular, we examine whether the two subdivisions have opposing roles in fear expression. Rats of three different age groups – infants, juveniles and adolescents – were exposed to an age-specific threatening or non-threatening stimulus and the function of the mPFC was assessed. We hypothesized that mPFC subdivisions differentially regulate amygdala and PAG activity and thus fear responsivity, and that the mPFC has this function in juvenile and adolescent rats but not in infant rats.

Material and Methods

Animals

Male Long-Evans hooded rats were bred and housed in standard laboratory cages, which were maintained in a colony room with a temperature of 22–24°C. The animals were given *ad libitum* access to food and water, and light in the room was set to a 12:12 h light/dark cycle with light onset at 6:00 A.M. Adult males and females were housed together for a three-week period and then the males were separated from the impregnated females. The cages of the impregnated females were checked daily, and rat pups found were considered as day 0. Offspring were weaned at 23 days of age and same sex littermates were kept together in groups of 2–4 until the completion of the experiments. The age groups used spanned across three stages of ontogeny: 14-day-old rats (P14) as preweaned infants, P26 as weaned juveniles, and P40-42 as adolescents.

Lactating females nursing infant pups, non-lactating females, and sexually experienced unfamiliar male rats were used as stimulus animals and were housed in the same room. All tests and treatment procedures were approved by and were in accordance with the Institutional Animal Care and Use Committee of New York State Psychiatric Institute.

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Surgery

One day before testing, four male rats from each tested litter were randomly selected for bilateral cannula intracranial implantation surgery. All rats undergoing surgery were anaesthetized with isoflurane (Henry Schein, Melville, NY) mixed with oxygen and nitrous oxide, which was regulated by an Open Tabletop Isoflurane Vaporizing System (Stoelting Company, Wood Dale, IL). For surgery, rats were placed in a stereotaxic apparatus (Stoelting Company, Wood Dale, IL), which was fitted with an adapter for infant and juvenile rats.

For infant rats, bilateral burr holes were drilled 1.6 mm anterior and 1.9 mm lateral to bregma for the infralimbic mPFC, and 1.6 mm anterior and 2.0 mm lateral to bregma for the prelimbic mPFC. 23-gauge guide cannulas were then inserted to a depth of 4.2 mm for the infralimbic and 3.2 mm for the prelimbic mPFC. For juvenile rats, bilateral burr holes were drilled 2.0 mm anterior and 2.0 mm lateral to bregma for the infralimbic mPFC, and 2.0 mm anterior and 2.2 mm lateral to bregma for the prelimbic mPFC. 23-gauge guide cannulas were then inserted to a depth of 4.2 mm for the infralimbic and 3.3 mm for the prelimbic mPFC. For adolescent rats, bilateral burr holes were drilled 2.6 mm anterior and 2.2 mm lateral to bregma for the infralimbic mPFC, and 2.6 mm anterior and 2.5 mm lateral to bregma for the prelimbic mPFC. 23-gauge guide cannulas were then inserted to a depth of 4.3 mm for the infralimbic and 3.5 mm for the prelimbic mPFC. Vertical insertion of the cannulas into the infralimbic mPFC may damage the prelimbic mPFC, and insertion into the prelimbic mPFC may damage the cingulate mPFC. Therefore, for all ages, guide cannulas were inserted at a 24° angle for the prelimbic mPFC and at 16° angle for the infralimbic mPFC to prevent damage to overlying mPFC structures. The cannulas were secured to the skull with dental cement. Rats were returned to their home cage and littermates after they had recovered from the anesthesia. Rats gained weight between surgery and testing the next day, which suggests that the recovery was successful.

Testing Procedure

Infant and juvenile rats were tested in groups of three to decrease isolation-induced stress, which itself can alter freezing (Hofer and Shair, 1980; Hennessy and Weinberg, 1990). At these ages a single pup of the group of three was identified a priori to provide a single data point. When the pups were in different experimental conditions, each contributed to the data. Adolescent rats were tested individually. Infusions were given immediately before the start of the testing. Cannulated rats were infused with either 200nl of 400ng GABA agonist, muscimol (Sigma-Aldrich, St. Louis, MO) or 200nl of vehicle solution (0.9% saline). The 30-gauge injection cannula was connected to a 10μl glass syringe (Hamilton Company, Reno, NV) operated by hand, and was left in the guide cannula for 30 seconds after the injection to permit maximal diffusion. Injection cannulas for all ages were constructed so that they extended 0.5 mm beyond the tip of the guide cannula. Previous radiolabeling results (Chen et al 2006) found that this volume of muscimol spreads to a maximum radius of 0.5 mm from the cannula tip, restricting the pharmacological inactivation effect to the mPFC subdivisions. Cannula placements were verified in later histology procedures (see below).

After the infusion, each group or animal was placed into one compartment of a testing cage. A wire-mesh screen divided the testing cage (46 × 25 × 21 cm) into two equal compartments. The compartment containing the rats had soiled bedding collected from the home cage just prior to the test. The other compartment was used to present threatening and non-threatening stimuli. Animals were given a 3 min adaptation period. Then the stimulus was placed in the experimental compartment for 5 min. In the control condition the

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experimental compartment was left empty. After testing, rats were kept in holding cages until perfusion.

Use of age-specific ecological threatening or non-threatening stimuli

We used different stimuli to provide age appropriate threats. Thus different stimuli were used at different ages. Had we used the same stimulus, for example the strange male, the resultant interaction would be different depending on age: the strange male is infanticidal for the 14 day old, neutral to the 26 day old, but a combatant for the adolescent. Similar changes occur in the response of the lactating female to different aged animals. Thus we chose "functionally" neutral stimuli based on ecological criteria.

For infant rats, an unfamiliar, unrelated adult male rat or a cat odor was used as a threatening stimulus. Adult males are a significant threat to infant rats, as they often kill unrelated offspring during the suckling period (Rosenberg, 1974; Brown, 1986; Mennella and Moltz, 1988). Cats pose a less likely threat to infant rats that remain largely in the burrow, but cat odor does elicit defensive behavior at this age (Wiedenmayer and Barr, 2001). Cat odor was derived from a cat pad. The fleece pad (38 × 61 cm, Flexi-mat, Chicago, IL) had been used by cats as resting place for approximately 12 months and probably contained fur, skin, and gland secretions. The pad was cut in pieces (9 × 10 cm) that were kept in zip-lock bags at −80° Celsius. Several hours before the training, the pad was thawed and kept in a closed container. At the start of the training session, the cover of the container was removed and the container was placed on the stimulus side of the cage behind the mesh. An unfamiliar lactating female rat was used as the non-threatening stimulus. Lactating females do not pose an infanticidal threat; in fact, lactating females behave maternally towards any pups (Beach and Jaynes, 1956).

For juvenile rats, an unfamiliar adult male rat was used as a non-threatening stimulus. The danger of infanticide stops at weaning (van Schaik and Janson, 2000) and adult male rats do not pose a threat or attack juvenile rats (Paul and Kupferschmidt, 1975). Cat odor was used as described above as the threatening stimulus because young rats are exposed to multiple predatory species (Caro, 2005).

