

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Neuroscience and Biobehavioral Reviews xx (2005) 1–12

---



---

 NEUROSCIENCE AND  
 BIOBEHAVIORAL  
 REVIEWS
 

---



---

[www.elsevier.com/locate/neubiorev](http://www.elsevier.com/locate/neubiorev)

Review

# TMT-induced autonomic and behavioral changes and the neural basis of its processing

Markus Fendt<sup>a,\*</sup>, Thomas Endres<sup>a</sup>, Catherine A. Lowry<sup>b</sup>,  
Raimund Apfelbach<sup>a</sup>, Iain S. McGregor<sup>c</sup>

<sup>a</sup>*Tierphysiologie, Zoologisches Institut, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany*<sup>b</sup>*Committee on Neurobiology, The University of Chicago, 940 E. 57th Street, Chicago, IL 60637, USA*<sup>c</sup>*School of Psychology, University of Sydney, Sydney, NSW 2006, Australia*


---

**Abstract**

One of the main interests in the field of neuroscience is the investigation of the neural basis of fear. During recent years, an increasing number of studies have used trimethylthiazoline (TMT), a component of red fox feces, as a stimulus to induce fear in predator naive rats, mice, and voles. The aim of the present review is to summarize these studies. We present an overview to the autonomic and behavioral changes that are induced by TMT exposure. Then, we summarize the small number of studies that have examined the neural processing of the TMT stimulus. Finally, we compare these studies with those using a natural predator or predator odor to induce fear and discuss the possible use of TMT exposure in rodents as an animal model of unconditioned fear in humans.

© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** Behavior; Cat odor; C-fos; Defensive behavior; Electrophysiology; Freezing; Fox odor; Innate fear; Lesions; Neuropharmacology; Predator odor; Trimethylthiazoline

---

**Contents**

1. Introduction	000
2. Autonomic and behavioral changes induced by TMT exposure	000
3. Neural processing of TMT	000
3.1. Neural activity measured by <i>c-fos</i> immunohistochemistry	000
3.2. Lesion studies	000
3.3. Neuropharmacology of TMT processing	000
4. Comparison with studies using natural predator odors	000
5. Possible use of TMT-induced changes as an animal model of fear	000
5.1. Is TMT a predator odor?	000
5.2. Criteria for animal models of fear	000
5.3. Conclusion	000
Acknowledgements	000
References	000

---

**1. Introduction**

Prey animals have developed many different adaptations to defend themselves from their predators (Kats and Dill, 1998). These adaptations can be morphological (e.g. armor spines or teeth), physiological (e.g. poisons, toxins), or behavioral (e.g. fighting, fleeing, and hiding). Since these defenses also have costs, prey animals often develop several

---

\* Corresponding author. Tel.: +49 7071 2975347; fax: +49 7071 292618.

E-mail address: [markus.fendt@uni-tuebingen.de](mailto:markus.fendt@uni-tuebingen.de) (M. Fendt).

ways to assess the risk of predation. For many species, olfaction plays a large role in risk assessment (Kats and Dill, 1998).

For rodents, the main predators are carnivores, including cats, dogs, mustelids, wolves, and foxes (Apfelbach et al. this issue; Gillies and Clout, 2003; Glowacinski and Profus, 1997; Goldyn et al., 2003; Masini et al., 2005). Several studies demonstrate that exposure to the odor of these predators induces species-specific behavioral antipredator responses (reviewed in Blanchard et al., 1990a; 2003a; Dielenberg and McGregor, 2001). Most of these studies have used feline odors to elicit defensive behaviors, including cat collars, cloths rubbed on cats, cat fur, cat bedding, cat urine, cat feces and soiled cat litter (e.g. Blanchard et al., 1990b; 2003b; Dielenberg and McGregor, 2001; Li et al., 2004; Zangrossi Jr. and File, 1992b). Some other studies have used odors from the red fox (Vernet-Maury et al., 1968). In 1980, Vernet-Maury reported that 2,4,5-Trimethyl-thiazoline (2,5-Dihydro-2,4,5-trimethylthiazole or TMT, for chemical structure see Fig. 1) is the most effective chemical constituent of the fox feces odor for inducing behavioral and autonomic antipredator responses in rats.

Since its discovery, TMT has been considered by many to be a specific olfactory cue associated with the red fox. TMT was not found in analyses of the volatile constituents of dog feces (Arnould et al., 1998) or in the anal gland secretions of the dog or coyote (Preti et al., 1976). However, this compound was first isolated from cooked beef (Mussinan et al., 1977), and is also found in wheat flour extrudates (Bredie et al., 2002). Thus, TMT may not necessarily be a specific predatory stimulus to rodents, although it could represent an ethologically-relevant odor. This information may help explain the varied results we report in this review.

Over the past 25 years, an increasing number of studies have used TMT to stimulate autonomic and behavioral changes in rats and mice, and to examine the neural basis of fear and antipredator behavior. The aim of the present review is to summarize these different studies and to attempt to gain an overall perspective on TMT. First, we present an overview of the autonomic and behavioral changes induced by TMT (summarized in Table 1). In the second part of this review, we examine the work done on the neural processing of the TMT stimulus. In the final section of this review, we compare the effects of TMT with those of other natural predator odors (feline and vulpine) and discuss the possible

use of TMT-induced autonomic and behavioral changes as an animal model of unconditioned fear.

## 2. Autonomic and behavioral changes induced by TMT exposure

Evelyne Vernet-Maury was the first to investigate the behavioral effects of TMT (Vernet-Maury, 1980; Vernet-Maury et al., 1984) in prey animals of the fox. In these studies, the behavior of laboratory rats, previously naive to TMT and fox odor, was observed. Odors were introduced into a large covered open field through a nozzle in the ceiling. TMT, as well as the original stimulus from which it is derived, i.e. fox feces (Vernet-Maury et al., 1968), induced a number of behavioral signs of fear: longer emergence time from the entry tunnel into the open field, less vertical and horizontal motor activity, fewer visits to the center of the open field, less grooming behavior, and more defecation and urination (Vernet-Maury et al., 1984). Such behavioral changes are usually observed in fearful or stressful situations (reviewed in Blanchard and Blanchard, 1969; Dielenberg and McGregor, 2001). In addition to the behavioral changes, the corticosterone levels measured in the rats' blood were correlated with the strength of the behavioral signs of fear they showed to TMT (Vernet-Maury et al., 1984). Similar results were later found testing wild-caught Norway rats (Vernet-Maury et al., 1992).

The primary result of Vernet-Maury's studies was that TMT induced avoidance behavior in rats. For some time, little or no further behavioral research was done with TMT. Then in 1997, Hotsenpiller and Williams demonstrated that TMT induces freezing and potentiates the analgesia expressed in the presence of a conditioned fear stimulus. Freezing behavior is the cessation of all movements except those that are necessary for breathing; analgesia is a suppression of pain processing (Bolles and Fanselow, 1980; Fendt and Fanselow, 1999). Like the parameters described above, analgesia and freezing are often used in anxiety research as indicators of fear (Fanselow and Helmstetter, 1988). These results have led to the increased use of TMT in preclinical fear and anxiety research in recent years.

While one other study also showed that TMT induces analgesia (Walf and Frye, 2003), the reports of freezing responses to TMT are quite varied. The fact that no freezing was observed in some cases is of special interest since freezing is one of the most prominent behavioral signs of fear in rats (Fendt and Fanselow, 1999). Morrow and colleagues did not see an induction of freezing during TMT exposure in a familiar, small, and dark open field (Morrow et al., 2000; McGregor et al., 2002), although they showed later that TMT is able to induce a small amount of freezing when presented in a brighter, larger, and novel open-field (Morrow et al., 2002). McGregor and colleagues found no reduction in locomotor activity in rats exposed to TMT in

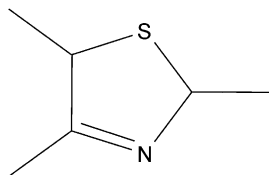


Fig. 1. Chemical structure of 2,4,5-Trimethylthiazoline (2,5-Dihydro-2,4,5-trimethylthiazole or TMT).

