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Acute and chronic effects of ferret odor exposure in Sprague–Dawley rats

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Abstract

This manuscript describes several behavioral and functional studies evaluating the capacity of ferret odors to elicit a number of acute and long-term responses in male Sprague–Dawley rats. Acute presentation elicits multiple responses, suggesting that ferret odor, likely from skin gland secretions, provides an anxiogenic-like stimulus in this strain of rats. Compared to cat odor, however, ferret odor did not produce rapid fear conditioning, a result perhaps attributable to methodological factors. Inactivation of the olfactory system and medial nucleus of the amygdala, combined with induction of the immediate-early gene *c-fos*, suggest the necessity of the accessory olfactory system in mediating the effects of ferret odor. Repeated exposures to ferret odor produce variable habituation of neuroendocrine and behavioral responses, perhaps indicative of the lack of control over the exact individual origin or concentration of ferret odor. Ferret odor induces rapid and long-term body weight regulation, thymic involution, adrenal hyperplasia and facilitation of the neuroendocrine response to additional challenges. It is argued that the use of such odors is exquisitely suited to investigate the brain regions coordinating anxiety-like responses and the long-term changes elicited by such stimuli.

Keywords

Predator; Odor; Ferret; Corticosterone; ACTH; Heart rate; Habituation; Sensitization; Olfactory bulb; Medial amygdala

1. Introduction

Predators, and some of the cues associated with them, have been employed over many years to study stress and anxiety-like processes in rodents (see Blanchard and Blanchard, 2003; Blanchard et al., 1990; Dielenberg and McGregor, 2001, for reviews). Cues associated with predators are particularly advantageous in the study of responses traditionally linked with anxiety as they usually produce their effects without clear pain or prior training. These conditions therefore provide an alternative to investigate the brain circuits and neurotransmitter systems activated during the elaboration of these responses. Predatory stimuli can judiciously be employed to test predictions from other traditional rodent anxiogenic models (for example, fear of open places studied with open fields and elevated mazes, and conditioned or learned fear obtained with Pavlovian techniques).

With this ultimate objective in mind, we have begun to employ ferret *odor* as a relatively simple and potent stimulus in laboratory rodents to investigate the brain regions and neurotransmitter

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systems directly associated with the constellation of behavioral, neuroendocrine, and autonomic responses it triggers. Our strategy is to activate the relatively discrete olfactory system with ferret odor to help pinpoint the brain regions that may control a number of effector systems traditionally associated with stress and anxiety responses. Although a number of regions are suspected in modulating and mediating anxiety responses, these regions are still only crudely defined (Brandao et al., 2008; Charney and Deutch, 1996). Here, we provide a synopsis of our behavioral and functional studies evaluating the effects of acute or chronic ferret odor exposure in male rats. Our findings generally point to similar responses and functional circuitry reported with the use of other rodent predators or their odors. Ferret odors thus provide an alternative potent stimulus in the elicitation of stress and anxiety-like responses in Sprague–Dawley rats. These are preliminary and necessary stages of a systematic study of the brain regions responsible for coordinating anxiety-like responses, and the determination of putative sites of plasticity associated with some of the long-term changes produced by predator odor exposures. The generality of these findings can then be tested against more complex situations more akin to human anxiety disorders.

2. Acute behavioral, endocrine, and autonomic responses to ferret odor

Ferret odor was examined as a naturalistic stressor in the rat. As a member of the carnivorous family *Mustelidae*, ferret (*Putorius putorius furo*) is a natural predator of rats (Apfelbach, 1978; Rusiniak et al., 1976). It was previously determined that rats exposed to live ferrets exhibit elevated levels of plasma corticosterone and adrenocorticotropin hormone (ACTH; Anisman et al., 1997; McIntyre et al., 1999; Plata-Salaman et al., 2000), and defensive behaviors (Plata-Salaman et al., 2000), indicative of a stress response. To our knowledge, however, ferret *odor* had not previously been used as a stressor. Ferret odor is generally collected by placing a small cotton hand towel in a cage with 1 male and 1 female adult ferret for approximately 1 month. Contrary to most ferret pets, these animals were “undescented”, in that their anal scent glands were not excised. The obtained towels are then cut into 5 cm × 5 cm square pieces, sealed in a plastic bag, and then placed in an –80°C freezer until used. Throughout most of our studies, control towels are obtained by soaking new cotton towels in 10% chlorine bleach, rinsing with water, air drying, and sealing in a plastic bag until used. Although the smell provided by this “control” procedure is likely novel to naïve male rats, many of our studies have also compared the effects of ferret odor with an additional novel odor, that of strawberry extract (McCormick & Co. Inc., Hunt Valley, MD), which is generally applied (50 µl) to the control towels described above. Control (bleached and/or strawberry) and ferret odor towels are transported to the experimental rooms immediately before testing inside sealed glass bell jars.

Our initial studies involved the independent determination of the effects of acute ferret odor presentations in a defensive withdrawal apparatus paradigm (see Fig. 1) and in a small “mouse” cage to evaluate ferret odor-induced behavioral and neuroendocrine responses, respectively (Masini et al., 2005). These independent determinations were important for several reasons. For behavioral determination, it was best to perform the defensive withdrawal test in the subjective day of the rats in order to capture the largest number of behaviors possible, but this particular time is also associated with higher basal levels of corticosterone and ACTH, making these neuroendocrine determinations less sensitive. In addition, the relative level of behavioral “control” (withdrawing to the small hide box or avoiding the odor-containing corner, for example) over the situation in the defensive withdrawal paradigm could lead to very different endocrine results when compared to a situation in which odors are relatively inescapable (Dielenberg et al., 2001b; File et al., 1993). For this reason, rats were housed in small mouse polycarbonate cages (28 cm × 18 cm × 14 cm) with their usual food and water supply overnight for morning endocrine determination following 30 min towel placement in the cage without additional handling (see Masini et al., 2005, for additional experimental details). Many

avoidance behaviors were observed in the defensive withdrawal apparatus when rats were exposed to ferret odor, as compared with control odors, for 10 min (see Masini et al., 2005, 2006a). Behaviors such as the number of visits, contacts and total time spent with odor towels are reliably reduced in the presence of ferret odors. On the other hand, time spent in the hide box and the number of scanning bouts (risk assessment behaviors) is increased in the presence of ferret odor as compared to control odors. A behavior that is not routinely observed in response to ferret odor is freezing, at least in the defensive withdrawal apparatus. Freezing may be expressed at a higher rate in the small cages, but this is difficult to accurately quantify given the low behavioral output even in control animals. An additional behavior noted but not quantified during studies in small cages, and more recently in home cages, containing “saw dust” bedding was a deliberate attempt at burying the towels with the bedding material, another index of defensive behavior.