For adolescent rats, cat odor was again used as a threatening stimulus. As rats of this age expand their home range and migrate away from their natal burrow environment, predation poses a significant threat to survival (Calhoun, 1963). A non-lactating female rat was used as a non-threatening stimulus. Lactating females were not used because they exhibit aggression towards intruders to protect their pups (Neumann et al., 2001; Deschamps et al., 2003), and adult male rats were not used because they react to intruding adolescent males with aggression and may even kill them (Thor and Flannelly, 1976).

Immunocytochemistry

Two hours after testing, when FOS expression levels peak (Morgan and Curran, 1991), the rats were removed from the holding cage and given an overdose volume of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) or a ketamine (100mg/kg) xylazine (15mg/kg) mixture. Transcardial perfusion of the rats was carried out using a 4% paraformaldehyde solution (Electron Microscopy Sciences, Hatfield, PA) as the fixative, and the brains were removed and stored in formalin overnight. The brains were placed in a 30% sucrose buffer for cryoprotection before they were frozen, and 30μm coronal sections were cut using a cryostat. Sections were collected for the prefrontal cortex, amygdala, and PAG in phosphate buffer solution (PBS). Free-floating brain sections were processed using a modified protocol provided by a commercial antibody staining kit (ABC kit, Vectastain Elite, Vector Laboratories, Burlingame, CA), which uses the diaminobenzedine-peroxidase

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method of visualizing antigen-binding sites. The sections were first incubated for 48 hours at 4°C in the primary antibody, rabbit anti-FOS (H-125, Santa Cruz Biotechnology Inc., Santa Cruz, CA), diluted 1:5,000 in PBS with Triton-X and 1% Normal Goat Serum. The sections were then rinsed and incubated with the secondary antibody (goat anti-rabbit, Vector Laboratories, Burlingame, CA) for one hour, and processed using the ABC kit protocol. Stained sections were mounted on vectabond-covered slides, dehydrated in alcohol, cleared in xylene, and cover slipped with DPX (Sigma Aldrich).

FOS Data Acquisition and Analysis

Positive labeled FOS like immunoreactive cells were visualized using a microscope (Nikon Labophot-2) with a 20x objective attached to a digital camera (Nikon DS-Fi1) connected to a computer. The subdivisions of the mPFC, amygdala, and PAG were determined with the cresyl violet stained sections using an atlas of the rat brain (Paxinos and Watson, 2007). All FOS positive cells were counted bilaterally in the relevant brain nuclei with NIS-Elements (Nikon, Melville, NY) by an experimenter blind to the condition of the specimen. For a cell to be considered FOS positive, it had to be distinct from the background regardless of the intensity of the staining. The profiles of FOS like immunoreactive cells in single optical planes were thus counted (Coggeshall and Lekan, 1996). Sections of different rats were matched for corresponding neuroanatomical levels and the mean number of cell counts per brain area was calculated by averaging the counts from all sections of each animal.

Histology

To verify cannula placements, 50μm coronalsections were taken through the mPFC, mounted on glass slides, stained with cresyl violet, and cover slipped. Cannula placement was determined for each rat using a brain atlas (Paxinos and Watson, 2007).

Behavioral Scoring

A digital camera (Sony DCR-SR45) was used to record behaviors. Video files were transferred to and scored in the Observer XT 7.0 (Noldus Information Technologies, Leesburg, VA). The scorer was blind to all conditions. The dependent variable quantifying fear was freezing. Freezing was defined as any posture in which the pup did not exhibit any movement except that necessary for respiration, and is expressed in the final analysis as a percentage of total observation time. In addition we measured other exploratory behaviors including: walking and sniffing around the cage, rearing (when they stand on their hind legs and sniff around), sniffing and walking around specifically the wire-mesh, rearing at the mesh and social interaction with littermates.

Statistical Analysis

All data were analyzed by factorial analyses of variance (ANOVA). For the freezing behavior and FOS cell counts in intact animals, one-way ANOVA’s were used with the stimulus type being the factor. Newman-Keuls tests were used for posthoc comparisons. In the studies where either the prelimbic or infralimbic structures were inactivated by muscimol injection, freezing and FOS counts were analyzed by two-way ANOVA’s with the factors being injection (vehicle/infralimbic, muscimol/infralimbic, vehicle/prelimbic, muscimol/prelimbic) and stimulus type. The Bonferroni method was used for posthoc multiple comparisons in these analyses.

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Results

Infant rats

Behavior—Infant rats with intact mPFCs were exposed to an age-specific threatening or non-threatening stimulus, an unfamiliar adult male, cat odor or a female rat. The behavioral response differed significantly across infant rats exposed to each of these stimuli **(ANOVA; $F(3,23) = 21.0$; $p < 0.001$).** Infant rats exposed to the male froze significantly more than rats exposed all other conditions and froze more to the cat odor more than to the female or the control condition (Newman-Keuls; $p < 0.001$ for the male vs control, female or cat odor; <. 05 for cat odor compared to the control and female, Figure 1A). Control rats did not differ from female exposed rats in their behavior.

FOS cell counts—A representative photomicrograph of FOS staining in the medial amygdala is shown in Figure 1B. The number of FOS positive cells in the infralimbic and prelimbic mPFC did not differ between control, female, cat odor or male exposed rats (Figure 1C). To map activation of other areas of the fear circuit, FOS expression was assessed in the amygdala and PAG (Figure 1D). Significant differences were found in the medial amygdala ($F(3,24) = 17.0$, $p < 0.001$), dPAG ($F(3,24=10.3$, $p<.001$) and vPAG ($F(3,24) = 23.20$, $p < 0.001$). Female and male exposure increased the number of FOS positive cells in the medial amygdala and dPAG compared to cat odor or controls ($p < 0.01$), which did not differ from each other. Male exposure increased FOS positive cells in the vPAG compared to cat odor or female exposed and control rats ($p <.001$ for each). Cat odor had higher levels of FOS positive cells than did the controls ($P<.05$) but not the female. Females and controls did not differ. The number of FOS positive cells did not differ across stimulus conditions in the basolateral/lateral amygdala.

Inactivation of the mPFC—To examine if the subdivisions of the mPFC are involved in fear regulation, the infralimbic and prelimbic mPFC were pharmacologically inactivated. Figure 2C shows the cannula placements for cannulas implanted in an angle to prevent damage to the overlying mPFC subdivisions. Cannula tips were located in the infralimbic and prelimbic mPFC (Figure 2C). Four rats were excluded because cannulas were located outside the target area. Inactivation of the infralimbic and prelimbic mPFC did not affect fear behavior (Figure 2A) and did not affect activation of the projection areas. The number of FOS positive cells in the amygdala and PAG did not differ between vehicle and muscimol injected rats (Figure 2B).