Table 1  
Overview of the different studies investigating the effects of TMT exposure on rats and mice

Observed measure	Apparatus	Species	References showing a ... fear-like effect	...no fear-like effect
Approach latency	Open field	<i>R. norvegicus</i>	Vernet-Maury, 1980 Vernet-Maury et al., 1984 <sup>a</sup>	
	Avoidance apparatus	<i>R. rattus</i> <i>R. norvegicus</i>	Burwash et al., 1998b Dielenberg et al., 1999 McGregor et al., 2002	
Contact duration	Long rectangular chamber	<i>R. norvegicus</i>	Blanchard et al., 2003b	
	Long rectangular chamber, home cage	<i>R. norvegicus</i>	Blanchard et al., 2003b	
Corticosterone release	Olfactory box	<i>M. musculus</i> <i>R. norvegicus</i>	Hebb et al., 2004	Lowry, unpublished observation
	Open field, olfactory boxes	<i>R. norvegicus</i>	Day et al., 2004 Holmes and Galea, 2002 Morrow et al., 2000; 2002 Vernet-Maury, 1980 Vernet-Maury et al., 1984	
Defecation	Large open field	<i>R. norvegicus</i>	Vernet-Maury, 1980 Vernet-Maury et al., 1984 <sup>a</sup>	Lowry, unpublished observation
Defensive burying	Open field, home cage	<i>R. norvegicus</i> <i>M. musculus</i>	Holmes and Galea, 2002 Hebb et al., 2004	
Eating behavior	Open field	<i>R. rattus</i>	Burwash et al., 1998b	
	Instrumental conditioning chamber	<i>R. norvegicus</i>	Endres et al., 2005	
Entries to a illuminated chamber	Light/dark test	<i>M. musculus</i>	Hebb et al., 2002; 2003a	
Freezing	Open field	<i>R. norvegicus</i>	Morrow et al., 2000; 2002 Vernet-Maury, 1980 Vernet-Maury et al., 1984	Rosen, 2004
	Small, familiar, and dark open field	<i>R. norvegicus</i>		Morrow et al., 2000
	Olfactory boxes, home cage	<i>R. norvegicus</i> <i>M. musculus</i>	Endres et al., 2005 Fendt et al., 2003 Rosen, 2004 Wallace and Rosen, 2000; 2001 Hebb et al., 2004	McGregor et al., 2002
Grooming	Open field	<i>R. norvegicus</i>	Vernet-Maury, 1980 Vernet-Maury et al., 1984 <sup>a</sup>	
Hiding	Olfactory box	<i>R. norvegicus</i>		Lowry, unpublished observation
	Avoidance apparatus	<i>R. norvegicus</i>		McGregor et al., 2002
Intracranial self stimulation	Skinner box	<i>M. musculus</i>		Hebb et al., 2003b
Motor activity	Open field, avoidance apparatus	<i>R. norvegicus</i>	Vernet-Maury, 1980	McGregor et al., 2002
	Open Field	<i>M. pennsylvanicus</i>	Vernet-Maury et al., 1984 <sup>a</sup> Perrot-Sinal et al., 1996; 2000	Morrow et al., 2000; 2002
Open arm entries Risk assessment	Elevated plus-maze	<i>R. norvegicus</i>	present paper	McGregor et al., 2002
	Avoidance apparatus	<i>R. norvegicus</i>		McGregor et al., 2002
	Olfactory box	<i>R. norvegicus</i>	Holmes and Galea, 2002	Lowry, unpublished observation
	Long rectangular chamber, home cage	<i>R. norvegicus</i>	Blanchard et al., 2003b	
Startle potentiation		<i>M. musculus</i>	Hebb et al., 2004	
	Olfactory boxes	<i>R. norvegicus</i>	Endres et al., 2005	
	Startle apparatus (after TMT exposure)	<i>M. musculus</i>	Hebb et al., 2003; 2003b	
Stress score <sup>b</sup>	Open field	<i>R. norvegicus</i>	Vernet-Maury, 1980 Vernet-Maury et al., 1984	
Urination	Open field	<i>R. norvegicus</i>	Vernet-Maury, 1980 Vernet-Maury et al., 1984 <sup>a</sup>	

<sup>a</sup> Subscorings (see the following footnote).

<sup>b</sup> Total sum of subscorings of emergence time (+), motor activity (-), visits of the open field center (-), grooming (-), defecation (+), and urination (+).

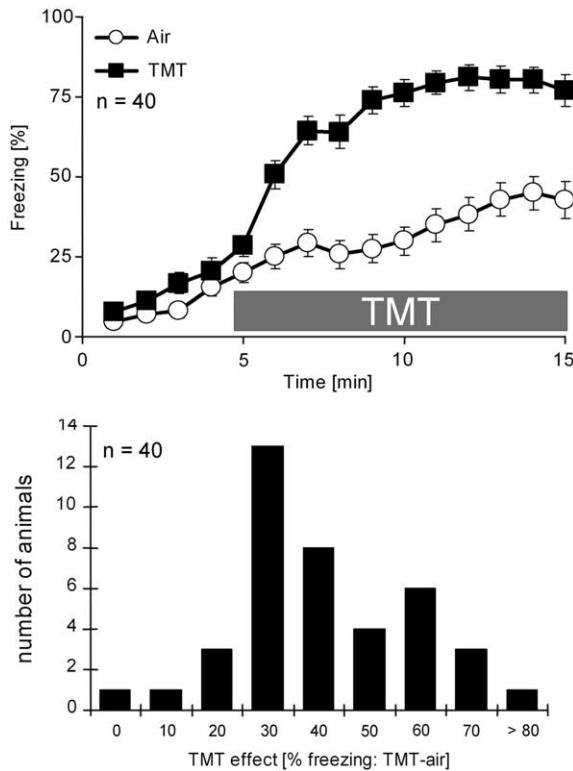


Fig. 2. TMT-induced freezing: Forty naive rats were put in an olfactory box. After 4 min, TMT (or fresh air as a control) was introduced. The freezing response of the rats were observed and calculated in percent freezing/minute. TMT highly increased the amount of freezing (two factor ANOVA: interaction odor x phase:  $F_{1,78}=69.68, p<0.0001$ ). A. Time course of the freezing response. B. Histogram of the 40 individual TMT effects on the freezing response (freezing during TMT exposure - freezing during exposure to fresh air). This experiment was carried out by T. Endres at the University of Tübingen.

several different types of test chambers (McGregor et al., 2002). Lowry and colleagues found no difference in freezing behavior for TMT relative to a large panel of other odorants tested (Lowry, unpublished observation). And although Hotsenpiller and Williams found increased freezing responses to TMT over no-odor controls, there was no difference in the freezing responses to TMT relative to butyric acid (Hotsenpiller and Williams, 1997), an unpleasant but non-predatory odor. Interestingly, a TMT-induced increase in corticosterone and adreno-corticotropin hormone release could be seen in naive rats even when freezing was not observed (Day et al., 2004; Morrow et al., 2000; 2002).

When small boxes or the home cage of the rodent was used for TMT exposure, higher levels of freezing have sometimes been reported (Endres et al., 2005; Fendt et al., 2003; Wallace and Rosen, 2000; 2001). In Fig. 2(A), the mean freezing response of 40 naive rats exposed to TMT is shown. As indicated in the histogram (Fig. 2(B)), 87.5% of these rats displayed an increase in freezing of more than 30% (percent time of freezing per minute) during TMT exposure, while 35% of the animals had an increase of more

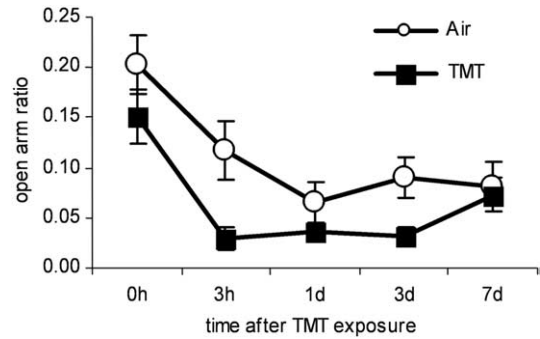


Fig. 3. TMT-induced behavioral changes on the elevated plus-maze: Naive rats were exposed to TMT ( $n=16$ ) or fresh air ( $n=14$ ). Directly after this exposure, as well as three hours, one, three, and seven days later, the animal were put on the elevated plus-maze for 5 min. Open arm ratio (time spent on open arms/total time) was decreased, as indicated by a significant effect of the factor odor (repeated-measure ANOVA:  $F_{1,132}=4.52, p=0.035$ , all time points are included in the analysis except 7d). This experiment was carried out by M. Fendt at the University of Tübingen.

than 50%. Hebb and colleagues observed substantial inter-individual variability in the expression of freezing during TMT exposure in mice (Hebb et al., 2004). This variability might help to explain the fact that some studies measured freezing intensities up to 80 percent of the test time (e.g. Fendt et al., 2003) whereas others report a complete lack of freezing during TMT exposure (e.g. McGregor et al., 2002).