As shown in Fig. 2, ferret odor additionally elicits very sizable and significant hypothalamo–pituitary–adrenocortical (HPA) axis activation with moderate levels of plasma ACTH and corticosterone, as compared with various control odors including isopentyl acetate and butyric acid ($n = 4$ per group; see also Masini et al., 2005), when presented in the small mouse cages described above. The novelty of even strong, rancid (butyric acid), odors is thus not sufficient to elicit HPA axis activation under these conditions. An additional experiment focused on HPA axis determination (Masini et al., 2005) was also conducted to determine more exactly the source of the anxiogenic ferret odor; the towels obtained likely contain a variety of molecules derived from feces, urine, anal scent gland, other skin glands and hair products, with any single one of those contributing uniquely, or perhaps in combination, to elicit the anxiogenic reactions observed in rats. Interestingly, only ferret hair, when tested independently, induced HPA axis activation (Masini et al., 2005). In that respect, this is very similar to the report that cat hair is more potent than their urine or feces at eliciting defensive responses and contextual conditioning (Blanchard et al., 2003c). Additionally, our results of weaker HPA axis activation with ferret hair (Masini et al., 2005) is also consistent with the report that cat hair is not as potent as worn cat collars (Dielenberg et al., 2004). These findings may suggest that excretions from predator skin glands are the main source of stress-inducing odorants in rats.

Autonomic activation has also been reported in the presence of cat odors in rats (Dielenberg et al., 2001a, 2004). In order to test putative autonomic activation by ferret odors, a recent study (Masini et al., 2006b) was devised in which rats were abdominally implanted with telemetric devices (E-Mitters—PDT-4000, Mini Mitter/Respironics Co., Bend, OR) allowing simultaneous measurement of heart rate and core body temperature. Following approximately 1 month’s recovery from surgery, implanted rats were moved, in their home cages (with food and water *ad lib*), in a different laboratory room equipped with individually ventilated and illuminated sound-attenuating boxes (double plywood boxes insulated with 1.5 cm Celotex™ material—internal diameter of 60 cm (w) $\times$ 38 cm (d) $\times$ 38 cm (h)) containing Mini Mitter ER4000 energizers (the devices that wirelessly “capture” the physiological data from the implanted E-Mitters) inside the boxes’ floor, overnight. The next morning, individual sound-attenuating boxes were opened and closed quietly and gently to hang ferret ($n = 16$) or strawberry control odor towels ($n = 16$) on each end of the rats’ home cages for 30 min. Physiological responses (heart rate and core body temperature) were recorded remotely on a computer via the VitalView acquisition system (Mini Mitter/Respironics) without disturbing the rats, every min for the duration of towel exposures, and 30 min prior to and following odor exposures, respectively. The baseline temperature and heart rate were not different between groups. As Fig. 3 indicates, ferret odor exposure induced a significant and sustained increase in body temperature compared to strawberry odor exposure during and after the odor exposure. Heart rate was also significantly accelerated during and after ferret odor compared to the novel control strawberry odor (Masini et al., 2006b). The size and the sustained characteristic of the heart rate and body temperature responses are reminiscent of the sustained arterial blood

pressure (Dielenberg et al., 2001a) and heart rate responses (Dielenberg et al., 2004) previously reported in response to cat odor exposures.

In summary, acute ferret odor exposure produces a collection of behavioral, autonomic and endocrine responses that are traditionally associated with stress and anxiety. This is similar to rat defensive behaviors reported in response to other predator odors (Dielenberg et al., 2001a; Dielenberg and McGregor, 2001; Hotsenpiller and Williams, 1997; Morrow et al., 2000; Wallace and Rosen, 2000). A critical test awaiting evaluation is the capacity of traditional anxiolytics, such as benzodiazepines, to reverse ferret odor-induced responses, as demonstrated with cat odors, at least on behavioral defensive responses (Blanchard et al., 1998a; Dielenberg et al., 1999; McGregor and Dielenberg, 1999; McGregor et al., 2004; Staples et al., 2005).

3. Acute regional brain responses to ferret odor

The brains of rats exposed to control or ferret odors for 30 min in small cages (see above) were collected immediately following towel exposures for determination of immediate-early genes (IEG—*c-fos*) activation (Masini et al., 2005). This strategy permitted an initial evaluation of the brain circuits activated by ferret odor, together with comparisons with similar studies using cat odors (Blanchard et al., 2005; Canteras et al., 1997; Canteras and Goto, 1999; Dielenberg et al., 2001b; McGregor et al., 2004), 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), a component of fox feces (Day et al., 2004; Funk and Amir, 2000; Redmond et al., 2002; Rosen et al., 2005), and some of our previous studies with the non-predatory loud noise stress (Burow et al., 2005; Campeau and Watson, 1997).

Many brain areas displayed higher levels of *c-fos* mRNA induction to ferret compared with control odor (see Table 1). Regions of the brain that are routinely associated with HPA axis activity express ferret odor-induced *c-fos* induction; among these, the bed nucleus of stria terminalis, cingulate cortex, lateral septum, and paraventricular nucleus of the hypothalamus were most prominent. As with a number of other stressors, ferret odor induces *c-fos* mRNA in a number of additional areas, including the orbitofrontal cortex, piriform cortex, anterodorsal preoptic, dorsomedial and ventromedial nuclei of the hypothalamus, the dorsal premammillary nucleus, supramammillary nucleus, basolateral and posterodorsal medial amygdala, and periaqueductal gray (Campeau et al., 1997; Campeau and Watson, 1997; Chen and Herbert, 1995; Cullinan et al., 1996, 1995; Dielenberg et al., 2001b; Imaki et al., 1993; Melia et al., 1994; Pace et al., 2005). In addition, the similarities between *c-fos* IEG induction between cat and ferret odors is striking; regions such as the lateral septum, dorsomedial hypothalamus, ventromedial hypothalamus, premammillary nucleus, posteroventral medial nucleus of the amygdala, and periaqueductal gray (dorsolateral, dorsomedial, and ventrolateral parts) all displayed high levels of cat and ferret odor induced *c-fos* (Dielenberg et al., 2001b). On the other hand, little induction was observed in the lateral hypothalamic area, anterodorsal medial, posteromedial cortical amygdala, and central amygdala (Dielenberg et al., 2001b).

Additional areas that displayed induction to ferret and cat odors, especially when rats could not hide or escape from the cat odors (McGregor et al., 2004), included the prelimbic cortex, basolateral amygdala, and cuneiform nucleus. Thus, in addition to being relatively similar to cat odor, ferret odor elicits a pattern of *c-fos* mRNA induction that includes many of the same brain regions activated by forced swim, restraint, and loud noise, especially in limbic areas and the hypothalamus (Burow et al., 2005; Campeau and Watson, 1997, 2000; Chowdhury et al., 2000; Cullinan et al., 1996, 1995; Melia et al., 1994). Although many of the same regions appear to be influenced by a variety of stress conditions, the extent to which the same cell populations are activated within any one region by different stressors is unknown, which could best be determined by detecting brain activity patterns from at least two different stressors in the same rats simultaneously. Nevertheless, these findings support the view that a relatively

similar set of brain regions are activated by different innate or unconditioned stress situations, which provides a neuroanatomical basis for the similar behavioral, autonomic, and neuroendocrine responses triggered by these very different stimuli.

4. Brain regions mediating acute ferret odor-induced stress responses

A number of laboratories have begun the identification of brain regions associated with defensive behavioral responses to predators or their odors. For instance, Fendt et al. (2003, 2005) have reported an important role of the anterior bed nucleus of the stria terminalis in some of the behavioral effects induced by TMT. Another structure often associated with behavioral responses to cat or cat odor exposures is the hypothalamic dorsal premammillary nucleus (Blanchard et al., 2005, 2003b; Markham et al., 2004). The periaqueductal gray region also appears to be associated with some behavioral and autonomic responses induced by cat odors (Dielenberg et al., 2004). A number of studies have also associated relatively specifically the medial amygdaloid nucleus in defensive behavioral responses induced by cat or cat odor, or TMT exposures (Blanchard et al., 2005; Li et al., 2004; Muller and Fendt, 2006). Given the emerging circuits controlling a variety of predator-induced defensive responses, we targeted the medial amygdaloid nucleus in our studies of the effects of ferret odors due to its well-known reception of odor information from a number of main and accessory olfactory regions, and its putative position to relay this information to regions controlling different effector response systems (Canteras et al., 1995).