Juvenile rats

Behavior—Juvenile rats with intact mPFCs responded differently to cat odor, a male rat or control (empty cage) ($F(2,18) = 6.5$; $p < 0.01$). Rats exposed to cat odor froze significantly more than rats exposed to the unfamiliar male rat or controls ($p < 0.01$ and <0.05 respectively, Figure 3A). Controls did not differ from male exposed rats.

FOS cell counts—Exposure to the three stimuli differentially activated the subdivisions of the mPFC. There was no effect on the infralimbic mPFC but there was a significant main effect for the prelimbic mPFC ($F(2,18) = 17.1$; $p < 0.001$). Cat odor exposed rats expressed significantly more FOS positive cells than male or control exposed rats ($p < 0.001$, Figure 3B). The number of cells did not differ between male exposed and control rats. FOS expression differed in the mPFC projection areas as well (Figure 3C). Significant differences were found in the medial amygdala ($F(2,18) = 65.2$, $p < 0.001$), basolateral/lateral amygdala ($F(2,18) = 7.3$, $p < 0.01$) and vPAG ($F(2,18) = 10.6$, $p < 0.01$). Cat odor exposed rats had significantly more FOS positive cells in the medial amygdala than male ($p < 0.001$) and control exposed ($p < 0.001$) rats, with male exposed rats having higher expression than

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controls ($p < 0.001$). Male exposed rats had significantly more FOS positive cells in the basolateral/lateral amygdala ($p < 0.01$). Cat odor exposed rats had significantly more FOS positive cells in the vPAG ($p < 0.01$) compared to control ($p < .001$) and male exposed ($p < .01$) rats. There were no significant differences in the dPAG.

Inactivation of the mPFC—To investigate the role of the mPFC in fear regulation in juvenile rats, the infralimbic and prelimbic subdivisions were inactivated. The tips of the intracranial cannulas were located within the infralimbic and the prelimbic mPFC (Figure 4C). Two animals were excluded because cannulas were placed outside target area. Muscimol or vehicle was infused and rats were exposed to the adult male rat or cat odor. Inactivation of the infralimbic and prelimbic mPFC did not affect fear behavior (Figure 4A) and did not affect levels of FOS expression in the projection areas (Figure 4B).

Adolescent rats

Behavior—Cat odor was used as threatening stimulus and an unfamiliar adult non-lactating female rat as a non-threatening stimulus. Adolescent rats with intact mPFCs responded differently to the female rat, cat odor, and control (an empty cage) ($F(2,15) = 16.2$; $p < 0.001$). Rats exposed to cat odor froze significantly more than rats exposed to the female or controls ($p < 0.001$, Figure 5A). Control rats did not differ from female-exposed rats.

FOS cell counts—Exposure to the three stimulus conditions differentially activated the subdivisions of the mPFC. There was no effect on the infralimbic mPFC but there was a significant main effect for the prelimbic mPFC ($F(2,15) = 17.7$; $p < 0.001$). In the prelimbic mPFC, cat odor exposed rats expressed significantly more FOS positive cells than female or control exposed rats ($p < 0.001$, Figure 5B). Number of cells did not differ between female exposed and control rats. Significant differences were found in the medial amygdala ($F(2,18) = 25.4$, $p < 0.001$), basolateral/lateral amygdala ($F(2,18) = 11.1$, $p < 0.001$) and vPAG ($F(2,18) = 22.7$, $p < 0.001$). Cat odor exposed rats had significantly more FOS positive cells in the medial amygdala than female ($p < 0.001$) and control exposed ($p < 0.001$) rats, and female exposed rats had significantly higher expression than controls ($p < 0.01$, Figure 5C). Cat odor exposed rats had significantly more FOS positive cells in the basolateral/lateral amygdala than controls ($p < 0.001$) and female exposed rats ($p < 0.01$). There were no differences between control and female exposed rats in the basolateral/lateral amygdala. Cat odor exposed rats had significantly more FOS positive cells in the vPAG ($p < 0.001$) compared to control and female exposed rats. There were no differences in the dPAG.

Inactivation of the mPFC—The infralimbic and prelimbic subdivisions were pharmacologically inactivated to assess their role in fear regulation. The tips of the intracranial cannulas were located within the infralimbic and the prelimbic mPFC (Figure 6C). Six animals were excluded because cannulas were placed outside the target area. Muscimol or vehicle was infused and rats were exposed to the adult female rat or cat odor. For the freezing behavior, there was a significant interaction between the treatment and the stimulus type ($F(3,48)=6.99$, $p < .001$). Post test comparisons showed that inactivation of the infralimbic mPFC did not affect fear behavior (Figure 6A whereas rats infused with muscimol into the prelimbic mPFC froze significantly less when exposed to cat odor than all other groups exposed to the cat odor ($p < 0.001$, Figure 6A). There was no significant difference in the female exposed rats. Inactivation of the prelimbic mPFC did not affect FOS expression in the amygdala or dPAG but reduced FOS expression in the vPAG in the cat odor condition ($F(3,50)=4.29$, $p < 0.01$; Figure 6B). In the vPAG, rats infused with muscimol into the prelimbic mPFC and exposed to the cat odor had significantly fewer FOS positive cells than all other infused groups exposed to the cat odor [posthoc comparisons: vs

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prelimbic vehicle ($p < 0.001$), vs infralimbic muscimol ($p < 0.001$), and vs infralimbic vehicle ($p < 0.01$)].

To test if muscimol injection into the prelimbic mPFC had unspecific effects, behavior during a three-minute period immediately before stimulus presentation (empty experimental compartment) was assessed in muscimol and vehicle infused rats. The two groups did not significantly differ in freezing (muscimol: 3.1% ± 1.2, vehicle: 3.8% ± 1.3) or explorative behavior (muscimol: 25.2% ± 3.3, vehicle: 30.4% ± 2.8).

Discussion

Although young rats respond to threatening stimuli throughout development, the prefrontal cortex does not participate in fear behavior at very young ages but rather becomes progressively functional as the rats mature. By adolescence, the prelimbic mPFC contributes to unlearned fear behavior through modulation of PAG activity.

Infants

Infant rats froze when exposed to an unfamiliar adult male or cat odor but not to the female rat. Male rats are infanticidal to infants and pose a significant threat but less so to the infant protected in the burrow (Rosenberg, 1974; Brown, 1986; Mennella and Moltz, 1988); lactating females however, behave maternally towards pups (Beach and Jaynes, 1956). Infant rats thus exhibit a fear response selectively to threatening stimuli and not the unthreatening stimuli. Because the infant rats had no experience with adult male rats or cat odor, their fear response is unlearned and provides immediate protection from high risk encounters (Wiedenmayer, 2009).

