Mouse alarm pheromone shares structural similarity with predator scents

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Sensing the chemical warnings present in the environment is essential for species survival. In mammals, this form of danger communication occurs via the release of natural predator scents that can involuntarily warn the prey or by the production of alarm pheromones by the stressed prey alerting its conspecifics. Although we previously identified the olfactory Grueneberg ganglion as the sensory organ through which mammalian alarm pheromones signal a threatening situation, the chemical nature of these cues remains elusive. We here identify, through chemical analysis in combination with a series of physiological and behavioral tests, the chemical structure of a mouse alarm pheromone. To successfully recognize the volatile cues that signal danger, we based our selection on their activation of the mouse olfactory Grueneberg ganglion and the concomitant display of innate fear reactions. Interestingly, we found that the chemical structure of the identified mouse alarm pheromone has similar features as the sulfur-containing volatiles that are released by predating carnivores. Our findings thus not only reveal a chemical Leitmotiv that underlies signaling of fear, but also point to a double role for the olfactory Grueneberg ganglion in intraspecies as well as interspecies communication of danger.

olfaction | animal communication | behavior | calcium imaging

In their continuous struggle for survival, predator and prey species interact to maximize the likelihood of finding food or avoiding being eaten. Preys have adapted to deal with dangerous encounters using specialized sensory systems. Detection of auditory signals, visual signs, and olfactory messages induces elaborate and innate survival behaviors in the prey, which can either hide, fight, or flee (1). Multiple hiding strategies (crypsis) coexist and are best when combined. Indeed, crypsis can be morphological when the prey uses its body shape/color to blend into the background (camouflage), it can be behavioral when the prey uses immobility or subtle steady movements to decrease the chance of being detected (freezing); or it can be chemical when the prey releases odorant molecules to mask its own odor (2, 3).

The olfactory messages communicating the presence of a danger to the prey are the scents naturally released in the environment by the predators, named kairomones (4, 5), and the alarm pheromones (APs) secreted by threatened or injured conspecifics (6–11). Intraspecies communication by APs is an evolutionarily widespread phenomenon, presumably occurring in all animal phyla. Social species of fish, insects, and mammals use AP secretion as an altruistic signal to protect their colony/group or family when in danger. These alert cues may derive from compounds that evolved to make the flesh unpalatable or toxic to predators. Their primary function could have been the control of skin pathogens (12, 13). In insects and fish, APs of variable chemical structures such as terpenes, hydrocarbons, ketones, or nitrogen/sulfated heterocyclic compounds have been identified (12–16). In mammals, the chemical structure of APs is still unknown but, to fulfill their sensory warning role, they should be volatile, hydrophilic, and short-lived molecules (17, 18). Their identification will be possible in rodents, for example, as they can be collected as a blend from the environment of a stressed animal and directly tested on a conspecific by physiological or behavioral experiments (17, 19–21).

Using CO_2 euthanasia known to cause a major alarm condition (21, 22), we previously collected mouse APs as a blend and identified the sensory system involved in their detection (21). We showed that the blend of mouse APs induces calcium transients specifically in the neurons of the Grueneberg ganglion (GG), an olfactory subsystem present at the tip of the nose, close to the opening of the naris (21, 23–27). The mouse GG is an arrow-shaped neuronal structure 750–1,000 μm in length. It lines both sides of the nasal septum and comprises 300–500 cells. Each cell sends out a single axon, and axons fasciculate immediately as they project caudally along the dorsal roof of the nasal cavity to the necklace complex in the olfactory bulb (24–26, 28, 29). The GG displays multimodal properties (21, 30–33) and starts developing around embryonic day 16. Contrary to the other olfactory subsystems, the GG appears to be complete and functional at birth (24–28), ensuring immediate AP sensing and increasing chances of survival in the wild. An intact GG is necessary to observe the fearful behavior of the recipient animals in the presence of APs (21).

In this study, we collect and chemically identify volatile mouse pheromones by a solid-phase microextraction followed by a gas chromatography and mass spectrometry method. We further assess their alarm sensory function by calcium imaging and behavioral experiments leading to the identification of a mouse alarm pheromone, 2-*sec*-butyl-4,5-dihydrothiazole. This alarm cue produced by both male and female mice under different alarm conditions resembles the sulfur-containing volatiles present in predator scents. Interestingly, this shared chemical feature that signals danger is specifically deciphered by the olfactory Grueneberg ganglion.