Interestingly, two studies have shown that the freezing response in rats did not habituate over a week of repeated daily TMT exposures (Endres et al., 2005; Wallace and Rosen, 2000). Furthermore, no contextual fear conditioning, i.e. an association of TMT-induced fear with the test apparatus, was found in several studies (Rosen, 2004; Wallace and Rosen, 2000). There is a hint, requiring further confirmation, that conditioned freezing after the pairings of TMT with a context is possible if larger test boxes are used (Rosen, 2004).

Several other species-specific behavioral defensive responses have been described during TMT exposure, though the results are similarly varied. A prolonged latency to approach TMT impregnated stimuli was observed under several different test conditions (Blanchard et al., 2003a; Dielenberg et al., 1999; McGregor et al., 2002). However, these studies differed in the ability of TMT to elicit risk assessment behavior. While McGregor and colleagues observed no changes in locomotor activity, hiding behavior, or risk assessment behavior (McGregor et al., 2002) in rats exposed to TMT, others found an increase in risk assessment (Blanchard et al., 2003b). To the best of our knowledge, only one study has investigated defensive burying (pushing or digging of bedding material towards or in the presence of aversive cues) during TMT exposure in rats. Here, a tremendous increase in the duration and frequency of defensive burying during TMT exposure was found (Holmes and Galea, 2002). There are also contradictory results in elevated plus-maze behavior after TMT exposure. Whereas McGregor and colleagues (2002) found



no behavioral changes on the plus-maze directly after TMT exposure, Fendt (see Fig. 3) observed slight but long-lasting changes in open arm entries. Additional studies from the latter group showed a potentiation of the startle response and a reduction of appetitive behavior (here, lever pressing for food) during TMT exposure (Endres et al., 2005).

The 'threshold' concentration of TMT required to induce behavioral and autonomic changes was measured in three studies which used small boxes for TMT exposure, freezing as the behavioral measure, and the release of stress hormones (corticosterone and adrenocorticotropin hormone) as an endocrine measure of stress (Day et al., 2004; Endres et al., 2005; Wallace and Rosen, 2000). The two published TMT concentrations that are required to induce a significant increase in freezing are very different: Wallace and Rosen (2000) observed freezing after exposure to 13.2  $\mu\text{l}$  TMT (concentration:  $4.8 \times 10^{-4}$  mol/l air), whereas Endres and colleagues (2005) found significantly enhanced freezing with 5  $\mu\text{l}$  of 0.001% TMT ( $1.8 \times 10^{-7}$  mol/l air). An increase in stress hormone release was found with exposure to 9.7  $\mu\text{l}$  TMT ( $3.5 \times 10^{-4}$  mol/l air (Day et al., 2004)). All these concentrations are much higher than the lowest concentration that can be perceived by rats (around  $3 \times 10^{-15}$  mol/l air; measured by an olfactory go/no/go discrimination paradigm (Laska et al., 2004)). Interestingly, the perceptual threshold for TMT appears much higher in animals that are not prey species of the red fox: In humans and several monkey species, thresholds between  $10^{-10}$  and  $10^{-12}$  mol/l were measured (Laska et al., 2004).

In the studies reviewed above, laboratory rats (*Rattus norvegicus*) were the subjects. In roof rats (*Rattus rattus*), a prolonged approach to food in close proximity to TMT was observed, as well as an inhibition of feeding behavior in that situation (Burwash et al., 1998b). An induction of freezing behavior during TMT exposure has also been seen in these animals [Siegl, Schauerman, Kollar and Fendt, unpublished observation]. In contrast, a field study by Burwash and colleagues found no avoidance behavior in roof rats exposed to TMT-prepared patches (Burwash et al., 1998a). The authors explain this surprising result by hypothesizing that the area used for their study (a macadamia orchard) has important positive habitat values (abundance of food, water, and cover) and these values might neutralize the negative value imparted by TMT (Burwash et al., 1998a).

As with laboratory rats, there is some variability in the fear behavior observed in laboratory mice (*Mus musculus*, CD-1 strain) after TMT exposure. Several behaviors have been measured in mice during and at several time points after TMT exposure. There was a long-lasting effect on the acoustic startle response: the startle magnitudes were potentiated for at least one week after TMT (Hebb et al., 2003a,b). In the light/dark box, the latency to enter the dark compartment was reduced (Hebb et al., 2002; 2003a), as was the total time spent in the light compartment and the number of transitions between the two compartments of

the apparatus (Hebb et al., 2002; 2004). An increase in risk assessment, measured by stretch attends, as well as an increase in defensive burying was found (Hebb et al., 2004). Interestingly, intracranial self-stimulation was not affected by TMT or by other aversive odors (Hebb et al., 2003b). To some extent, this is in contrast to the study of Endres and colleagues showing that (in rats) lever pressing for food is reduced during TMT exposure (Endres et al., 2005). Furthermore, TMT-induced freezing was observed in mice (Hebb et al., 2004; Fendt, unpublished observations). Hebb and colleagues observed in mice a great inter-individual variability in the expression of freezing during TMT exposure (Hebb et al., 2004). This variability might help to explain why some studies of rodents measured freezing intensities up to 80 percent of the test time whereas others report a complete lack of freezing during TMT exposure (e.g. McGregor et al., 2002).

Behavioral responses to TMT were not only measured in the typical laboratory rodents: rats and mice. Perrot-Sinal and colleagues studied laboratory-fostered meadow voles (*Microtus pennsylvanicus*) and found reduced motor activity during TMT exposure (Perrot-Sinal et al., 1996; 1999). This behavioral change was more prominent in reproductive males. In addition, the authors suggested that testosterone levels in the blood might be reduced by TMT exposure (Perrot-Sinal et al., 1999).

Taken together, it appears that many of the defensive responses of rodents seen in traditional unconditioned fear paradigms are also observed during and after exposure to TMT. However, there are clearly some exceptions to this, as some studies report an absence of specific defensive responses. In a number of the studies discussed here, TMT is compared with plain air, rather than other odors. There are very few studies in which the effects of TMT have been easily differentiated from the effects of other noxious, but non-predator derived, odors. Further comparisons between TMT and other odors may help to tease apart why TMT elicits some defensive behaviors and not others. Also, we have noted that in some cases (e.g. McGregor et al., 2002; Morrow et al., 2002) it appears that the context in which TMT is delivered is crucial to the induction of defensive behaviors. This may be an important consideration for future studies to consider.

### 3. Neural processing of TMT

#### 3.1. Neural activity in the olfactory system

Neural activity in the mammalian olfactory system is characterized by oscillations of the local field potential (LFP), ranging in frequency from 1 to 100 Hz (Adrian, 1950). These oscillations are most prominent in the olfactory bulb (OB), which receives input directly from the nasal epithelium and projects widely throughout the limbic system. The OB is also the recipient of multiple

feedback loops from these limbic brain regions (Shipley and Adamek, 1984). This architecture enables strong modulation of primary sensory processing by internal states (Kay and Freeman, 1998; Kay and Laurent, 1999).

In response to TMT, increased bursts of oscillation in the beta frequency band (15–32 Hz) have been observed in the OB, piriform cortex (PC), and dentate gyrus (DG) of rats (Heale and Vanderwolf, 1994; Vanderwolf and Zibrowski, 2001; Zibrowski and Vanderwolf, 1997). It was proposed that these beta oscillations may be correlated with a specific predator response, despite evidence that TMT was unable to produce beta oscillations in the PC of the meadow vole (Vanderwolf et al., 2002) and that several other chemicals (non-predator odors) were found to also elicit beta oscillations in rats (Heale et al., 1994; Heale and Vanderwolf, 1994; Vanderwolf and Zibrowski, 2001; Zibrowski et al., 1998; Zibrowski and Vanderwolf, 1997). Toluene, in particular, has been shown to elicit the same beta bursts as TMT when presented in short duration (~1 s) to awake (Chapman et al., 1998) and anesthetized rats (Neville and Haberly, 2003). No chemical or perceptual similarity has yet been found between the odorants eliciting the beta oscillations in these studies.