Infant rats detect conspecifics by olfaction (Hepper, 1986; Chen et al., 2006). Chemosensory information is relayed to the amygdala, which is critically involved in the detection and assessment of threatening stimuli (Davis, 2000; LeDoux, 2000; Fanselow and Gale, 2003; Rosen, 2004). Male and female rat exposure activated the medial amygdala in infant rats but cat odor did not. It has been hypothesized that the medial amygdala processes socially relevant stimuli and assesses potential threats; it is considered the major amygdaloid nucleus for social recognition in mammals (Brennan and Kendrick, 2006; Sanchez-Andrade and Kendrick, 2009). Here we found specificity in the medial nucleus, which was activated by a conspecific independently of threat potential, but not activated by the cat odor. Whether this is a function of a live stimulus versus an odor or conspecific versus non-conspecific stimuli is not known. Nonetheless, these data imply a developmental continuity in the function of the medial amygdala. The amygdala projects to the PAG, which organizes defensive responding (LeDoux, 2000; Price, 2005). The ventrolateral columns of the PAG mediate passive defensive behaviors including freezing whereas the dorsolateral columns mediate active defensive behaviors such as flight (Keay and Bandler, 2001). Male and cat odor exposure increased FOS expression in the vPAG, consistent with the induction of freezing; lesions of the vPAG decrease male-induced freezing in infant rats (Wiedenmayer et al., 2000). In contrast there was a small increase in the number of FOS positive cells in the dorsal PAG, replicating the pattern seen in the medial amygdala.

The two subdivisions of the mPFC were not activated by female or male exposure and pharmacological inactivation did not affect FOS expression in the amygdala and PAG, or the behavioral response. Thus mPFC does not play a role in the regulation of unlearned fear in infant rats.

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Juveniles

Adult male rats do not pose an infanticidal threat to young rats after weaning (Paul and Kupferschmidt, 1975). Juvenile rats did not show fear during male exposure but did freeze when exposed to the odor of a natural predator, a cat. Under feral conditions, juvenile rats leave the natal burrow for brief periods of time (Boice, 1977; Galef, 1981), increasing predation risk (Caro, 2005). Juvenile rats thus respond appropriately to the threat of predation, as do adult animals (Apfelbach et al., 2005).

Cat odor increased FOS expression in the same regions of the amygdala and PAG as the adult male did for infant rats and as predator odor does in adult rats (Dielenberg et al., 2001; McGregor et al., 2004; Campeau et al., 2008), indicating developmental continuity in processing threatening stimuli. As in infant rats, the non-threatening stimulus activated the medial amygdala, demonstrating its role in the processing of ecologically relevant stimuli such as unfamiliar conspecifics. Unexpectedly, the adult male also induced FOS expression in the basolateral amygdala, an area typically involved in association and discrimination learning (Gall et al., 1998; Tronel and Sara, 2002; Kippin et al., 2003; Reijmers et al., 2007). Thus, basolateral activation may reflect the interaction of weaned rats with other colony members as they leave the natal burrow (Calhoun, 1963).

Exposure to cat odor activated the prelimbic but not infralimbic mPFC. This represents a significant change from infant rats and indicates that the mPFC is engaged in stimulus processing at this later age. However, the mPFC does not seem to play a role in fear behavior because mPFC inactivation did not affect amygdala or PAG activity or the behavioral response. Whether this is due to immaturity of the mPFC, its projections to the amygdala and PAG, or the inability of these latter two structures to respond to mPFC inputs is not known.

Adolescents

Adolescent wild rats leave the nest site to forage in novel environments and eventually disperse from the natal area (Calhoun, 1963; Galef, 1981). This transition is facilitated by increased motivation for exploration, novelty seeking and risk-taking (Spear, 2000), but increases vulnerability to predation (Caro, 2005). However, increased activity during adolescence brings young rats in contact with conspecifics other than mother and siblings (Calhoun, 1963; Galef, 1981). Accordingly, adolescent rats froze when exposed to cat odor but not to an unfamiliar female rat, demonstrating the ability to discriminate between threatening and non-threatening stimuli.

Like juveniles, cat odor exposure increased FOS expression in the prelimbic mPFC, medial amygdala and vPAG. Similar to infants and juveniles, exposure to the non-threatening stimulus activated the medial amygdala, again indicating that this nucleus processes not only aversive but more generally ecologically relevant stimuli.

In contrast to infant and juvenile rats, inactivation of the prelimbic mPFC decreased freezing to threat. Prelimbic mPFC inactivation also reduced FOS expression in the vPAG. Adult animals exposed to predator odor show similar activation patterns (Dielenberg et al., 2001; McGregor et al., 2004; Campeau et al., 2008). There are, however, also differences: predator odor induced FOS expression in the infralimbic mPFC (Staples et al., 2008) and the basolateral amygdala (Campeau et al., 2008; Staples et al., 2008) in the adult but not the adolescent. Therefore, the adolescent mPFC is not fully mature.

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Fear regulation across ages

The mPFC progressively matures during early ontogeny and becomes functional in adolescence where it mediates unlearned fear response to an ecologically relevant threat. Our data suggest a nuanced process by which the mPFC that continues to mature even through adolescence to engage more caudal structures and to modify freezing. Few other studies have examined the role of the mPFC in unlearned fear and those findings are not conclusive. Inactivation of the prelimbic mPFC by tetrodotoxin infusion in adult rats through a cannula placed between the two hemispheres did not affect freezing to a cat or open field activity (Corcoran and Quirk, 2007). This inactivation may be sufficient to block learned but not unlearned fear. Lesion studies have also produced mixed results. Prelimbic mPFC lesion in rats reduced (Maaswinkel et al., 1996) or increased anxiety in the elevated plus-maze (Jinks and McGregor, 1997), increased anxiety in the open field test (Jinks and McGregor, 1997) or had no effect (Burns et al., 1996; Maaswinkel et al., 1996). Lesion of both the infralimbic and prelimbic mPFC decreased fear behavior in the elevated plus-maze and open field (Lacroix et al., 2000). It remains to be investigated what features of a threatening situation are processed by the mPFC and what response patterns are regulated by it.

The accepted model of fear regulation is based on adult animals that possess a fully matured mPFC. In young animals, the mPFC is maturing and the existent neural circuitry regulates behavior specific for that age and ontogenetic niche. Components are added as the animal matures, leaves the protection of the nest and requires more complicated circuitry to allow for survival.

The mPFC did not play a role in unlearned fear in infant or juvenile rats. Even in the absence of a fully functional mPFC, pups froze to the threat on first exposure. Other areas of the fear circuit must mediate differential fear responsiveness at these ages. A possible mPFC-independent mechanism could involve subpopulations of neurons within the medial amygdala. Female and male odors activate different subdivisions of the medial amygdala in male rodents, indicating regional specialization of neurons in odor classification (Samuelsen and Meredith, 2009; Donato et al., 2010). Alternatively, other areas that were not examined here but have been implicated in unlearned fear (Mongeau et al., 2003; Engin and Treit, 2008) could contribute to the expression of freezing.