An initial study evaluated the effects of large medial amygdaloid nucleus excitotoxic lesions on HPA axis activation and behaviors in the defensive withdrawal paradigm in response to ferret odors. Excitotoxic lesions were targeted at the medial nucleus of the amygdala (3.0 mm anterior, 3.3 mm lateral, and 8.3 mm ventral to Bregma) via injections of 0.3 μ l/side of ibotenic acid in 30 male Sprague–Dawley rats (Harlan), with additional control animals ($n = 21$) receiving a similar volume of ACSF in the same location. After at least 1 week recovery, rats were first tested in the defensive withdrawal paradigm, as described above during their subjective day time (7:00–10:00 p.m.), in a counterbalanced design in which some lesioned and control rats were presented with either strawberry or ferret odors. Twenty-four to forty-eight hours later, the same rats were transported to the laboratory the night before exposure to strawberry or ferret odors in their home cages the next morning. Following 30 min exposure to control or ferret odors, rats were immediately sacrificed, with trunk blood and brains being collected for analysis of plasma corticosterone and ACTH. Lesion location was assessed following brain sectioning and immunohistochemical detection of NeuN in the region of the medial nucleus of the amygdala. As shown in Fig. 4, complete bilateral medial amygdala lesions (see Fig. 5) significantly disrupted behavioral defensive responses to ferret odors, without modifying behaviors elicited by strawberry odor. Likewise, these lesions prevented the sizable corticosterone and ACTH release normally elicited by ferret odor, as displayed in Fig. 6. Together with findings from cat and TMT odor exposures, these results suggest that the medial nucleus of the amygdala is part of a necessary circuit mediating the effects of predator odors on multiple response systems.

In order to determine the necessary source of olfactory information ultimately reaching the medial amygdaloid nucleus, we have begun an evaluation of the relative contributions of the main and accessory olfactory pathways in transmitting the signal elicited by ferret odor-containing towels. Although recent evidence (Pro-Sistiaga et al., 2007) suggests that the termination of the main and accessory olfactory systems are not as segregated as previously argued (Scalia and Winans, 1975; Winans and Scalia, 1970), subregions of the medial amygdaloid nucleus are still reported to receive relatively specific afferent innervation from the accessory olfactory bulb (Mohedano-Moriano et al., 2007). Prior work by Dielenberg et al. (2001b), Dielenberg and McGregor (2001), and McGregor et al. (2004) indicated that the

posteroventral nucleus of the medial amygdala is preferentially activated by cat odors, a finding that we partially replicated with ferret odors, although additional regions of the medial amygdala (posterodorsal nucleus) and posterolateral cortical amygdala also displayed reliable *c-fos* mRNA induction (Masini et al., 2005). Interestingly, McGregor's group has recently reported specific activation of the *posterior* aspect of the accessory olfactory bulb (McGregor et al., 2004), which, in addition to projecting to the posteroventral and -dorsal medial amygdaloid nuclei, is also reported to project more specifically to the dorsal anterior amygdala, deep cell layers of the bed nucleus of the accessory olfactory tract and the anteroventral medial amygdaloid nucleus (Mohedano-Moriano et al., 2007). These results may indicate that the accessory olfactory system, via vomeronasal stimulation, is responsible for the detection of cat and, presumably, ferret odors, as previously suggested (Dielenberg et al., 2001b; Dielenberg and McGregor, 2001; McGregor et al., 2004). However, the main olfactory bulb is consistently activated by these odors as well (Dielenberg et al., 2001b; Masini et al., 2005; McGregor et al., 2004), making a clear distinction between the main and accessory olfactory system difficult on the basis of immediate-early genes activation alone.

To test the relative contributions of the main and accessory olfactory systems, we have recently inactivated the main olfactory bulb by injecting 20 male Sprague–Dawley rats (Harlan Co.) with 10% zinc sulfate intranasally, until two drops were seen exiting the nose. Zinc sulfate is immediately lethal if the solution reaches the airways, but otherwise, the animals recover from this procedure without obvious signs of discomfort or illness, which is essential for the behavioral testing. Importantly, this procedure rendered most treated rats anosmic, as indicated by a complete failure to find a piece of Oreo™ cookie buried under 2 cm of saw dust in a clean cage 24 h after treatment, while all control rats found the palatable food within 2 min. However, these anosmic rats still displayed a very robust and similar corticosterone response to a 30-min ferret odor exposure in their home cages, as Fig. 7 demonstrates. These same rats were sacrificed immediately following towel exposure so as to verify main and accessory bulb *c-fos* mRNA induction, and as indicated in Fig. 7, rats treated with zinc sulfate had a nearly complete lack of induction in the main olfactory bulb as compared to saline-treated rats. The only *c-fos* mRNA induction detectable in the olfactory bulb of zinc sulfate-treated rats was in the region of the accessory olfactory bulb (AOB—Fig. 7). These results are consistent with the hypothesis that the accessory olfactory system is sufficient to mediate the effects of predator odors, which we are currently testing by removing the vomeronasal organ in male rats. However, these preliminary results do not rule out a potential role of the main olfactory system just yet.

5. Subacute and chronic effects of ferret odor on neuroendocrine and physiologic responses

Prior work with predators and their odors in rodents indicates that repeated (sometimes single) exposures to these stimuli produce a number of effects ranging from habituation and sensitization, to conditioned fear (Adamec et al., 1998, 2005; Adamec and Shallow, 1993; Blanchard et al., 2003a,c, 1990a,c, 1998b, 2001; Dielenberg and McGregor, 1999; Endres and Fendt, 2007; Figueiredo et al., 2003; File et al., 1993; McGregor et al., 2002; Takahashi et al., 2005; Wallace and Rosen, 2000; Zangrossi and File, 1994, 1992), that have been shown to be sustained and long-lasting in some instances. While repeated exposure to live cat is reported to produce little behavioral habituation in rats (Blanchard et al., 1998b; Figueiredo et al., 2003), their odors, which are thought to provide only a “partial predator stimulus” (Dielenberg and McGregor, 2001), produce variable development of clear behavioral (Blanchard et al., 1990; Dielenberg and McGregor, 1999; File et al., 1993; Takahashi et al., 2005; Zangrossi and File, 1994, 1992) or neuroendocrine habituation (File et al., 1993). It should be noted that in cases where behavioral and neuroendocrine response habituation to cat odors were observed (Dielenberg and McGregor, 1999; File et al., 1993), rats often had control over the odor