Results

Chemical Extraction of Mouse Volatiles. We used solid-phase microextraction (SPME) fibers to adsorb the volatile mouse APs released under a CO_2 alarm condition. After extraction, these molecules were analyzed by gas chromatography coupled to mass spectrometry (GC/MS) (Fig. 1*A* and Fig. S1). A total of 44 mouse volatiles were detected (Fig. 1*B*) among which we found pheromones important for social interactions such as 2-heptanone (34) or α- and β-farnesene (35) (Fig. S2). Thirty-two of these 44 volatile chemical cues were also emitted by control mice (under a non-alarm condition). Of the 12 chemicals appearing only under alarm condition (Fig. 1*B*, in red), 8 were of transient nature, thereby fulfilling an important chemical criterion of volatile alarm cues.

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The authors declare no conflict of interest.

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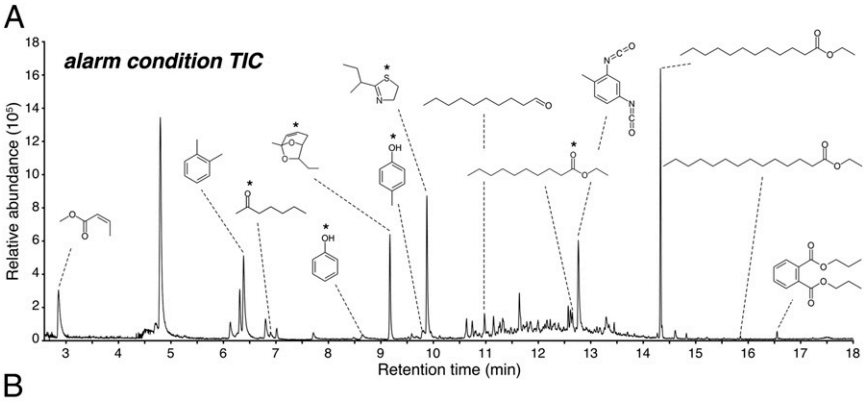

B

Identification of mice volatile chemical cues by SPME-GC/MS

RT	Molecule	RT	Molecule
Alarm condition			
2.935	Ethane, 1,1-diethoxy- (# Acetal)	9.791	Phenol, 4-methyl- (# *p*-Cresol)
3.395	2-Pentanone, 4-methyl-	9.823	Benzoic acid, methyl ester (# Methyl benzoate)
3.848	1,2-Propanediol (# Propylene glycol) †	9.875	2-*sec*-butyl-4,5-dihydrothiazole (# SBT) †
4.433	1-Pentanol (# Amylol)	9.938	Unidentified compound
4.781	1-Butyric acid (# Butyric acid) †	10.217	Acetic acid, 2-ethylhexyl ester
4.819	Unidentified compound †	10.419	Octanoic Acid (# Caprylic acid)
5.286	Hexanal	10.554	5-Decanone
6.432	Unidentified compound	10.738	Octanoic acid, ethyl ester
6.658	Pentanoic acid (# Valeric acid) †	11.254	2,4-Nonadienal
6.940	2-Heptanone †	11.289	Unidentified compound
7.083	Unidentified compound †	11.271	2-Propanol, 1-[1-methyl-2-(2-propenyloxy)ethoxy]-
7.156	Heptanal	11.568	6-Undecanone
7.697	3-Cyclohexene-1-methanol †	12.378	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methyl)propyl ester
8.138	Hexanoic acid	12.515	6-Dodecanone
8.367	5-Hepten-2-one, 6-methyl-	12.574	Unidentified compound †
8.656	Phenol	12.642	Decanoic acid, ethyl ester
9.120	3,5-Octadien-2-ol	12.654	Unidentified compound
9.172	exo-7-Ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]oct-3-ene (# DHB) †	13.166	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene- (# β-farnesene)
9.325	Unidentified compound	13.444	Nonadecane
9.492	2-Octenal	13.618	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl- (# α-farnesene)
9.631	2-Butoxyethyl acetate	13.231	5,9-Undecadien-2-one, 6,10-dimethyl- (# geranyl acetone)
9.683	Benzenamine, 4-methyl-	16.850	Hexanedioic acid, bis(2-ethylhexyl) ester †

Fig. 1. Chemical identification of candidate mouse alarm pheromones. (*A*) Representative total ion chromatogram (TIC) of volatiles emitted by mice under alarm conditions obtained by SPME-GC/MS. Chemical formulas of a selection of volatiles emitted by mice (*) or background volatiles are indicated above peaks. A mix of five adult male and female (3:2) mice under lethal CO_2 stress was used for this experiment. (*B*) Forty-four mouse volatiles were detected in our experimental conditions. The volatiles are listed according to their retention time (RT). Both the chemical nomenclature and the usual names (#) are mentioned. The names of the compounds detected but not found in the Wiley7N or the NIST08 libraries are listed as "Unidentified compound." Red type indicates the 12 chemicals appearing only under alarm conditions. Short-lived molecules (compounds not generated 30 min after the death of the mice) are indicated by "†."