Finally, the model of mPFC fear regulation predicts that fear is promoted or inhibited through mPFC effects on amygdala, which in turn activates the PAG to co-ordinate both the physiological and behavioral responses to potential threats (An et al., 1998; Floyd et al., 2000; Keay and Bandler, 2001; Gabbott et al., 2005). The PAG matures within the developmental time frame that requires the animal to engage in defensive behavior. Lesions of the ventral PAG but not the lateral PAG suppressed freezing to a unfamiliar male rat at 14 days of age and injection of either kainate or a kappa opioid agonists enhanced defensive behavior at 14 but not 7 days of age, (Goodwin and Barr, 1998; Wiedenmayer et al., 2000; Goodwin and Barr, 2005). Here, the PAG showed higher levels of FOS expression in the infants to the appropriate threat, consistent with prior work (Wiedenmayer and Barr, 2001). Therefore we hypothesize that the PAG is poised first to engage in defense by the end of the second week of life.

In conclusion, the “fear” circuit develops in a complex stepwise fashion with the amygdala and PAG serving this function in infants. The prelimbic mPFC is activated for the first time in juvenile animals but does not participate fully in the circuit until adolescence.

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References

An X, Bandler R, Öngür D, Price JL. Prefrontal cortical projections to longitudinal columns in the midbrain periaqueductal gray in macaque monkeys. J Comp Neurol. 1998; 401:455–479. [PubMed: 9826273]

Apfelbach R, Blanchard DC, Blanchard RJ, Hayes RA, McGregor IS. The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neurosci Biobehav Rev. 2005; 29:1123–1144. [PubMed: 16085312]

Beach FA, Jaynes J. Studies of maternal retrieving in rats I: Recognition of young. J Mammal. 1956; 37:177–180.

Benes FM, Taylor JB, Cunningham MC. Convergence and plasticity of monoaminergic systems in the medial prefrontal cortex during the postnatal period: Implications for the development of psychopathology. Cereb Cortex. 2000; 10:1014–1027. [PubMed: 11007552]

Boice R. Burrows of wild and albino rats: effects of domestication, outdoor raising, age, experience, and maternal state. J Comp Physiol Psychol. 1977; 91:649–661. [PubMed: 559696]

Brennan PA, Kendrick KM. Mammalian social odours: attraction and individual recognition. Phil Trans R Soc B. 2006; 361:2061–2078. [PubMed: 17118924]

Bronson GW. The development of fear in man and other animals. Child Dev. 1968; 39:409–431. [PubMed: 4967765]

Brown RE. Social and hormonal factors influencing infanticide and its suppression in adult male Long-Evans rats. J Comp Psychol. 1986; 100:155–161. [PubMed: 3720284]

Burns BJ, Annett L, Kelley AE, Everitt BJ, Robbins TW. Effects of lesions to amygdala, ventral subiculum, medial prefrontal cortex, and nuccleus accumbens on the reaction to novelty: implication for limbic-striatal interactions. Behav Neurosci. 1996; 110:60–73. [PubMed: 8652073]

Calhoun, JB. The Ecology and Sociology of the Norway Rat. U.S. Department of Health, Education, and Welfare; Bethesda, MD: 1963. p. 288

Campeau S, Nyhuis TJ, Sasse SK, Day HEW, Masini CV. Acute and chronic effects of ferret odor exposure in Sprague-Dawley rats. Neurosci Biobehav Rev. 2008; 32:1277–1286. [PubMed: 18573530]

Canteras NS, Goto M. Fos-like immunoreactivity in the periaqueductal gray of rats exposed to a natural predator. NeuroReport. 1999; 10:413–418. [PubMed: 10203345]

Caro, T. Antipredator Defenses in Birds and Mammals. Chicago: The University of Chicago Press; 2005.

Chen SWC, Shemyakin A, Wiedenmayer CP. The role of the amygdala and olfaction in unconditioned fear in developing rats. J Neurosci. 2006; 26:233–240. [PubMed: 16399692]

Coggeshall RE, Lekan HA. Methods for determining numbers of cells and synapses: A case for more uniform standards of review. J Comp Neurol. 1996; 364:6–15. [PubMed: 8789272]

Corcoran KA, Quirk GJ. Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. J Neurosci. 2007; 27:840–844. [PubMed: 17251424]

Curio, E. Proximate and developmental aspects of antipredator behavior. In: Slater, PJB.; Rosenblatt, JS.; Snowdon, CT.; Milinski, M., editors. Advances in the Study of Behavior. Academic Press; 1993. p. 135-238.

Davis, M. The role of the amygdala in conditioned and unconditioned fear and anxiety. In: Aggleton, JP., editor. The Amygdala. 2. Oxford: Oxford University Press; 2000. p. 213-287.

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Deschamps S, Woodside B, Walker C-D. Pups presence eliminates the stress hyporesponsiveness of early lactating females to a psychological stress representing a threat to the pups. J Neuroendocrinol. 2003; 15:486–497. [PubMed: 12694374]

Dielenberg RA, Hunt GE, McGregor IS. 'When a rat smells a cat': The distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. Neuroscience. 2001; 104:1085–1097. [PubMed: 11457592]

Donato J, Cavalcante JC, Silva RJ, Teixeira AS, Bittencourt JC, Elias CF. Male and female odors induce Fos expression in chemically defined neuronal population. Physiol Behav. 2010; 99:67–77. [PubMed: 19857504]

Engin E, Treit D. The effects of intra-cerebral drug infusions on animals' unconditioned fear reactions: A systematic review. Prog Neuro-Psychopharmacol Biol Psychiatry. 2008; 32:1399–1419.

Fanselow MS, Gale GD. The amygdala, fear, and memory. Ann NY Acad Sci. 2003; 985:125–134. [PubMed: 12724154]

Floyd NS, Price JL, Ferry AT, Keay KA, Bandler R. Orbitomedial prefrontal cortical projections to distinct longitudinal columns of the periaqueductal gray in the rat. J Comp Neurol. 2000; 422:556–578. [PubMed: 10861526]

Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ. Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol. 2005; 492:145–177. [PubMed: 16196030]

Galef, BG. The ecology of weaning. In: Gubernick, DJ.; Klopfer, PH., editors. Parental Care in Mammals. New York: Plenum Press; 1981. p. 211-241.

Gall CM, Hess US, Lynch G. Mapping brain networks engaged by, and changed by, learning. Neurobiol Learn Memory. 1998; 70:14–36.

Goodwin GA, Barr GA. Behavioral and heart rate effects of infusing kainic acid into the dorsal midbrain during early development in the rat. Dev Brain Res. 1998; 107:11–20. [PubMed: 9602029]

Goodwin GA, Barr GA. Developmental changes in the behavioral and autonomic effects of kappa opioid receptor stimulation of the midbrain periaqueductal gray. Dev Psychobiol. 2005; 46:47–56. [PubMed: 15690388]

Hennessy MB, Weinberg J. Adrenocortical activity during conditions of brief social separation in preweaning rats. Behav Neural Biol. 1990; 54:42–55. [PubMed: 2378605]

Hepper PG. Parental recognition in the rat. Quart J Exp Psychol. 1986; 38B:151–160.