exposures by being allowed to “hide” in a box as part of the experimental apparatus, thus perhaps avoiding the predator odor. Our initial studies with repeated ferret odor exposures suggested limited, if any, behavioral and neuroendocrine response habituation (Masini et al., 2006a). As discussed above, the element of control was limited in our neuroendocrine determination by presenting ferret odor in small mouse cages that made the odors difficult to avoid. More recently, additional repeated ferret odor exposures were conducted in male Sprague–Dawley (Harlan) rats (11 consecutive 30 min daily exposures to ferret odor or strawberry towels in large home cages), but in this case, plasma corticosterone and ACTH release did show reliable habituation (Fig. 8, n 's = 8 per group, $p < 0.05$). In addition, these repeated ferret odor exposures induced reliable physiologic changes in body, thymus, and adrenal gland weights (see Table 2) that are rapidly produced and relatively long-lasting (see below), as reported with cat odors (Blanchard et al., 1998b). The variability in neuroendocrine habituation may be related to the specific stimulus employed. The towels that are obtained from the ferret breeders or owners, over time, contain scent from a number of different ferrets. Some of the recent results from Ian McGregor's laboratory (see accompanying manuscript in this issue) indicate that rats are able to distinguish odors from different cats. It is therefore conceivable that if our towels contain scent from multiple ferrets that are presented randomly, these may be less conducive to habituation, whereas if towels were derived from the same ferret, habituation may take place more readily. Alternatively, it is conceivable that the concentration of ferret scents varies between different experiments, such that smaller concentrations of ferret odors might be more conducive to habituation than higher concentrations, as demonstrated with cat odors (Takahashi et al., 2005). These hypotheses need to be tested, at least partially, by keeping track of the specific origin (i.e., ferret) of the towels obtained and employing towels from the same or different ferrets repeatedly.

Live predators are powerful stimuli that even with short exposures can lead to reliable and relatively long-lasting changes (often seen as facilitation or sensitization) in behavioral and neuroendocrine responses in a variety of situations (Adamec et al., 1998, 2007, 2005, 1999; Adamec and Shallow, 1993; Blanchard et al., 1998b; Blundell et al., 2005; Figueiredo et al., 2003). Whether such sensitizing actions take place with predator odors alone has not been investigated extensively, although some reports with cat odor (Adamec et al., 2006) and our work with ferret odor (Masini et al., 2006a) suggest that this might be the case. For instance, a single exposure to ferret odor led to higher plasma corticosterone and ACTH release in response to an additional 30 min exposure to ferret odor 11 days later, compared to a group of rats exposed to ferret odor for the first time and another group of rats repeatedly exposed (seven daily sessions of 30 min each) to ferret odor (Masini et al., 2006a). Because some habituation to the ferret odor might have counteracted a putative sensitizing effect in the group receiving additional (6) ferret odor exposures (homotypic challenge), we have revisited this effect using a different challenge stressor (heterotypic challenge) following ferret odor exposures; the possible confounding effects of habituation to the homotypic challenge stimulus was thus avoided.

In this recent study, male Sprague–Dawley (Harlan) rats were exposed to control or ferret odors ($n = 8$ per group) for four successive 60-min daily sessions in their home cages. Body weights were measured prior to any odor exposure and again after each daily exposure. One day after the last ferret or control (strawberry) odor exposure, rats were placed in a novel environment (open white round 5-gallons bucket) for 30 min, and were then rapidly sacrificed to collect trunk blood for measurement of plasma corticosterone and ACTH, and the thymi and adrenal glands were dissected for weighing. As Table 3 indicates, a reliably higher level of corticosterone was observed in rats previously experiencing the ferret odor repeatedly, suggesting facilitation or sensitization of the HPA axis. The apparent plasma ACTH difference did not reach statistical significance. In addition, by the second exposure, ferret odor-exposed rats weighed reliably less than strawberry odor-exposed rats, and this weight difference was

maintained up to seven days following the last exposure in a subsequent study (data not shown). It should be noted that ferret- or control-treated non-stress groups on the last experimental day were not included in the study reported in Table 3, as these groups were observed in other pilot studies not to differ on basal hormonal measures (data not shown). The above long-lasting effects on weight gain that outlast stress exposure previously were reported in response to a relatively short-term (3×3 h) regimen of restraint (Harris et al., 2004; Harris et al., 2006). An additional index of physiologic challenge was a reliable thymic involution in rats pre-exposed to ferret odor (see Table 3). The apparent increase in adrenal weight in this study did not reach statistical significance. These organ differences are observed in pre-exposed rats independent of stressor presentation on the sacrifice day (data not shown). Similar physiologic regulation have been observed in different prey species to a variety of predator odors, in addition to changes in reproductive hormones, reproductive success and developmental maturation, although most of these results were observed with much longer-term treatments (weeks to months—see Apfelbach et al., 2005, for a review). Clearly, additional parametric studies are necessary to tease apart the emergence of various physiologic effects of ferret odors and their association with the type and duration of facilitated responses. Importantly, relatively short-term predator odors exposure appears sufficient to produce effects that were mostly reported with live predators or with long-term predator odor exposures; use of relatively short-term, specific and unimodal olfactory cues may thus facilitate the delineation of the neurocircuitry and molecular mechanisms of response sensitization.

One curious feature of ferret odor has been our lack of evidence of contextual *conditioned* effects (Masini et al., 2006a), which are relatively widespread in response to cat odors (Blanchard et al., 2003c, 2001; Dielenberg et al., 1999; Hubbard et al., 2004; McGregor et al., 2002; Staples et al., 2005; Staples and McGregor, 2006; Takahashi et al., 2005), and more limited in response to TMT (Endres and Fendt, 2007; Rosen, 2004). A potential explanation for the apparent lack of ferret odor-induced context conditioning may have to do with the exact experimental conditions employed in our laboratory. Indeed, one of the possible problems with demonstrating conditioning with TMT has been the size and type of apparatus employed in producing conditioning (Endres and Fendt, 2007; Rosen, 2004). For instance, sizable conditioned freezing in rats was observed in a large defensive withdrawal apparatus 24 h following exposure to TMT or cat odor, but not when the exposure and test took place in a smaller environment (Rosen, 2004). As discussed above, there was very little freezing observed in the defensive withdrawal paradigm in response to ferret odor. Additional apparatus configurations will therefore need to be tested to evaluate more fully the putative conditioning properties of ferret odor.

6. Conclusions

Work in our laboratory has demonstrated that the skin/fur odor of ferrets elicits behavioral, neuroendocrine, and autonomic responses indicative of stress and anxiety-like states. This olfactory stimulus provides an alternative to cat odor, as these appear to produce variable or weaker responses, especially in the Sprague–Dawley strain of rats (Staples and McGregor, 2006), which initially motivated us to look for different predator odors due to our inconsistent results with cat scent. The similarities between the behavioral, neuroendocrine, autonomic and brain immediate-early genes responses, and neural structures mediating the effects of cat and ferret odors are remarkable and strongly suggest that these stimuli act in very similar ways on the nervous system. Thus, although the issue of rat strain susceptibility to different predator odors needs to be kept in mind in the design of any study (Rosen et al., 2006), these differences in no way invalidate the usefulness of predator odors. Furthermore, peripheral olfactory factors (olfactory receptor expression) are likely the source of these differences, as predator odor responders and non-responders from the same strain do not differ on other tests used to model anxiety (Hogg and File, 1994). The most notable difference observed between cat and ferret

odors thus far is the rapid conditioning property of cat odor that we have failed to observe with ferret odor. Given some of the methodological issues regarding conditioning that are emerging with other putative predator odors (Endres and Fendt, 2007; Rosen, 2004), this question clearly needs additional attention. It will also be important to determine if the various effects of ferret odor are sensitive to anxiolytic agents, given the variable effects of benzodiazepines in different laboratories to seemingly similar cat odors (see Dielenberg and McGregor, 2001, for discussion).