They could thus be considered as AP candidates. Among these eight AP candidates, we found five compounds listed in the chemical libraries—namely, 1,2-propanediol, butyric acid (36) (BA), pentanoic acid, dehydro-*exo*-brevicomin (37) (DHB) (Fig. S2), and 2-*sec*-butyl-4,5-dihydrothiazole (37) (SBT) (Fig. S3 *A* and *B*)—and three unlisted chemicals. The identified chemicals were then purchased if commercially available or synthesized in house (Fig. S3*C*) to test by calcium imaging whether they could activate mouse GG neurons.

Identification of a Mouse Alarm Pheromone. We performed calcium imaging experiments on GG coronal slices (21) of a particular gene-targeted mouse strain called OMP-GFP (olfactory marker protein-green fluorescent protein) in which all olfactory neurons express GFP as a reporter gene (38, 39). Tissue slices were incubated in the calcium-sensitive dye Fura-2 acetoxymethyl ester. GG neurons were identified by the intrinsic green fluorescence of GFP in their cell bodies and by their specific morphology (Fig. 2*A*). The uptake of the dye was confirmed by fluorescence measurements (Fig. 2*B*). Candidate alarm cues were delivered in oxycarbonated artificial cerebrospinal fluid (ACSF) on the tissue slices in the imaging chamber (Fig. 2*C*). Four of five AP candidates did not activate GG neurons (1,2-propanediol, n = 0/28 neurons tested; BA, n = 0/19; pentanoic acid, n = 0/27; DHB, n = 0/53). Perfusion of SBT, on the other hand, induced in GG neurons, in both female and male mice tissue slice preparations, reversible and reproducible calcium transients (n = 36/50) (Fig. 2 *C* and *D*; Fig. S4 *A*–*C*). In a separate set of experiments, we could verify that SBT activated the same GG neurons as the blend of mouse APs (n = 9/9) (Fig. S4 *D* and *E*). Calcium responses were observed at different concentrations of SBT (Fig. 2*E*), allowing the estimation of an EC_{50} value in the micromolar range (n = 7) (Fig. 2*F*). Interestingly, SBT was produced by mice undergoing different alarm conditions: a lethal CO_2 stress, a confinement stress, or a cold temperature stress (40–42) (Fig. S5 *A*–*C*). The estimated SBT concentration released during these different alarm conditions was also in the micromolar range (Fig. S5*C*). In addition, we find SBT to be released under alarm conditions by both male and female mice (Fig. S5 *D*–*H*). Together, these data suggested that SBT could be a candidate molecule for carrying the danger signal.

We tested this hypothesis by exposing adult male and female mice to this putative alarm cue and found that it induced a significant elevation of their plasma corticosterone levels, indicating a systemic stress response (43) (Fig. 2*G*). Indeed, SBT alone could fully reproduce, in both genders (Fig. S6), the systemic stress response that was also generated by the blend of APs collected under the alarm condition (Fig. 1*A*). Thus, from these experiments, SBT emerged as our best candidate mouse alarm pheromone.