Oscillations in the beta frequency range (15–32 Hz in rats) have recently received attention for their possible role in odor processing. Olfactory learning induces changes in OB beta band responses, suggesting that this oscillation is involved in odor processing and recognition (Martin et al., 2004; Ravel et al., 2003). Further evidence of an odor-behavior role of beta oscillations is provided in a study which showed a food odor, and not other odors, increased beta activity in the OB of food deprived rats (Chabaud et al., 2000). Also, a connection between the OB and the PC is necessary for beta oscillations to occur (Neville and Haberly, 2003). These studies also indicate that beta oscillations may be more predominant during the onset of an odor.

However, in a recent examination of the first 20 s of two-minute odor exposures, beta oscillations in response to TMT were no different to that obtained with other odors (Lowry et al., unpublished observation). Interestingly, the oscillatory responses to another putative predator odor (fox urine) also did not differ. The differences studying these results to previous studies (Heale et al., 1994; Heale and Vanderwolf, 1994; Vanderwolf et al., 2002; Vanderwolf and Zibrowski, 2001; Zibrowski et al., 1998; Zibrowski and Vanderwolf, 1997) could be due to different odor exposure procedures. Lowry and colleagues (Lowry et al., unpublished observation), diffused TMT into the air of an enclosed chamber, while in the other studies TMT was presented on a cotton swab (Heale et al., 1994; Heale and Vanderwolf, 1994; Vanderwolf et al., 2002; Vanderwolf and Zibrowski, 2001; Zibrowski et al., 1998; Zibrowski and Vanderwolf, 1997). This, combined with the behavioral results above, could indicate that the method of odor delivery—or the context in

which an animal encounters an odor—may be an important key to understanding how rodents perceive odors.

It appears then that TMT, as well as fox urine, does not induce electrophysiological responses in the olfactory bulb that are readily differentiated from non-predator odors.

### 3.2. Neural activity measured by *c-fos* immunohistochemistry

As with the electrophysiological data mentioned above, immunohistochemical studies using the *c-fos* technique have as yet been unable to demonstrate unique effects of TMT in the olfactory system relative to other odorants. A recent study by Illig and Haberly (2003) found that TMT (diluted at 1:10,000) and presented to rats in an olfactometer caused robust patterns of activation in the glomerular and granular layers of the olfactory bulb and in the posterior and anterior piriform cortex. Interestingly, in this study 4 of the 9 rats given TMT showed apparent attempts to escape from the chamber in which TMT was presented. However, the pattern of neural activation in the olfactory system of ‘escape attempting’ rats could not be differentiated from the other rats presented with TMT.

A recent study investigated the neural activation in the whole brain induced by TMT exposure using the *c-fos* technique. Day and colleagues (2004) found a TMT-specific (compared with the control odors water and butyric acid) and partly dose-dependent increase of *c-fos* mRNA within several brain regions: anteromedial nucleus of the stria terminalis, the septohypothalamic nucleus, the lateral septum, the anterodorsal, anteroventral, and medial preoptic nuclei, the lateral hypothalamic area, and the lateral external parabrachial nucleus. The activity pattern of these brain sites had a high correlation with the activity within the paraventricular nucleus of the hypothalamus, the activity of which was closely correlated with the blood level of adrenocorticotropin hormone. The authors suggested that the *c-fos* activation seen across these brain sites reflects the activation of the hypothalamic-pituitary-adrenal-axis.

TMT-induced *c-fos* mRNA was also evident across another subgroup of brain nuclei consisting of the oval nucleus of the bed nucleus of the stria terminalis, the medial anterodorsal nucleus of the amygdala, the supramammillary hypothalamic nucleus, the external parabrachial nucleus, and the nucleus of the solitary tract. It was hypothesized that activity in this circuit is principally related to activation of the central nucleus of the amygdala (CEA) and reflects the presence of fear and of associated defensive behaviors (Campeau et al., 1997; Radulovic et al., 1998). However the CEA does not appear to be activated by other predator odors such as cat odor and, interestingly, is also activated by anxiolytic agents such as midazolam (Dielenberg et al., 2001). These observations are hard to reconcile with the authors suggestion that CEA activation by TMT reflects the recruitment of fear pathways.

Several studies have reported on TMT-induced *c-fos* expression in specific brain regions of interest. In mice, increased *fos-related antigen* after TMT exposure was found in the prelimbic and intralimbic cortex, in the shell and the core region of the nucleus accumbens, and in the ventral tegmental area (Hebb et al., 2004). However, no TMT-induced increase was found in the different subnuclei of the amygdaloid complex, namely the basolateral, central and medial nuclei of the amygdala (Hebb et al., 2004). Interestingly, mice showing a very intense freezing response or a weak freezing response to TMT could be differentiated by the extent of *c-fos* activation in the shell region of the nucleus accumbens: High-responders to TMT had double number of *c-fos* immunoreactive neurons in this region compared to low-responders (Hebb et al., 2004). Furthermore, those animals showing a high amount of anxiety, measured by the light/dark box, after TMT exposure had a more intense activation of the prelimbic cortex. However, further studies need to address the question of whether the differences in brain activation seen in these two studies from the same group (Day et al., 2004) reflect the different species or different methodologies used.

It should be noted that an activation of *c-fos* expression within a specific brain area by TMT is only a first hint of an involvement of this brain area in the processing of TMT or the mediation or modulation of TMT-induced behavioral and autonomic changes. The exact functionality of these brain areas can be further investigated with further methodologies, e.g. lesion studies.

### 3.3. Lesion studies

Wallace and Rosen (2001) were the first to investigate the neural basis of TMT-induced behavioral changes using lesion techniques. Since the observed behavioral and autonomic changes during TMT exposure are fear-like, they hypothesized that the amygdala is involved in the processing of TMT-induced behavioral changes. This hypothesis was based on the well-accepted concept that the amygdala is the central structure within a brain circuitry processing fear and inducing the autonomic and behavioral changes during fear (Davis et al., 1993; Fendt and Fanselow, 1999; LeDoux, 2000; Rosen, 2004). Wallace and Rosen (2001) found that electrolytic lesions of the lateral region of the amygdala blocked TMT-induced freezing whereas neurotoxic lesions of the amygdala did not affect TMT-induced freezing. This rather surprising result was interpreted as indicating that the electrolytic lesions affect critical fibers of passage whereas the neurotoxic lesions spare these fibers. The result that the lateral region of the amygdala plays no role in TMT-induced freezing was further confirmed in a study using temporary inactivation of this region by local injections of the GABA<sub>A</sub>-agonist muscimol (Fendt et al., 2003). In contrast, inactivation of the BNST results in a blockade of TMT-induced freezing (Fendt et al., 2003). The BNST is hypothesized as being

important for anxiety whereas the amygdala is more important for fear (Walker et al., 2003); other studies showed a crucial role for the BNST in fear induced by a context in which shock has been previously received (Sullivan et al., 2004).

Unpublished studies have further demonstrated that other regions of the amygdala are involved in the processing of TMT-induced freezing: Injections of muscimol into the medial nucleus of the amygdala completely block freezing observed during TMT exposure (Müller and Fendt, unpublished observations).

### 3.4. Neuropharmacology of TMT processing

During TMT exposure, an increase in dopamine release within the prefrontal cortex can be observed using microdialysis. The magnitude of this increase of dopamine release is about 50–100% (Wu et al., 2003); a similar increase of dopamine is found using *post mortem* neurochemical techniques in mice (Morrow et al., 2000; 2002). This dopamine release is caused by an activation of dopaminergic neurons within the ventral tegmental area (Redmond et al., 2002). Other dopaminergic neurons originating from the substantia nigra do not appear to be activated by TMT (Redmond et al., 2002).

Increased dopamine during TMT exposure was also found in the amygdala of mice (Morrow et al., 2000; Morrow et al., 2002). It should be noted that in contrast to the prefrontal cortex there is no increased dopamine release within the amygdala after presentation of a conditioned fear stimulus (Morrow et al., 2000). Furthermore, the mRNA levels of cholecystokinin (CCK, a neuropeptide which plays an important role in fear, anxiety, and stress (Charney, 2003)) were enhanced within the medial nucleus of the amygdala and ventral segmental area, but not in the basolateral complex of the amygdala after TMT exposure (Hebb et al., 2003a). However, the CCK mRNA increase in the medial nucleus of the amygdala was also observed during exposure to a control odor, here clean wood shavings (Hebb et al., 2003a). The authors interpret these findings with the hypothesis that the CCK increase within the amygdala is associated with enhanced vigilance and arousal, whereas the increase within the prefrontal cortex is associated with changes in the motivational and emotional state of the animal (Hebb et al., 2003a).