The utility of predator odors, as compared with live predators, goes well beyond the impracticality of housing prey and predator species under the same roof, and ensuring similar behaviors from predators toward prey. For instance, being able to focus on the olfactory system as the route of entry of the response-generating stimulus, as compared to what may possibly be a much more complex, multisensory, stimulus in the case of live predators, makes the delineation of the central neural structures associated with this specific stimulus somewhat simpler. So far, the pattern of immediate-early genes and connectivity strongly suggest that the accessory olfactory system is sufficient to mediate HPA axis activation by ferret odor, although this does not yet rule out the main olfactory system. Our results, and those of others using cat odor, are consistent with the hypothesis that one of the major recipients of accessory olfactory projections, the “vomeronasal” medial amygdala, is also necessary for the behavioral and neuroendocrine responses induced by predator odor exposures. Although the medial amygdala is not reported to project directly to many, if any, of the effector/motor centers controlling behavioral, autonomic, and neuroendocrine responses (Canteras et al., 1995), it nevertheless projects to many regions such as the anterior bed nucleus of the stria terminalis, medial preoptic region, and a number of hypothalamic nuclei (dorsomedial, ventromedial, and premammillary divisions), that have those characteristics. Current anatomico-functional studies are under way to precisely define the input–output relationships of the medial amygdala with a specific focus on the vomeronasal subdivisions. One or a small number of these regions may thus function to integrate incoming information not only from the medial amygdaloid nucleus, but from a number of additional regions implicated in the perception of internal/external challenging situations, and coordinate activity in multiple effector/motor centers. The “medial hypothalamic defense system” has been suggested as one such putative integrative region, but has been tested only with behavioral responses (Blanchard et al., 2005; Canteras and Goto, 1999; Markham et al., 2004). Use of predator odors can thus precisely direct the generation of specific hypotheses concerning the brain regions likely to organize complex coordinated responses based on precise anatomical connections, the generality of which can further be tested to more complex, non-predatory, stimuli (Blanchard et al., 2005). Given the additional capacity of predator odors to produce relatively long-term response changes, this system is further amenable to investigate the putative plastic sites mediating long-term changes, from the accessory olfactory bulb all the way to specific effector/motor centers, with a minimum of *a priori* biases. Such an approach holds realistic promises to help better define the etiology and management of anxiety-related disorders.

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References

- Adamec R, Head D, Blundell J, Burton P, Berton O. Lasting anxiogenic effects of feline predator stress in mice: sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. *Physiol Behav* 2006;88(1–2):12–29. [PubMed: 16624347]

- Adamec R, Kent P, Anisman H, Shallow T, Merali Z. Neural plasticity, neuropeptides and anxiety in animals—implications for understanding and treating affective disorder following traumatic stress in humans. *Neurosci Biobehav Rev* 1998;23(2):301–318. [PubMed: 9884124]
- Adamec R, Muir C, Grimes M, Pearcey K. Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. *Behav Brain Res* 2007;179(2):192–207. [PubMed: 17335916]
- Adamec RE, Blundell J, Burton P. Neural circuit changes mediating lasting brain and behavioral response to predator stress. *Neurosci Biobehav Rev* 2005;29(8):1225–1241. [PubMed: 16099042]
- Adamec RE, Burton P, Shallow T, Budgell J. NMDA receptors mediate lasting increases in anxiety-like behavior produced by the stress of predator exposure—implications for anxiety associated with posttraumatic stress disorder. *Physiol Behav* 1999;65(4–5):723–737. [PubMed: 10073474]
- Adamec RE, Shallow T. Lasting effects on rodent anxiety of a single exposure to a cat. *Physiol Behav* 1993;54(1):101–109. [PubMed: 8327588]
- Anisman H, Lu ZW, Song C, Kent P, McIntyre DC, Merali Z. Influence of psychogenic and neurogenic stressors on endocrine and immune activity: differential effects in fast and slow seizing rat strains. *Brain Behav Immun* 1997;11(1):63–74. [PubMed: 9193768]
- Apfelbach R. Instinctive predatory behavior of the ferret (*Putorius putorius furo* L.) modified by chlordiazepoxide hydrochloride (Librium). *Psychopharmacology (Berl)* 1978;59(2):179–182. [PubMed: 103120]
- Apfelbach R, Blanchard CD, 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(8):1123–1144. [PubMed: 16085312]
- Blanchard DC, Canteras NS, Markham CM, Pentkowski NS, Blanchard RJ. Lesions of structures showing FOS expression to cat presentation: effects on responsivity to a cat, cat odor, and nonpredator threat. *Neurosci Biobehav Rev* 2005;29(8):1243–1253. [PubMed: 16084591]
- Blanchard DC, Griebel G, Blanchard RJ. Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion. *Prog Neuro-Psychopharm Biol Psychiatry* 2003a;27(8):1177–1185.
- Blanchard DC, Griebel G, Rodgers RJ, Blanchard RJ. Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. *Neurosci Biobehav Rev* 1998a;22(5):597–612. [PubMed: 9662722]
- Blanchard DC, Li CI, Hubbard D, Markham CM, Yang M, Takahashi LK, Blanchard RJ. Dorsal premammillary nucleus differentially modulates defensive behaviors induced by different threat stimuli in rats. *Neurosci Lett* 2003b;345(3):145–148. [PubMed: 12842277]
- Blanchard DC, Markham C, Yang M, Hubbard D, Madarang E, Blanchard RJ. Failure to produce conditioning with low-dose trimethylthiazoline or cat feces as unconditioned stimuli. *Behav Neurosci* 2003c;117(2):360–368. [PubMed: 12708532]
- Blanchard RJ, Blanchard DC. Bringing natural behaviors into the laboratory: a tribute to Paul MacLean. *Physiol Behav* 2003;79(3):515–524. [PubMed: 12954446]
- Blanchard RJ, Blanchard DC, Rodgers J, Weiss SM. The characterization and modelling of antipredator defensive behavior. *Neurosci Biobehav Rev* 1990;14(4):463–472. [PubMed: 2287483]
- Blanchard RJ, Nikulina JN, Sakai RR, McKittrick C, McEwen B, Blanchard DC. Behavioral and endocrine change following chronic predatory stress. *Physiol Behav* 1998b;63(4):561–569. [PubMed: 9523899]
- Blanchard RJ, Yang M, Li CI, Gervacio A, Blanchard DC. Cue and context conditioning of defensive behaviors to cat odor stimuli. *Neurosci Biobehav Rev* 2001;25(7–8):587–595. [PubMed: 11801284]
- Blundell J, Adamec R, Burton P. Role of NMDA receptors in the syndrome of behavioral changes produced by predator stress. *Physiol Behav* 2005;86(1–2):233–243. [PubMed: 16102786]
- Brandao ML, Zanoveli JM, Ruiz-Martinez RC, Oliveira LC, Landeira-Fernandez J. Different patterns of freezing behavior organized in the periaqueductal gray of rats: association with different types of anxiety. *Behav Brain Res* 2008;188(1):1–13. [PubMed: 18054397]
- Burow A, Day HE, Campeau S. A detailed characterization of loud noise stress: intensity analysis of hypothalamo–pituitary–adrenocortical axis and brain activation. *Brain Res* 2005;1062(1–2):63–73. [PubMed: 16256084]