Hofer MA, Shair H. Sensory processes in the control of isolation-induced ultrasonic vocalization by 2-week-old rats. J Comp Physiol Psychol. 1980; 94:271–279. [PubMed: 7364999]

Jinks AL, McGregor IS. Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. Brain Res. 1997; 772:181–190. [PubMed: 9406971]

Keay KA, Bandler R. Parallel circuits mediating distinct emotional coping reactions to different types of stress. Neurosci Biobehav Rev. 2001; 25:669–678. [PubMed: 11801292]

Kim JH, Richardson R. New findings on extinction of conditioned fear early in development: Theoretical and clinical implications. Biol Psychiatry. 2010; 67:297–303. [PubMed: 19846065]

Kim JH, Hamlin AS, Richardson R. Fear extinction across development: The involvement of the medial prefrontal cortex as assessed by temporary inactivation and immunohistochemistry. J Neurosci. 2009; 29:10802–10808. [PubMed: 19726637]

Kippin TE, Cain SW, Pfaus JG. Estrous odors and sexually conditioned neutral ordors activate separate neural pathways in the male rat. Neuroscience. 2003; 117:971–979. [PubMed: 12654349]

Lacroix L, Spinelli S, Heidbreder CA, Feldon J. Differential role of the medial and lateral prefrontal cortices in fear and anxiety. Behav Neurosci. 2000; 114:1119–1130. [PubMed: 11142644]

LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci. 2000; 23:155–184. [PubMed: 10845062]

Maaswinkel H, Gispen W-H, Spruijt BM. Effects of an electrolytic lesion of the prelimbic area on anxiety-related and cognitive tasks in the rat. Behav Brain Res. 1996; 79:51–59. [PubMed: 8883816]

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McGregor IS, Hargreaves GA, Apfelbach R, Hunt GE. Neural correlates of cat odor-induced anxiety in rats: Region-specific effects of the benzodiazepine midazolam. J Neurosci. 2004; 24:4134–4144. [PubMed: 15115808]

Mennella JA, Moltz H. Infanticide in rats: male strategy and female counter-strategy. Physiol Behav. 1988; 42:19–28. [PubMed: 3387474]

Mongeau R, Miller GA, Chiang E, Anderson DJ. Neural correlates of competing fear behaviors evoked by an innately aversive stimulus. J Neurosci. 2003; 23:3855–3868. [PubMed: 12736356]

Morgan JI, Curran T. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Annu Rev Neurosci. 1991; 14:421–451. [PubMed: 1903243]

Neumann ID, Toschi N, Ohl F, Torner L, Krömer SA. Maternal defence as an emotional stressor in female rats: correlation of neuroendocrine and behavioural parameters and involvement of brain oxytocin. Eur J Neurosci. 2001; 13:1016–1024. [PubMed: 11264675]

Öhman A, Mineka S. Fears, phobias, and preparedness: towards an evolved module of fear and fear learning. Biol Rev. 2001; 108:483–522.

Paré D, Quirk GJ, LeDoux JE. New vistas on amygdala networks in conditioned fear. J Neurophysiol. 2004; 92:1–9. [PubMed: 15212433]

Paul L, Kupferschmidt J. Killing of conspecific and mouse young by male rats. J Comp Physiol Psychol. 1975; 88:755–763.

Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates. 6. San Diego: Academic Press; 2007.

Peters J, Kalivas PW, Quirk GJ. Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Memory. 2009; 16:279–288.

Price JL. Free will versus survival: Brain systems that underlie intrinsic constraints on behavior. J Comp Neurol. 2005; 493:132–139. [PubMed: 16255003]

Reijmers LG, Perkins BL, Matsuo N, Mayford M. Localization of a stable neural correlate of associative memory. Science. 2007; 317:1230–1233. [PubMed: 17761885]

Rosen JB. The neurobiology of conditioned and unconditioned fear: A neurobehavioral system analysis of the amygdala. Behav Cogn Neurosci Rev. 2004; 3:23–41. [PubMed: 15191640]

Rosenberg KM. Effects of pre- and postpubertal castration and testosterone on pup-killing behavior in the male rat. Physiol Behav. 1974; 13:159–161. [PubMed: 4859306]

Samuelsen CL, Meredith M. Categorization of biologically relevant chemical signals in the medial amygdala. Brain Res. 2009; 1263:33–42. [PubMed: 19368822]

Sanchez-Andrade G, Kendrick KM. The main olfactory system and social learning in mammals. Behav Brain Res. 2009; 200:323–335. [PubMed: 19150375]

Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev. 2000; 24:417–463. [PubMed: 10817843]

Staples LG, McGregor IS, Apfelbach R, Hunt GE. Cat odor, but not trimethylthiazoline (fox odor), activates accessory olfactory and defense-related brain regions in rats. Neuroscience. 2008; 151:937–947. [PubMed: 18201833]

Thor DH, Flannelly KJ. Age of intruder and territorial-elicited aggression in male Long-Evans rats. Behav Biol. 1976; 17:237–241. [PubMed: 986812]

Tronel S, Sara SJ. Mapping of olfactory memory circuits: Region-specific c-fos activation after odor-reward associative learning or after its retrieval. Learn Memory. 2002; 9:105–111.

Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. Nat Rev Neurosci. 2009; 10:397–409. [PubMed: 19469025]

van Eden CG, Kros JM, Uylings HBM. The development of the rat prefrontal cortex. Prog Brain Res. 1990; 85:169–183. [PubMed: 2094893]

van Schaik, CP.; Janson, CH., editors. Infanticide by Males and Its Implications. Cambridge: Cambridge University Press; 2000.

Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ. Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn Memory. 2006; 13:728–733.

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Wiedenmayer CP. Plasticity of defensive behavior and fear in early development. Neurosci Biobehav Rev. 2009; 33:432–441. [PubMed: 19073211]

Wiedenmayer CP, Barr GA. Developmental changes in c-fos expression to an age-specific social stressor in infant rats. Behav Brain Res. 2001; 126:147–157. [PubMed: 11704260]

Wiedenmayer CP, Goodwin GA, Barr GA. The effect of periaqueductal gray lesions on responses to age-specific threats in infant rats. Dev Brain Res. 2000; 120:191–198. [PubMed: 10775771]

Wiedenmayer CP, Magarinos AM, McEwen BS, Barr GA. Age-specific threats induce CRF expression in the paraventricular nucleus of the hypothalamus and hippocampus of young rats. Horm Behav. 2005; 47:139–150. [PubMed: 15664017]

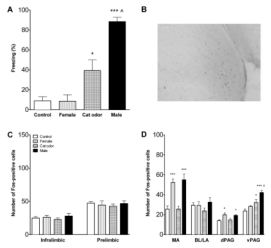

Figure 1.