Hebb and colleagues (2004) investigated whether enkephalin neurons are activated by TMT. Increased *c-fos* activation in enkephalin-positive neurons was observed in the nucleus accumbens (shell and core) but not in other brain sites after TMT exposure. Furthermore, high-responders to TMT (freezing) had a larger activation of enkephalin neurons in the shell region of the nucleus accumbens than low-responders (Hebb et al., 2004). These high-responders also had a lower number of activated enkephalin-positive neurons within the central nucleus of the amygdala. Responsiveness to TMT was measured with



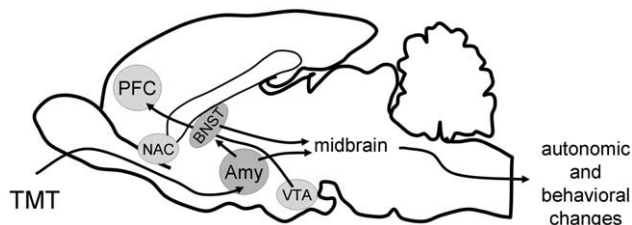


Fig. 4. First draft of a hypothetical neural pathway of TMT-induced fear. Abbreviations: Amy, amygdala; BNST, bed nucleus of the stria terminalis; NAC, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area.

a light/dark test after TMT exposure: high responders showed greater activation of enkephalin neurons within the basolateral and central nucleus of the amygdala. Furthermore, the number of enkephalin-positive neurons was increased in these two brain areas, as well as in the medial nucleus of the amygdala, whereas it was decreased in the core region of the nucleus accumbens (Hebb et al., 2004).

In summary, the nucleus accumbens and the amygdala may be involved in the behavioral responses that lead to an avoidance of further threats during and after TMT exposure (cf. Fig. 4). While an increase in the activity of neurons containing CCK, an anti-opioid peptide, was seen in the amygdala, activated neurons containing enkephalin, an opioid agonist, were seen in the nucleus accumbens.

#### 4. Comparison with studies using natural predator odors

At the outset it might be noted that a systematic comparison of the few studies using TMT with the studies using natural odors (e.g. cat, dog, fox or ferret odor) is very difficult. Much more work has to be done involving within-experiment comparison of these odors. For example, we do not know definitively whether TMT is a specific predatory cue. Furthermore, most of the TMT studies have exposed rats to very intense concentrations of TMT. Thus, many of the observed responses could be due to an aversive nature of the concentrated chemical. TMT is an extremely repugnant odor to humans, capable of inducing nausea (personal observation; Blanchard et al., 2003b). Therefore, more studies using lower TMT concentrations would be helpful. So, comparison should proceed cautiously with the caveat that the concentrations used in experiments with natural predator odors and with TMT may not be directly comparable.

There are only few studies which have tested the effects of natural fox odor on the behavior of rodents. In two field experiments, Dickman and colleagues showed that the capture success of wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*), field vole (*Microtus agrestis*) as well as house mice (*Mus domesticus*) in traps bearing fox feces is significantly less than in traps bearing control odors (Dickman, 1992; Dickman and Doncaster,

1984). This has been supported in the laboratory setting; Cattarelli and colleagues have seen laboratory rats express more freezing behavior, more avoidance behavior, increased vigilance, less grooming behavior, more defecation and urination, as well as an analgesic response when they are exposed to fox feces (Cattarelli and Chanel, 1979; Cattarelli and Vigouroux, 1981). In addition, an appetitive conditioned response towards food was strongly inhibited by exposure to fox feces (Cattarelli and Vigouroux, 1981).

In mice, fox feces odor was approached with significantly greater latency than other odors in two strains (CD-1 and C57/BL6), and in another strain (CBA) fox feces caused an increase of defensive burying and sniffing as well as a decrease in rearing (Dell'omo et al., 1994).

Most studies using natural predator odors have used the odor of cat fur as the stimulus. Some, but not all, of the defensive behaviors which are observed after exposure to cat odor are also seen after exposure to TMT (e.g. Blanchard et al., 2003b; McGregor et al., 2002; Zangrossi Jr. and File, 1992a; see section 2 of this article), a notable exception being the lack of 'head out' risk assessment behavior reported by McGregor and colleagues (2002) during TMT exposure.

Some defensive behaviors seen in response to TMT can be more intense than those seen in response to cat odor, although similar to the responses to other aversive odorants (McGregor et al., 2002; Hebb et al., 2002; but see also Hebb et al., 2003a; 2003b; 2004; Morrow et al., 2000; Wallace and Rosen, 2000). Defensive behaviors to TMT can be similar to behaviors observed with cat odor if lower concentrations of TMT are used. For example, lower levels of freezing (similar to the freezing durations observed during exposure to cat odor) are generally observed with lower concentrations of TMT (Blanchard et al., 2003b; Endres et al., 2005; Wallace and Rosen, 2000). Conversely, higher levels of freezing can be seen to cat odor if a larger piece of cat odor impregnated cloth is used (see Takahashi et al., this issue). Furthermore, the lower risk assessment behavior observed with TMT relative to cat odor might be explained by the use of very high TMT concentrations. It is hypothesized that risk assessment behavior is maximal during a medium perceived intensity of threat (Blanchard and Blanchard, 1990), so one would expect more risk assessment behavior in experiments with weak TMT concentrations. Indeed, there are some hints that risk assessment behavior (e.g. curve approach and curve duration) is more pronounced with lower rather than higher TMT concentrations (Blanchard et al., 2003b).

Some studies did not observe any typical fear behavior after exposure to TMT (e.g. McGregor et al., 2002) while there are also laboratories observing no behavioral and autonomic fear responses to cat odor (e.g. Masini and Campeau, unpublished observations quoted in Day et al., 2004). One hypothesis to explain these differences could be that other environmental stimuli are necessary in addition to the TMT or predator odor exposure to induce a full fear



response. Such stimuli might include lighting conditions and size and design of the testing apparatus. Furthermore, differences in the strain and species of test animals used could be responsible for different results. For example, PVG hooded rats express far more freezing during exposure to a cat than Sprague-Dawley rats (Farook et al., 2001). Further experiments have to be carried out to investigate possible effects of additional environmental stimuli or genetic background of the test animals on TMT or predator odor induced fear behavior.

One salient and highly reproducible difference between TMT and cat odor (from cat fur) is the inability of TMT, to date, to produce contextual conditioning (reviewed in Blanchard et al., 2003a). This result suggests a fundamental difference between these two stimuli. In an interesting analysis, Blanchard and colleagues (2003b) found that both cat urine and cat feces also failed to support conditioned contextual fear. The authors note that cat feces/anal gland odors and cat fur/skin odors differ in the extent to which they predict predatory immanence: 'scatological' stimuli such as feces and urine signify that a predator was historically present, while skin-derived odors suggest that a cat is currently nearby. Thus the failure urine and feces-derived predator stimuli (including TMT) to support contextual conditioning may relate to the fact that they are relatively unreliable signals of actual predatory threat.

Nonetheless, there is an overlap in the brain areas that are activated during or after exposure to natural predator odor or to TMT. *c-fos* and lesion studies demonstrated an important role of the amygdala complex, as well as of the bed nucleus of the stria terminalis in the processing of fear induced by TMT (Day et al., 2004; Fendt et al., 2003; Müller et al., 2003) or cat odor (Dielenberg et al., 2001; Li et al., 2004). Furthermore, the prelimbic cortex, as well as several midbrain structures mediating defensive responses, may be important for both TMT and cat odor-induced fear behavior, particularly when the stimulus is inescapable (McGregor et al., 2004).

Until recently, there were no published studies directly comparing the neural effects of natural predator odors and TMT in the same experiment, using the same test apparatus, test design, and the same animals. Furthermore, as stated above, TMT is mostly used in much more intense concentrations than cat odor, so that any observed differential activation patterns may be related to different concentrations.