- Campeau S, Falls WA, Cullinan WE, Helmreich DL, Davis M, Watson SJ. Elicitation and reduction of fear: behavioural and neuroendocrine indices and brain induction of the immediate-early gene *c-fos*. *Neuroscience* 1997;78(4):1087–1104. [PubMed: 9174076]
- Campeau S, Watson SJ. Neuroendocrine and behavioral responses and brain pattern of *c-fos* induction associated with audiogenic stress. *J Neuroendocrinol* 1997;9:577–588. [PubMed: 9283046]
- Campeau S, Watson SJ. Connections of some auditory-responsive posterior thalamic nuclei putatively involved in activation of the hypothalamo–pituitary–adrenocortical axis in response to audiogenic stress in rats: an anterograde and retrograde tract tracing study combined with Fos expression. *J Comp Neurol* 2000;423:474–491. [PubMed: 10870087]
- Canteras NS, Chiavegatto S, Do Valle LER, Swanson LW. Severe reduction of rat defensive behavior to a predator by discrete hypothalamic chemical lesions. *Brain Res Bull* 1997;44(3):297–305. [PubMed: 9323445]
- Canteras NS, Goto M. Fos-like immunoreactivity in the periaqueductal gray of rats exposed to a natural predator. *Neuroreport* 1999;10(2):413–418. [PubMed: 10203345]
- Canteras NS, Simerly RB, Swanson LW. Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. *J Comp Neurol* 1995;360:213–245. [PubMed: 8522644]
- Charney DS, Deutch A. A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorders. *Crit Rev Neurobiol* 1996;10:419–446. [PubMed: 8978989]
- Chen X, Herbert J. Regional changes in *c-fos* expression in the basal forebrain and brainstem during adaptation to repeated stress: correlations with cardiovascular, hypothermic and endocrine responses. *Neuroscience* 1995;64(3):675–685. [PubMed: 7715780]
- Chowdhury GM, Fujioka T, Nakamura S. Induction and adaptation of Fos expression in the rat brain by two types of acute restraint stress. *Brain Res Bull* 2000;52(3):171–182. [PubMed: 10822158]
- Cullinan WE, Helmreich DL, Watson SJ. Fos expression in forebrain afferents to the hypothalamic paraventricular nucleus following swim stress. *J Comp Neurol* 1996;368:88–99. [PubMed: 8725295]
- Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* 1995;64(2):477–505. [PubMed: 7700534]
- Day HEW, Masini CV, Campeau S. The pattern of brain *c-fos* mRNA induced by a component of fox odor, 2,5-dihydro-2,4,5-Trimethylthiazoline (TMT), in rats, suggests both systemic and processive stress characteristics. *Brain Res* 2004;1025(1–2):139–151. [PubMed: 15464754]
- Dielenberg RA, Arnold JC, McGregor IS. Low-dose midazolam attenuates predatory odor avoidance in rats. *Pharmacol Biochem Behav* 1999;62(2):197–201. [PubMed: 9972683]
- Dielenberg RA, Carrive P, McGregor IS. The cardiovascular and behavioral response to cat odor in rats: unconditioned and conditioned effects. *Brain Res* 2001a;897(1–2):228–237. [PubMed: 11282383]
- 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* 2001b;104(4):1085–1097. [PubMed: 11457592]
- Dielenberg RA, Leman S, Carrive P. Effect of dorsal periaqueductal gray lesions on cardiovascular and behavioral responses to cat odor exposure in rats. *Behav Brain Res* 2004;153(2):487–496. [PubMed: 15265647]
- Dielenberg RA, McGregor IS. Habituation of the hiding response to cat odor in rats (*Rattus norvegicus*). *J Comp Psychol* 1999;113(4):376–387. [PubMed: 10608561]
- Dielenberg RA, McGregor IS. Defensive behavior in rats towards predatory odors: a review. *Neurosci Biobehav Rev* 2001;25(7–8):597–609. [PubMed: 11801285]
- Endres T, Fendt M. Conditioned behavioral responses to a context paired with the predator odor trimethylthiazoline. *Behav Neurosci* 2007;121(3):594–601. [PubMed: 17592951]
- Fendt M, Endres T, Apfelbach R. Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks freezing induced by trimethylthiazoline, a component of fox feces. *J Neurosci* 2003;23(1):23–28. [PubMed: 12514197]
- Fendt M, Siegl S, Steiniger-Brach B. Noradrenaline transmission within the ventral bed nucleus of the stria terminalis is critical for fear behavior induced by trimethylthiazoline, a component of fox odor. *J Neurosci* 2005;25(25):5998–6004. [PubMed: 15976089]

- Figueiredo HF, Bodie BL, Tauchi M, Dolgas CM, Herman JP. Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis. *Endocrinology* 2003;144(12):5249–5258. [PubMed: 12960031]
- File SE, Zangrossi H Jr, Sanders FL, Mabbutt PS. Dissociation between behavioral and corticosterone responses on repeated exposures to cat odor. *Physiol Behav* 1993;54(6):1011–1109. [PubMed: 8248365]
- Funk D, Amir S. Circadian modulation of fos responses to odor of the red fox, a rodent predator, in the rat olfactory system. *Brain Res* 2000;866(1–2):262–267. [PubMed: 10825502]
- Harris RBS, Gu H, Mitchell TD, Endale L, Russo M, Ryan DH. Increased glucocorticoid response to a novel stress in rats that have been restrained. *Physiol Behav* 2004;81(4):557–568. [PubMed: 15178148]
- Harris RBS, Palmondon J, Leshin S, Flatt WP, Richard D. Chronic disruption of body weight but not of stress peptides or receptors in rats exposed to repeated restraint stress. *Horm Behav* 2006;49(5):615–625. [PubMed: 16423352]
- Hogg S, File SE. Responders and nonresponders to cat odor do not differ in other tests of anxiety. *Pharmacol Biochem Behav* 1994;49(1):219–222. [PubMed: 7816877]
- Hotsenpiller G, Williams JL. A synthetic predator odor (TMT) enhances conditioned analgesia and fear when paired with a benzodiazepine receptor inverse agonist (FG-7142). *Psychobiology* 1997;25(1):83–88.
- Hubbard DT, Blanchard DC, Yang M, Markham CM, Gervacio A, Chun-I L, Blanchard RJ. Development of defensive behavior and conditioning to cat odor in the rat. *Physiol Behav* 2004;80(4):525–530. [PubMed: 14741237]
- Imaki T, Shibasaki T, Hotta M, Demura H. Intracerebroventricular administration of corticotropin-releasing factor induces *c-fos* mRNA expression in brain regions related to stress responses: comparison with pattern of *c-fos* mRNA induction after stress. *Brain Res* 1993;616:114–125. [PubMed: 8358602]
- Li CI, Maglinao TL, Takahashi LK. Medial amygdala modulation of predator odor-induced unconditioned fear in the rat. *Behav Neurosci* 2004;118(2):324–332. [PubMed: 15113258]
- Markham CM, Blanchard DC, Canteras NS, Cuyno CD, Blanchard RJ. Modulation of predatory odor processing following lesions to the dorsal pre-mammillary nucleus. *Neurosci Lett* 2004;372(1–2):22–26. [PubMed: 15531081]
- Masini CV, Sauer S, Campeau S. Ferret odor as a processive stress model in rats: neurochemical, behavioral, and endocrine evidence. *Behav Neurosci* 2005;119(1):280–292. [PubMed: 15727532]
- Masini CV, Sauer S, White J, Day HE, Campeau S. Non-associative defensive responses of rats to ferret odor. *Physiol Behav* 2006a;87(1):72–81. [PubMed: 16183085]
- Masini, CV.; Srinidhi, SK.; Day, HEW.; Campeau, S. Exposure to predator odor increases heart rate, temperature, and activity measures in rats. Atlanta, GA: Society for Neuroscience; 2006b. Program No. 59.19. 2006 Neuroscience Meeting Planner 2006. Online
- McGregor IS, Dielenberg RA. Differential anxiolytic efficacy of a benzodiazepine on first versus second exposure to a predatory odor in rats. *Psychopharmacology (Berl)* 1999;147(2):174–181. [PubMed: 10591885]
- 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(17):4134–4144. [PubMed: 15115808]
- McGregor IS, Schrama L, Ambermoon P, Dielenberg RA. Not all ‘predator odours’ are equal: cat odour but not 2,4,5 trimethylthiazoline (TMT; fox odour) elicits specific defensive behaviours in rats. *Behav Brain Res* 2002;129(1–2):1–16. [PubMed: 11809490]
- McIntyre DC, Kent P, Hayley S, Merali Z, Anisman H. Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats. *Brain Res* 1999;840(1–2):65–74. [PubMed: 10517953]
- Melia KR, Ryabinin AE, Schroeder R, Bloom FE, Wilson MC. Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress. *J Neurosci* 1994;14(10):5929–5938. [PubMed: 7931554]