Fear response and fos expression in infant rats with intact mPFC. **(A)** Percent time freezing (mean ± SEM) during exposure to an adult female rat, **cat fur odor**, or an adult male rat. Controls were exposed to an empty cage. Male-exposed rats froze significantly more than cat odor, female or control exposed rats ($p<.001$ for all). Pups exposed to the cat odor froze more than pups exposed to either the control or female ($p<.05$ for each). **(B)** Representative micrograph to show FOS staining in the medial amygdala of a male exposed 14 day old pup. **(C)** FOS expression in the mPFC subdivisions in infant rats with intact mPFC. There were no differences in the number of FOS positive cells (mean ± SEM) in infralimbic and prelimbic mPFC. **(D)** Activation of the amygdala and PAG in infant rats with intact mPFC. Exposure to the non-threatening stimulus (adult female) or the threatening stimulus (adult male), but not the cat odor, increased the number of FOS positive cells in the medial amygdala and dPAG. Exposure to the adult male and to a lesser extent the cat odor increased FOS in the ventrolateral PAG. MA: medial amygdala, BL/LA: basolateral/lateral amygdala, dPAG: dorsolateral PAG, vPAG: ventrolateral PAG. N=6–9 for all conditions. *** $p < 0.001$, **$P<.01$ compared to controls; ^ $p<.05$ compared to control or female exposed. Note the Y-axis scale differs for Figures 1, 3, and 5 for the prefrontal cortex FOS counts.

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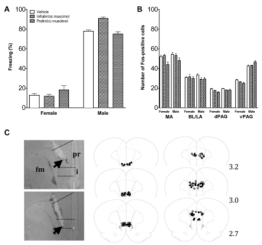

Figure 2.

Fear response and brain activation in infant rats infused with muscimol or vehicle in mPFC. **(A)** Percent time freezing in infant rats infused with muscimol or vehicle in mPFC and exposed to a non-threatening female or threatening male rat. Mean ± SEM. **(B)** Number of FOS positive cells in the amygdala and PAG of infant rats injected with vehicle or muscimol into infralimbic and prelimbic mPFC and exposed to a non-threatening female or threatening male rat. Mean ± SE. N = 6–9 in each condition. **(C)** Cannula placement in mPFC of infant rats. Photographs of cresyl violet-stained sections with placement in prelimbic (pr) and infralimbic (i) mPFC; fm: forceps minor corpus callosum; arrow: cannula tip. Drawing of placement in infralimbic (middle) and prelimbic mPFC (right). Open circles represent the location of cannula tips in rats infused with vehicle; filled circles represent the location of cannula tips in rats infused with muscimol. Numbers indicate the distance in millimeters from bregma.

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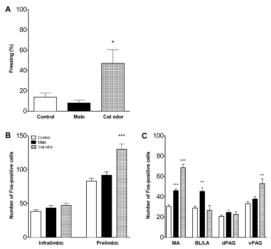

Figure 3.

Fear response and FOS expression in juvenile rats with intact mPFC. **(A)** Percent time freezing (mean ± SE) during exposure to an adult male rat or cat fur odor. Controls were exposed to an empty cage. Cat odor exposed rats froze significantly more than male or control exposed rats. **(B)** Activation of the mPFC in juvenile rats with intact mPFC. Number of FOS positive cells (mean ± SE) was significantly increased in prelimbic mPFC of rats exposed to the threatening stimulus (cat odor). **(C)** Activation of the amygdala and PAG in juvenile rats with intact mPFC. Exposure to the threatening stimulus (cat odor) increased the number of FOS positive cells (mean ± SE) in the medial amygdala and ventrolateral PAG. Exposure to the non-threatening stimulus (adult male) increased FOS in the medial and basolateral/lateral amygdala. MA: medial amygdala, BL/LA: basolateral/lateral amygdala, dPAG: dorsolateral PAG, vPAG: ventrolateral PAG, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared to control; ^p<.01 vs male exposure. N = 6–7 in each condition

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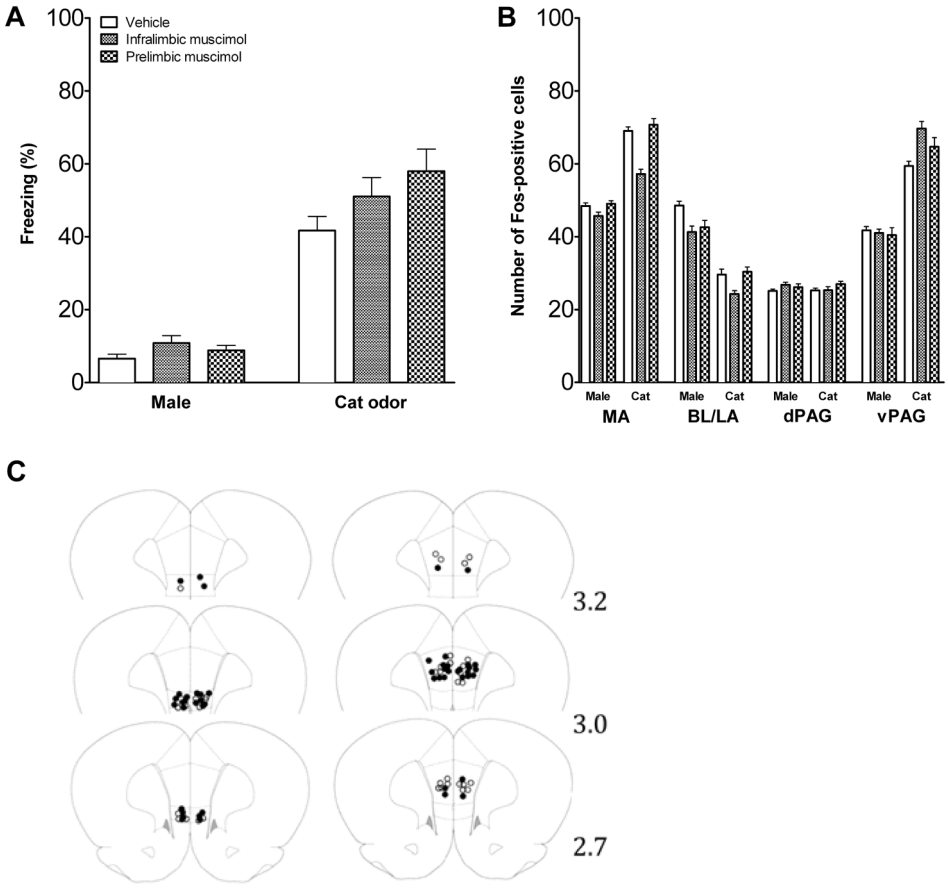

Figure 4.

Fear response and brain activation in juvenile rats infused with muscimol or vehicle in mPFC. **(A)** Percent time freezing in juvenile rats infused with vehicle or muscimol in mPFC and exposed to a non-threatening male or threatening cat odor. Mean ± SE. **(B)** Number of FOS positive cells in the amygdala and PAG of juvenile rats injected with vehicle or muscimol into infralimbic and prelimbic mPFC and exposed to a non-threatening male or threatening cat odor. Mean ± SE. N = 6–7 in each condition. **(C)** Cannula placement in infralimbic (left) and prelimbic mPFC (right) of juvenile rats. Open circles represent the location of cannula tips in rats infused with vehicle, and filled circles represent the location of cannula tips in rats infused with muscimol. Numbers indicate the distance in millimeters from bregma.