In recent unpublished work, McGregor and colleagues have made a direct comparison between TMT and cat odor (Hunt et al., 2004). One very salient difference was that cat odor is processed in a pheromone-like fashion, with pronounced activation in the accessory olfactory bulb and vomeronasal projection areas such as the medial amygdala (McGregor et al., 2004). In contrast, no accessory olfactory bulb activation could be seen in TMT exposed rats, despite robust activation in the main olfactory bulb, similar to that described by Illig and Haberly (2003). Another intriguing

difference is the failure of cat odor to activate the central nucleus of the amygdala, which is a key region activated by TMT (see above). Finally, TMT, but not cat odor, appears to activate taste and visceral-related regions of the brainstem such as the nucleus of the solitary tract and parabrachial nucleus, perhaps reflecting the repugnant nature of the TMT stimulus.

## 5. Possible use of TMT-induced changes as an animal model of fear

### 5.1. Is TMT a predator odor?

The question of whether or not TMT is a predator odor is a difficult one to answer. TMT is a component of the fox fecal odor (Vernet-Maury, 1980) and foxes are predators of rodents (Goldyn et al., 2003). Furthermore, TMT appears to be the most effective component of fox feces in inducing behavioral changes (Vernet-Maury, 1980) almost to the level than the concentrated fox-dropping solvent extract does (Vernet-Maury, 1980; Vernet-Maury et al., 1984). However, other components of fox feces were also able to induce such fear-like behavioral changes but with less efficacy.

We have discussed here clear evidence that TMT elicits a number of autonomic and behavioral changes that are indicative of fear. However, there is also evidence that TMT is not very different from other aversive or noxious odors in its ability to elicit behavioral and neurophysiological changes (Lowry et al., 2005; McGregor et al., 2002; Wallace and Rosen, 2000). One hypothesis, first suggested by McGregor (McGregor et al., 2002), is that TMT is not a natural predator stimulus but rather is a generalized noxious stimulus which elicits fear responses, particularly when presented in a confined space where its noxious effects cannot be escaped. The fact that TMT clearly elicits classic fear behaviors may suggest that the rodent olfactory system is attuned to noxious odors, perhaps as a warning system of possible toxicity. However, the observation that even very low TMT concentrations induce specific behavioral and autonomic changes (Blanchard et al., 2003b; Endres et al., 2005, Fendt, unpublished observation) argues against this hypothesis, although this in turn raises difficult questions as to the subjective intensity of odors in rodent species.

Without doubt, TMT is a component of a predator-related stimulus (fox feces). Mostly, however, TMT is presented in a concentration that probably does not correspond to the concentration of a predator odor in natural conditions. In such high concentrations, TMT may act more as a noxious stimulus than a predator stimulus. However, the question whether TMT reflects a natural predator stimulus is not critical for the question whether TMT-induced changes could be used as an animal model of fear. Many fear models use an electric foot shock to induce fear, a stimulus which is demonstrably unnatural. But like a

foot shock, TMT is a stimulus whose intensity can be well controlled by the experimenter. This is probably not the case with the use of exposure to a cat or cat collar (although see Takahashi et al., this issue).

### 5.2. Criteria for animal models of fear

Animal models of fear should fulfill most of the criteria used to evaluate the validity of an animal model. In particular, this includes face validity, construct validity, and predictive validity (Hogg, 1996; Rodgers, 1997). Face validity means that the behavioral and autonomic signs of fear are similar in humans and in the animal model. Clearly, the changes induced by TMT exposure in rodents include behavioral and autonomic signs of fear that are very similar to those reflecting fear in humans. Furthermore, the brain structures processing and/or inducing these changes should be the same in animals and humans (construct validity). Despite the relatively few studies on the neural basis of TMT-induced effects, there are convincing data showing that the limbic system (including the amygdala and its extended parts (i.e. BNST), the septum, and the hypothalamus) as well as further brain sites, are important for the mediation of TMT-induced autonomic and behavioral changes.

One very critical criterion for an animal model of fear is that drugs which are known to treat human fear work also in the animal model (predictive validity; see Gray, 1988). Up to now, there is only one published study showing that the benzodiazepine receptor agonist midazolam is not effective in reducing TMT-induced behavioral changes (Dielenberg et al., 1999). In this study, a dose of 0.5 mg/kg midazolam was used. Despite behavioral changes to cat odor being completely blocked by midazolam in this study, this dose may have been too weak to affect TMT-induced changes since this dose was not effective in other well established animal models of fear (Hijzen and Slangen, 1989; Pain et al., 2002; Savic et al., 2004). Other anxiolytic agents such diazepam, imipramine, buspirone, and antalarmin have also been tested for their efficacy in reducing TMT-induced behavioral changes and all are reported as ineffective (mentioned in Blanchard et al., 2003a). However, these results are as yet unpublished.

### 5.3. Conclusion

Taken together, there is considerable evidence that TMT induces behavioral and autonomic signs of fear in naive rats and mice (see Table 1). Differences in the test apparatus and behavioral procedures used, the strain and species of animal used, and the strength and method of TMT presentation could be responsible for the fact that only weak or negligible behavioral changes were observed in some experiments. Initial studies on the neural basis of TMT-induced behavioral changes showed that brain structures previously implicated in the processing of fearful stimuli are also activated during

TMT exposure (see figure 4). The areas of the brain activated by TMT indicate that this natural chemical may induce fear in a similar manner to other innate fear stimuli. At this time, there are no comprehensive studies published investigating whether anxiolytic drugs are able to block TMT-induced fear behavior. Initial, preliminary data have questioned this. If further studies can show that relevant doses of anxiolytic drugs are able to block TMT-induced behavioral changes, then TMT-induced fear behavior could serve as a model of innate fear. TMT could then serve as a more natural stimulus than the often-used electric shock, and would also have the advantage of being more easily controlled in its intensity than exposure to a cat or to cat odor.

### Acknowledgements

Supported by the Deutsche Forschungsgemeinschaft (SFB 550/C8) to MF and TE, by an Australian Research Council grant to ISM, and by a Brain Research Foundation Fay/Frank Seed grant to Leslie M. Kay. The helpful comments of Matthias Laska and the collaboration of Robert Dielenberg and Glenn Hunt are gratefully acknowledged. We would also like to thank J.P. LaFontaine for his consultations on the chemistry behind TMT.

### References

- Adrian, E.D., 1950. The electrical activity of the mammalian olfactory bulb. *Electroencephalogr. Clin. Neurophysiol.* 2, 377–388.
- Arnould, C., Malosse, C., Signoret, J.P., Descoins, C., 1998. Which chemical constituents from dog feces are involved in its food repellent effect in sheep? *J. Chem. Ecol.* 24, 559–576.
- Blanchard, R.J., Blanchard, D.C., 1969. Passive and active reactions to fear-eliciting stimuli. *J. Comp. Physiol. Psychol.* 68, 129–135.
- Blanchard, R.J., Blanchard, D.C., 1990. An ethoexperimental analysis of defense, fear, and anxiety. In: McNaughton, N., Andrews, G. (Eds.), *Anxiety*. University of Otago Press, Dunedin, pp. 124–133.
- Blanchard, R.J., Blanchard, D.C., Rodgers, J., Weiss, S.M., 1990a. The characterization and modelling of antipredator defensive behavior. *Neurosci. Biobehav. Rev.* 14, 463–472.
- Blanchard, R.J., Blanchard, D.C., Weiss, S.M., Meyer, S., 1990b. The effects of ethanol and diazepam on reactions to predatory odors. *Pharmacol. Biochem. Behav.* 35, 775–780.
- Blanchard, D.C., Griebel, G., Blanchard, R.J., 2003a. Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 27, 1177–1185.
- Blanchard, D.C., Markham, C., Yang, M., Hubbard, D., Madarang, E., Blanchard, R.J., 2003b. Failure to produce conditioning with low-dose trimethylthiazoline or cat feces as unconditioned stimuli. *Behav. Neurosci.* 117, 360–368.
- Bolles, R.C., Fanselow, M.S., 1980. A perceptual-defensive-recuperative model of fear and pain. *Behav. Brain Sci.* 3, 291–301.
- Bredie, W.L., Mottram, D.S., Guy, R.C., 2002. Effect of temperature and pH on the generation of flavor volatiles in extrusion cooking of wheat flour. *J. Agric. Food Chem.* 50, 1118–1125.
- Burwash, M.D., Tobin, M.E., Woolhouse, A.D., Sullivan, G.M., 1998a. Field testing synthetic predator odors for roof rats (*Rattus rattus*) in Hawaiian macadamia nut orchards. *J. Chem. Ecol.* 24, 603–630.