- Mohedano-Moriano A, Pro-Sistiaga P, Ubeda-Banon I, Crespo C, Insausti R, Martinez-Marcos A. Segregated pathways to the vomeronasal amygdala: differential projections from the anterior and posterior divisions of the accessory olfactory bulb. *Eur J Neurosci* 2007;25(7):2065–2080. [PubMed: 17419754]
- Morrow BA, Redmond AJ, Roth RH, Elsworth JD. The predator odor, TMT, displays a unique, stress-like pattern of dopaminergic and endocrinological activation in the rat. *Brain Res* 2000;864(1):146–151. [PubMed: 10793199]
- Muller M, Fendt M. Temporary inactivation of the medial and basolateral amygdala differentially affects TMT-induced fear behavior in rats. *Behav Brain Res* 2006;167(1):57–62. [PubMed: 16213603]
- Pace TWW, Gaylord R, Topczewski F, Girotti M, Rubin B, Spencer RL. Immediate-early gene induction in hippocampus and cortex as a result of novel experience is not directly related to the stressfulness of that experience. *Eur J Neurosci* 2005;22(7):1679–1690. [PubMed: 16197508]
- Plata-Salaman CR, Ilyin SE, Turrin NP, Gayle D, Flynn MC, Bedard T, Merali Z, Anisman H. Neither acute nor chronic exposure to a naturalistic (predator) stressor influences the interleukin-1beta system, tumor necrosis factor-alpha, transforming growth factor-beta1, and neuropeptide mRNAs in specific brain regions. *Brain Res Bull* 2000;51(2):187–193. [PubMed: 10709966]
- Pro-Sistiaga P, Mohedano-Moriano A, Ubeda-Bañon I, del mar Arroyo-Jimenez M, Marcos P, Artacho-Pérua E, Crespo C, Insausti R, Martinez-Marcos A. Convergence of olfactory and vomeronasal projections in the rat basal telencephalon. *J Comp Neurol* 2007;504(4):346–362. [PubMed: 17663431]
- Redmond AJ, Morrow BA, Elsworth JD, Roth RH. Selective activation of the A10, but not A9, dopamine neurons in the rat by the predator odor, 2,5-dihydro-2,4,5-trimethylthiazoline. *Neurosci Lett* 2002;328(3):209–212. [PubMed: 12147308]
- Rosen JB. The Neurobiology of conditioned and unconditioned fear: a neurobehavioral system analysis of the amygdala. *Behav Cogn Neurosci Rev* 2004;3(1):23–41. [PubMed: 15191640]
- Rosen JB, Adamec RE, Thompson BL. Expression of *egr-1* (*zif268*) mRNA in select fear-related brain regions following exposure to a predator. *Behav Brain Res* 2005;162(2):279–288. [PubMed: 15970222]
- Rosen JB, West EA, Donley MP. Not all rat strains are equal: differential unconditioned fear responses to the synthetic fox odor 2,4,5-trimethylthiazoline in three outbred rat strains. *Behav Neurosci* 2006;120(2):290–297. [PubMed: 16719693]
- Rusiniak KW, Gustavson CR, Hankins WG, Garcia J. Preylithium aversions. II Laboratory rats and ferrets. *Behav Biol* 1976;17(1):73–85. [PubMed: 938413]
- Scalia F, Winans SS. The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. *J Comp Neurol* 1975;161(1):31–55. [PubMed: 1133226]
- Staples LG, Hunt GE, Cornish JL, McGregor IS. Neural activation during cat odor-induced conditioned fear and 'trial 2' fear in rats. *Neurosci Biobehav Rev* 2005;29(8):1265–1277. [PubMed: 16111751]
- Staples LG, McGregor IS. Defensive responses of Wistar and Sprague–Dawley rats to cat odour and TMT. *Behav Brain Res* 2006;172(2):351–354. [PubMed: 16712971]
- Takahashi LK, Nakashima BR, Hong H, Watanabe K. The smell of danger: a behavioral and neural analysis of predator odor-induced fear. *Neurosci Biobehav Rev* 2005;29(8):1157–1167. [PubMed: 16095694]
- Wallace KJ, Rosen JB. Predator odor as an unconditioned fear stimulus in rats: elicitation of freezing by trimethylthiazoline, a component of fox feces. *Behav Neurosci* 2000;114(5):912–922. [PubMed: 11085605]
- Winans SS, Scalia F. Amygdaloid nucleus: new afferent input from the vomeronasal organ. *Science* 1970;170(955):330–332. [PubMed: 5460037]
- Zangrossi H Jr, File SE. Habituation and generalization of phobic responses to cat odor. *Brain Res Bull* 1994;33(2):189–194. [PubMed: 8275338]
- Zangrossi JH, File SE. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. *Brain Res Bull* 1992;29(3–4):381–388. [PubMed: 1393611]

Fig. 1. Defensive withdrawal apparatus, as seen from above (camera angle). The black rectangle inside the apparatus depicts the hide box, with the X representing the opening on the side of the box. The small white square diagonally opposite the hide box represents the location where the ferret or control odor towels are taped down.

Fig. 2.

(A) Mean plasma levels of corticosterone (CORT; $\pm$ S.E.M.) for rats exposed to ferret odor ($n = 4$), control odor ($n = 4$), isopentyl acetate ($n = 4$), or butyric acid ($n = 4$) for 30 min at the trough of the circadian cycle. (B) Mean plasma levels of adrenocorticotropin hormone (ACTH; $\pm$ S.E.M.) for the same rats as in (A). Asterisks indicate a significant difference from all other groups ($p < 0.05$).

Fig. 3. (A) Mean heart rate (beat per min $\pm$ S.E.M.) and (B) core body temperature (degree C $\pm$ S.E.M.) of rats exposed to ferret (rhombus shape) or strawberry (square shape) odors for 30 min (grey bars).