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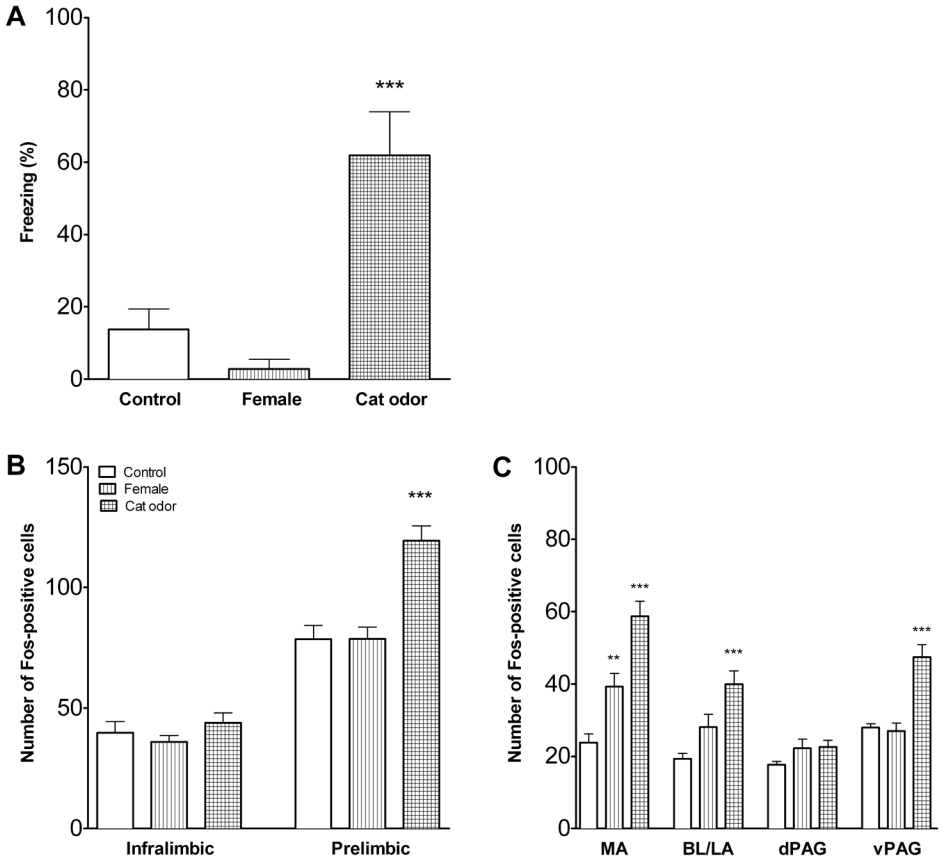

Figure 5.

Fear response and brain activation in adolescent rats with intact mPFC. **(A)** Percent time freezing in adolescent rats exposed to an adult female rat or cat fur odor. Controls were exposed to empty cage. Cat odor exposed rats froze significantly more than female or control exposed rats. N = 6–7 in each condition. **(B)** Activation of the mPFC in adolescent rats with intact mPFC. Number of FOS positive cells was significantly increased in prelimbic mPFC of rats exposed to the threatening stimulus (cat odor). N = 6 in each condition. **(C)** Activation of the amygdala and PAG in adolescent rats with intact mPFC. Exposure to the threatening stimulus (cat odor) increased the number of FOS positive cells in the medial and basolateral/lateral amygdala and vPAG and exposure to the non-threatening stimulus (adult female) increased FOS in the medial amygdala. MA: medial amygdala, BL/LA: basolateral/lateral amygdala, dPAG: dorsolateral PAG, vPAG: ventrolateral PAG, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, N = 7 in each condition.

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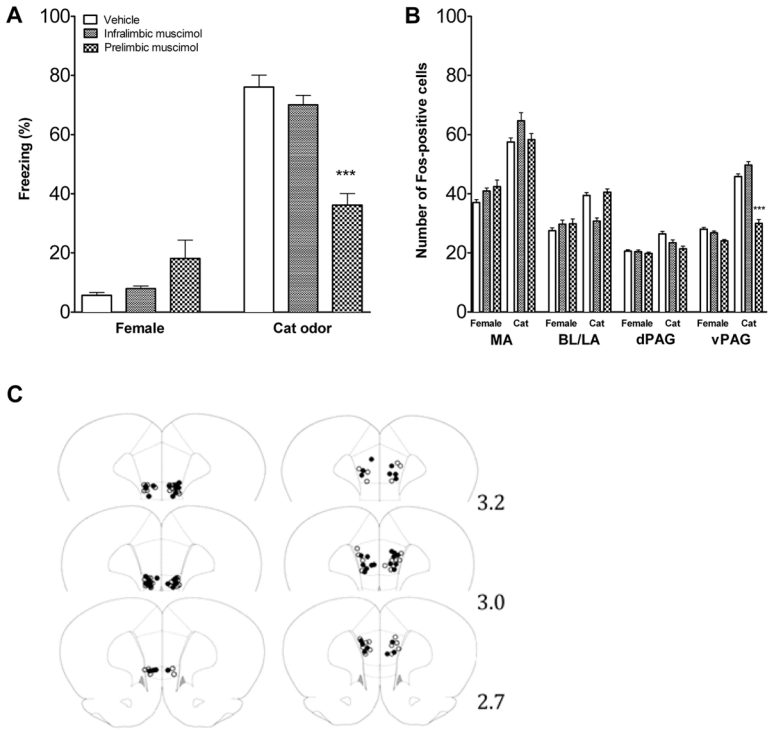

Figure 6.

Fear response and brain activation in adolescent rats infused with muscimol or vehicle in mPFC. **(A)** Percent time freezing in adolescent rats infused with vehicle or muscimol in the infralimbic and prelimbic mPFC and exposed to a non-threatening female or threatening cat odor (Mean ± SEM). There were no significant differences for rats infused in the infralimbic mPFC but when infused into the Prelimbic mPFC, freezing to the cat was significantly reduced ($p<.001$). **(B)** Number of FOS positive cells in the amygdala and PAG of adolescent rats injected with vehicle or muscimol into infralimbic or prelimbic mPFC and exposed to a non-threatening female or threatening cat odor. Mean ± SEM. FOS expression was selectively reduced in the vPAG only by prelimbic infustion ($p < 0.01$). N = 6–8 in each condition. **(C)** Cannula placement in infralimbic (left) and prelimbic mPFC (right) of adolescent rats. Open circles represent the location of cannula tips in rats infused with vehicle, and filled circles represent the location of cannula tips in rats infused with muscimol. Numbers indicate the distance in millimeters from bregma.