- Burwash, M.D., Tobin, M.E., Woolhouse, A.D., Sullivan, T.P., 1998b. Laboratory evaluation of predator odors for eliciting an avoidance response in roof rats (*Rattus rattus*). *J. Chem. Ecol.* 24, 49–65.
- Campeau, S., Falls, W.A., Cullinan, W.E., Helmreich, D.L., Davis, M., Watson, S.J., 1997. Elicitation and reduction of fear: behavioural and neuroendocrine indices and brain induction of the immediate-early gene *c-fos*. *Neuroscience* 78, 1087–1104.
- Cattarelli, M., Chanel, J., 1979. Influence of some biologically meaningful odorants on the vigilance states of the rat. *Physiol Behav.* 23, 831–838.
- Cattarelli, M., Vigouroux, M., 1981. Evaluation of emotional reactions of the rat elicited by biologically meaningful odorants during an operant behavior (author's transl). *Physiol. Behav.* 27, 445–455.
- Chabaud, P., Ravel, N., Wilson, D.A., Mouly, A.M., Vigouroux, M., Farget, V., Gervais, R., 2000. Exposure to behaviourally relevant odour reveals differential characteristics in rat central olfactory pathways as studied through oscillatory activities. *Chem. Senses* 25, 561–573.
- Chapman, C.A., Xu, Y., Haykin, S., Racine, R.J., 1998. Beta-frequency (15–35 Hz) electroencephalogram activities elicited by toluene and electrical stimulation in the behaving rat. *Neuroscience* 86, 1307–1319.
- Charney, D.S., 2003. Neuroanatomical circuits modulating fear and anxiety behaviors. *Acta Psychiatr. Scand. Suppl.*, 38–50.
- Davis, M., Falls, W.A., Campeau, S., Kim, M., 1993. Fear-potentiated startle: a neural and pharmacological analysis. *Behav. Brain Res.* 58, 175–198.
- Day, H.E., Masini, C.V., Campeau, S., 2004. 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.* 1025, 139–151.
- Dell'Omio, G., Fiore, M., Alleva, E., 1994. Strain differences in mouse response to odours of predators. *Behav. Proc.* 32, 105–116.
- Dickman, C.R., 1992. Predation and habitat shift in the house mouse, *Mus domesticus*. *Ecology* 73, 313–322.
- Dickman, C.R., Doncaster, C.P., 1984. Responses of small mammals to Red fox (*Vulpes vulpes*) odour. *J. Zool.* 204, 521–531.
- Dielenberg, R.A., McGregor, I.S., 2001. Defensive behavior in rats towards predatory odors: a review. *Neurosci. Biobehav. Rev.* 25, 597–609.
- Dielenberg, R.A., Arnold, J.C., McGregor, I.S., 1999. Low-dose midazolam attenuates predatory odor avoidance in rats. *Pharmacol. Biochem. Behav.* 62, 197–201.
- Dielenberg, R.A., Hunt, G.E., McGregor, I.S., 2001. 'When a rat smells a cat': the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. *Neuroscience* 104, 1085–1097.
- Endres, T., Apfelbach, R., Fendt M., 2005. Behavioral changes induced in rats by exposure to Trimethylthiazoline, a component of fox odor. *Behav. Neurosci.* 1119, in press.
- Fanselow, M.S., Helmstetter, F.J., 1988. Conditional analgesia, defensive freezing, and benzodiazepines. *Behav. Neurosci.* 102, 233–243.
- Farook, J.M., Zhu, Y.Z., Wang, H., Moochhala, S., Lee, L., Wong, P.T., 2001. Strain differences in freezing behavior of PVG hooded and Sprague-Dawley rats: differential cortical expression of cholecystokinin2 receptors. *NeuroReport* 12, 2717–2720.
- Fendt, M., Fanselow, M.S., 1999. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci. Biobehav. Rev.* 23, 743–760.
- Fendt, M., Endres, T., Apfelbach, R., 2003. 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.* 23, 23–27.
- Gillies, C., Clout, M., 2003. The prey of domestic cats (*Felis catus*) in two suburbs of Auckland City, New Zealand. *J. Zool.* 259, 309–315.
- Glowacinski, Z., Profus, P., 1997. Potential impact of wolves *Canis lupus* on prey populations in eastern Poland. *Biol. Conserv.* 80, 99–106.
- Goldyn, B., Hromada, M., Surmacki, A., Tryjanowski, P., 2003. Habitat use and diet of the red fox *Vulpes vulpes* in an agricultural landscape in Poland. *Zeitschrift für Jagdwissenschaft* 49, 191–200.
- Gray, J.A., 1988. Behavioural and neural-system analyses of the actions of anxiolytic drugs. *Pharmacol. Biochem. Behav.* 29, 767–769.
- Heale, V.R., Vanderwolf, C.H., 1994. Dentate gyrus and olfactory bulb responses to olfactory and noxious stimulation in urethane anaesthetized rats. *Brain Res.* 652, 235–242.
- Heale, V.R., Vanderwolf, C.H., Kavaliers, M., 1994. Components of weasel and fox odors elicit fast wave bursts in the dentate gyrus of rats. *Behav. Brain Res.* 63, 159–165.
- Hebb, A.L., Zacharko, R.M., Dominguez, H., Trudel, F., Laforest, S., Drolet, G., 2002. Odor-induced variation in anxiety-like behavior in mice is associated with discrete and differential effects on mesocorticolimbic cholecystokinin mRNA expression. *Neuropsychopharmacology* 27, 744–755.
- Hebb, A.L., Zacharko, R.M., Dominguez, H., Laforest, S., Gauthier, M., Levac, C., Drolet, G., 2003a. Changes in brain cholecystokinin and anxiety-like behavior following exposure of mice to predator odor. *Neuroscience* 116, 539–551.
- Hebb, A.L., Zacharko, R.M., Gauthier, M., Drolet, G., 2003b. Exposure of mice to a predator odor increases acoustic startle but does not disrupt the rewarding properties of VTA intracranial self-stimulation. *Brain Res.* 982, 195–210.
- Hebb, A.L., Zacharko, R.M., Gauthier, M., Trudel, F., Laforest, S., Drolet, G., 2004. Brief exposure to predator odor and resultant anxiety enhances mesocorticolimbic activity and enkephalin expression in CD-1 mice. *Eur. J. Neurosci.* 20, 2415–2429.
- Hijzen, T.H., Slangen, J.L., 1989. Effects of midazolam, DMCM and lindane on potentiated startle in the rat. *Psychopharmacology* 99, 362–365.
- Hogg, S., 1996. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol. Biochem. Behav.* 54, 21–30.
- Holmes, M.M., Galea, L.A.M., 2002. Defensive behavior and hippocampal cell proliferation: differential modulation by naltrexone during stress. *Behav. Neurosci.* 116, 160–168.
- Hotsenpiller, G., Williams, J.L., 1997. A synthetic predator odor (TMT) enhances conditioned analgesia and fear when paired with a benzodiazepine receptor inverse agonist (FG-7142). *Psychobiology* 25, 83–88.
- Hunt, G.E., Apfelbach, R., Staples, L., McGregor, I.S., 2004. Cat odor, but not trimethylthiazoline (TMT, fox odor), activates accessory olfactory and defense-related brain regions in rats. *Soc. Neurosci. Abstr.* 782.13.
- Illig, K.R., Haberly, L.B., 2003. Odor-evoked activity is spatially distributed in piriform cortex. *J. Comp. Neurol.* 457, 361–373.
- Kats, L.B., Dill, L.M., 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5, 361–394.
- Kay, L.M., Freeman, W.J., 1998. Bidirectional processing in the olfactory-limbic axis during olfactory behavior. *Behav. Neurosci.* 112, 541–553.
- Kay, L.M., Laurent, G., 1999. Odor- and context-dependent modulation of mitral cell activity in behaving rats. *Nat. Neurosci.* 2, 1003–1009.
- Laska, M., Fendt, M., Wieser, A., Endres, T., Hernandez Salazar, L.T., Apfelbach, R., 2004. Detecting danger—or just another odorant? Olfactory sensitivity for the fox odor component 2,4,5-trimethylthiazoline in four species of mammals. *Physiol. Behav.* 84, 211–215.
- LeDoux, J.E., 2000. Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184.
- Li, C.I., Maglinao, T.L., Takahashi, L.K., 2004. Medial amygdala modulation of predator odor-induced unconditioned fear in the rat. *Behav. Neurosci.* 118, 324–332.
- Martin, C., Gervais, R., Hugues, E., Messaoudi, B., Ravel, N., 2004. Learning modulation of odor-induced oscillatory responses in the rat olfactory bulb: a correlate of odor recognition? *J. Neurosci.* 24, 389–397.
- Masini, C.V., Sauer, S., Campeau, S., 2005. Ferret odor as a processive stress model in rats: neurochemical, behavioral, and endocrine evidence. *Behav. Neurosci.* 119, 280–292.