Fig. 4.

Effect of control (strawberry—STR) or ferret (FER) odor in rats sustaining either complete bilateral medial amygdala ibotenic acid (IBO) cell loss or sham (SHAM) lesions on mean time ($\pm$ S.E.M.) spent in the towel corner (A), in the hide chamber (B), or the number of visits to the towel corner (C). Note that the IBO/FER rats respond in a way more similar to rats presented with control (IBO/STR, SHAM/STR) than normal rats presented with ferret odor (SHAM/FER). * $p < 0.05$ compared to all other groups (Tukey post hoc multiple means comparisons).

Fig. 5. Immunohistochemical detection of the neuronal specific marker NeuN indicates the extent of neuron loss in the region of the medial amygdaloid nucleus (MeA—black arrow, right) following ibotenic acid injection, compared to an injection of ACSF (left).

Fig. 6.

Effect of control (strawberry—STR) or ferret (FER) odor in rats sustaining either complete bilateral medial amygdala ibotenic acid (IBO) cell loss or sham (SHAM) lesions on mean levels of plasma corticosterone (A) and ACTH (B). Note that the IBO/FER rats respond in a way more similar to rats presented with control (IBO/STR, SHAM/STR) than normal rats presented with ferret odor (SHAM/FER). * $p < 0.05$ compared to all other groups (Tukey post hoc multiple means comparisons).

Fig. 7.

(A) Mean circulating plasma corticosterone ($\mu\text{g/dl} \pm \text{S.E.M.}$) in response to ferret (FER) or control (clean towel—CON) odor following intranasal saline (SAL) or a 10% solution of zinc sulfate (10%) in male rats ($n = 8$ for SAL/FER, $n = 13$ for 10% ZnSu, $n = 6$'s for both SAL/CON and 10%/CON, respectively). There was no statistical difference between the SAL/FER and 10%/FER groups. (B) *c-fos* mRNA induction at the level of the olfactory bulb in control (saline) or 10% zinc sulfate (ZnSu)-treated rats in response to ferret odor. Note the *c-fos* mRNA induction greatly limited to the accessory olfactory bulb (AOB) in ZnSu-treated rats as compared to a much larger induction throughout the main olfactory bulb (MOB) of saline-treated rats.

Fig. 8. Reliable plasma corticosterone (A) and ACTH (B) response habituation (respective ANOVAs over time, reliable group $\times$ time effect, $p < 0.05$) to 11 consecutive 30 min daily exposures to ferret or control odor towels hung in the home cages ($n = 8$ per group).

Table 1
Mean integrated densities/100 ($\pm$ S.E.M.) of *c-fos* mRNA expression

Region	Control odor		Ferret odor	
	M	S.E.M.	M	S.E.M.
Forebrain				
BNST fusiform nucleus **	1.4	8.8	46.8	27.5
BNST oval nucleus **	3.1	4.0	16.8	8.8
Caudate/putamen **	105.8	219.4	777.0	486.6
Lateral septum **	69.1	87.8	829.4	403.3
Olfactory granular layer	188.8	24.1	188.5	37.1
Cortex				
Cingulate cortex **	319.4	140.6	1026.4	794.7
Motor cortex **	663.2	288.8	1742.2	884.1
Orbitofrontal cortex **	401.5	118.0	916.9	95.2
Piriform cortex **	546.9	137.9	991.0	182.2
Prelimbic cortex **	107.1	44.2	557.1	97.3
Preoptic and hypothalamic areas				
Anterodorsal preoptic area **	6.7	8.2	92.9	35.5
Dorsomedial n. of hypothalamus **	67.7	50.5	305.1	95.6
Lateral n. of hypothalamus	15.4	4.9	32.0	5.3
Paraventricular n. hypothalamus **	66.8	15.0	209.6	135.5
Posterior n. of hypothalamus **	23.1	6.7	80.3	14.0
Premammillary nucleus (dorsal) **	27.0	9.7	205.5	19.7
Supramammillary nucleus **	90.5	27.9	320.8	46.0
Ventromedial n. hypothalamus **	46.4	12.9	289.4	29.6
Hippocampus				
CA1	88.1	17.2	119.7	26.9
CA2	5.6	1.4	15.3	5.0
CA3 **	32.6	5.2	68.3	11.6
Dentate gyrus	19.4	5.0	28.1	8.1
Amygdala				
Anterodorsal medial nucleus	79.4	3.3	153.7	24.3
Basolateral nucleus **	49.8	10.7	116.0	19.9
Central nucleus	22.3	3.3	42.9	8.5
Posterodorsal medial **	8.5	2.0	45.5	9.1
Posterolateral cortical **	57.0	11.3	138.0	19.1
Posteromedial cortical	75.9	20.8	132.4	10.3
Posteroventral medial **	88.2	27.4	216.3	20.2
Periaqueductal gray				
Cuneiform nucleus	2.8	0.4	18.9	5.9
Dorsolateral PAG **	7.3	1.8	78.6	8.8

Region	Control odor		Ferret odor	
	M	S.E.M.	M	S.E.M.
Dorsomedial PAG ^{**}	20.4	5.8	194.1	18.6
Ventrolateral PAG ^{**}	12.4	2.4	55.5	9.6
Brainstem				
Nucleus of tractus solitarius ^{**}	3.3	0.7	10.4	1.7
Spinal trigeminal nucleus	36.2	3.0	24.6	4.3

Note: mRNA = messenger RNA; BNST = bed nucleus of the stria terminalis; n. = nucleus; PAG = periaqueductal gray. Copyright © 2005 by the American Psychological Association. Reproduced with permission. The official citation that should be used in referencing this material is Masini et al. (2005). The use of APA information does not imply endorsement by APA. Reproduced with author's permission.

^{**} $p < 0.01$, significant difference between groups.

Table 2

Body weight (BW) change and organ weights corrected for final body weights (g/100 g BW) in response to repeated (11 × 30 min daily) ferret odor exposures

	Control odor	Ferret odor
	Mean (S.E.M.)	Mean (S.E.M.)
Body weight change	60.0 (6.32)	41.13 (2.81) *
Thymus weights	0.1772 (0.0146)	0.146 (0.0051) *
Adrenal weights	0.0153 (0.0013)	0.0171 (0.0005) *

Note: Body weight change was calculated by subtracting initial body weight (24 h prior to first ferret towel exposure) from final body weight (24 h prior to sacrifice).

* Statistically reliable difference between control- and ferret-exposed rats ($p < 0.05$).

Table 3

Plasma corticosterone, ACTH, body weight (BW) change and organ weights corrected for final body weights (g/100 g BW) in response to a novel environmental challenge 24 h following control or ferret odor preexposure (4 × 60 min daily)

	Control odor	Ferret odor
	Mean (S.E.M.)	Mean (S.E.M.)
Plasma corticosterone	4.50 (1.02)	11.60 (2.14)*
Plasma ACTH	78.59 (7.72)	98.59 (20.64)
Body weight change (g)	35.88 (2.30)	8.63 (3.61)*
Thymus weights	0.168 (0.004)	0.135 (0.008)*
Adrenal weights	0.0130 (0.0016)	0.0154 (0.0011)

Note: Body weight change was calculated by subtracting initial body weight (24 h prior to first ferret towel exposure) from final body weight (24 h prior to sacrifice).

* Statistically reliable difference between control- and ferret-exposed rats ($p < 0.05$).