- McGregor, I.S., Schrama, L., Ambernoon, P., Dielenberg, R.A., 2002. 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.* 129, 1–16.
- McGregor, I.S., Hargreaves, G.A., Apfelbach, R., Hunt, G.E., 2004. Neural correlates of cat odor-induced anxiety in rats: region-specific effects of the benzodiazepine midazolam. *J. Neurosci.* 24, 4134–4144.
- Morrow, B.A., Redmond, A.J., Roth, R.H., Elsworth, J.D., 2000. The predator odor, TMT, displays a unique, stress-like pattern of dopaminergic and endocrinological activation in the rat. *Brain Res.* 864, 146–151.
- Morrow, B.A., Elsworth, J.D., Roth, R.H., 2002. Fear-like biochemical and behavioral responses in rats to the predator odor, TMT, are dependent on the exposure environment. *Synapse* 46, 11–18.
- Müller, M., Apfelbach, R., Fendt, M., 2003. Temporary inactivation of the medial amygdala blocks freezing in rats induced by trimethylthiazoline, a component of fox feces. In: Elsner, N., Zimmermann, H. (Eds.), *Göttingen Abstract*. Georg Thieme Verlag, Stuttgart.
- Mussinan, C.J., Wilson, R.A., Katz, I., Hruza, A., Vock, M.H., 1977. Identification and flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef American Chemical Society Symposium Series, Phenolic, Sulfur, and Nitrogen Compounds 1977 pp. 133–145.
- Neville, K.R., Haberly, L.B., 2003. Beta and gamma oscillations in the olfactory system of the urethane-anesthetized rat. *J. Neurophysiol.* 90, 3921–3930.
- Pain, L., Launoy, A., Fouquet, N., Oberling, P., 2002. Mechanisms of action of midazolam on expression of contextual fear in rats. *Br. J. Anaesth.* 89, 614–621.
- Perrot-Sinal, T.S., Heale, V.R., Ossenkopp, K.P., Kavaliers, M., 1996. Sexually dimorphic aspects of spontaneous activity in meadow voles (*Microtus pennsylvanicus*): effects of exposure to fox odor. *Behav. Neurosci.* 110, 1126–1132.
- Perrot-Sinal, T., Ossenkopp, K.P., Kavaliers, M., 1999. Effects of repeated exposure to fox odor on locomotor activity levels and spatial movement patterns in breeding male and female meadow voles (*Microtus pennsylvanicus*). *J. Chem. Ecol.* 25, 1567–1584.
- Preti, G., Muetterties, E.L., Furman, J.M., Kennelly, J.J., Johns, B.E., 1976. Volatile constituents of dog (*Canis familiaris*) and coyote (*Canis latrans*) anal sacs. *J. Chem. Ecol.* 2, 186.
- Radulovic, J., Kammermeier, J., Spiess, J., 1998. Relationship between Fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. *J. Neurosci.* 18, 7452–7461.
- Ravel, N., Chabaud, P., Martin, C., Gaveau, V., Hugues, E., Tallon-Baudry, C., Bertrand, O., Gervais, R., 2003. Olfactory learning modifies the expression of odour-induced oscillatory responses in the gamma (60–90 Hz) and beta (15–40 Hz) bands in the rat olfactory bulb. *Eur. J. Neurosci.* 17, 350–358.
- Redmond, A.J., Morrow, B.A., Elsworth, J.D., Roth, R.H., 2002. 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.* 328, 209–212.
- Rodgers, R.J., 1997. Animal models of 'anxiety': where next? *Behav. Pharmacol.* 8, 477–496.
- Rosen, J.B., 2004. The Neurobiology of Conditioned and Unconditioned Fear: A Neurobehavioral System Analysis of the Amygdala. *Behav. Cogn Neurosci. Rev.* 3, 23–41.
- Savic, M.M., Obradovic, D.I., Ugresic, N.D., Cook, J.M., Yin, W., Bokonjic, D.R., 2004. Bidirectional effects of benzodiazepine binding site ligands in the elevated plus-maze: differential antagonism by flumazenil and beta-CCT. *Pharmacol. Biochem. Behav.* 79, 279–290.
- Shiple, M.T., Adamek, G.D., 1984. The connections of the mouse olfactory bulb: a study using orthograde and retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase. *Brain Res. Bull.* 12, 669–688.
- Sullivan, G.M., Apergis, J., Bush, D.E., Johnson, L.R., Hou, M., LeDoux, J.E., 2004. Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. *Neuroscience* 128, 7–14.
- Vanderwolf, C.H., Zibrowski, E.M., 2001. Pyriform cortex beta-waves: odor-specific sensitization following repeated olfactory stimulation. *Brain Res.* 892, 301–308.
- Vanderwolf, C.H., Zibrowski, E.M., Wakarchuk, D., 2002. The ability of various chemicals to elicit olfactory beta-waves in the pyriform cortex of meadow voles (*Microtus pennsylvanicus*) and laboratory rats (*Rattus norvegicus*). *Brain Res.* 924, 151–158.
- Vernet-Maury, E., 1980. Trimethyl-thiazoline in fox feces: A natural alarming substance for the rat. *Olfaction Taste VII*, 407.
- Vernet-Maury, E., Le Magnen, J., Chanel, J., 1968. Comportement émotif chez le rat, Influence de l'odeur d'un prédateur et d'un non-prédateur. *C.R. Acad. Sci. Paris* 267, 331–334.
- Vernet-Maury, E., Polak, E.H., Demael, A., 1984. Structure-activity relationship of stress-inducing odorants in the rat. *J. Chem. Ecol.* 10, 1007–1018.
- Vernet-Maury, E., Constant, B., Chanel, J., 1992. Repellent effect of trimethyl thiazoline in the wild rat *Rattus norvegicus* Berkenhout. In: Doty, R.L., Muller-Schwarze, D. (Eds.), *Chemical Signals in Vertebrates VI*. Plenum Press, New York, pp. 305–310.
- Walf, A.A., Frye, C.A., 2003. Anti-nociception following exposure to trimethylthiazoline, peripheral or intra-amygdala estrogen and/or progesterone. *Behav. Brain Res.* 144, 77–85.
- Walker, D.L., Toufexis, D.J., Davis, M., 2003. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *Eur. J. Pharmacol.* 463, 199–216.
- Wallace, K.J., Rosen, J.B., 2000. Predator odor as an unconditioned fear stimulus in rats: elicitation of freezing by trimethylthiazoline, a component of fox feces. *Behav. Neurosci.* 114, 912–922.
- Wallace, K.J., Rosen, J.B., 2001. Neurotoxic lesions of the lateral nucleus of the amygdala decrease conditioned fear but not unconditioned fear of a predator odor: comparison with electrolytic lesions. *J. Neurosci.* 21, 3619–3627.
- Wu, W.R., Li, N., Sorg, B.A., 2003. Prolonged effects of repeated cocaine on medial prefrontal cortex dopamine response to cocaine and a stressful predatory odor challenge in rats. *Brain Res.* 991, 232–239.
- Zangrossi Jr., H., File, S.E., 1992a. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. *Brain Res. Bull.* 29, 381–388.
- Zangrossi Jr., H., File, S.E., 1992b. Chlordiazepoxide reduces the generalised anxiety, but not the direct responses, of rats exposed to cat odor. *Pharmacol. Biochem. Behav.* 43, 1195–1200.
- Zibrowski, E.M., Vanderwolf, C.H., 1997. Oscillatory fast wave activity in the rat pyriform cortex: relations to olfaction and behavior. *Brain Res.* 766, 39–49.
- Zibrowski, E.M., Hoh, T.E., Vanderwolf, C.H., 1998. Fast wave activity in the rat rhinencephalon: elicitation by the odors of phytochemicals, organic solvents, and a rodent predator. *Brain Res.* 800, 207–215.