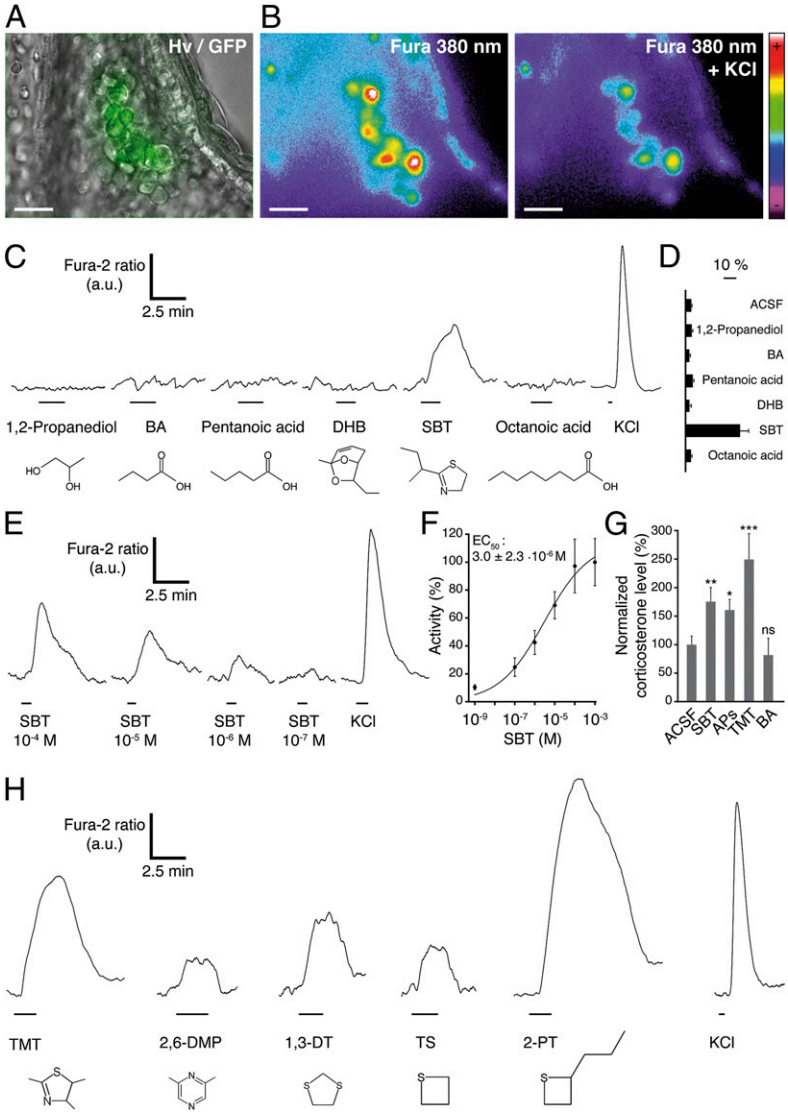

Fig. 2. GG neurons are activated by alarm pheromones and predator kairomones that share a common chemical structure. (*A*) GG coronal slice from an OMP-GFP mouse where GG neurons can be observed with their intrinsic GFP fluorescence. (*B*) Uptake of Fura-2AM into GG neurons measured at 380 nm in color-coded map for unbound Fura before and at the peak of an intracellular calcium increase induced by a control pulse of KCl (20 mM). (*C*) SBT (10^{-4} M) induced a reversible calcium transient in GG neurons. The other identified volatile chemical cues emitted by mice 1,2-propanediol, BA, pentanoic acid, DHB, and octanoic acid (used as a long-lasting volatile control) perfused at the same concentration had no effect. (*D*) SBT emerged as the best candidate alarm pheromone from the normalized responses to KCl (20 mM). (*E*) SBT responses were observed over a broad range of concentrations (shown here from 10^{-4} to 10^{-7} M). (*F*) Dose–response representation of the neuronal percentage of activity for SBT; EC_{50} value for SBT calculated from the Hill equation: $3.0 \pm 2.3 \cdot 10^{-6}$ M ($n = 7$). (*G*) Exposure to SBT and a blend of APs and TMT, a component of fox feces, induces systemic stress responses measured by the elevation of the plasma corticosterone level normalized to the control ACSF solution (62.8 ± 7.3 ng/mL). BA, used here as an aversive control substance, did not induce any hormonal increase. Error bars: SEM of $n = 6$–15 observations; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant. (*H*) Representative calcium transients induced by five volatile predator kairomones (TMT; 2,6-DMP; 1,3-DT; TS; and 2-PT; here shown at 10^{-4} M) in mouse GG neurons from male and female mice. Corresponding chemical formulas are indicated below the names of the compounds. Fura-2 ratio is indicated with arbitrary units (a.u.). Perfusion times are indicated by horizontal bars. (Scale bars, 20 μm.)

Alarm Pheromones and Predator Scents Sharing a Common Chemical Structure Activate Grueneberg Ganglion Neurons. Interestingly, SBT shares its chemical structure with a different class of odorant molecules also involved in danger communication: the molecules found in rodent predator scents (44–46) (Fig. 2 *C* and *H*). These molecules are often the only warning a prey has that a predator is nearby. They include a class of heterocyclic sulfur or nitrogen-containing compounds generated by meat digestion (47). To these belongs 2,4,5-trimethylthiazoline (TMT), a component of fox (*Vulpes vulpes*) feces (48) that is well known for its fear-inducing properties (49, 50), (Fig. 2*G*).

Using calcium imaging, we found that TMT also induced an intracellular calcium increase in GG neurons of both female and male mice ($n = 33/34$; Fig. 2*H*). We further tested other representatives of this class of molecules at 10^{-3} to 10^{-9} M and found that each one activated GG neurons. Among these were dimethylpyrazines (DMP) [2,3-DMP ($n = 39/39$) or 2,6-DMP ($n = 39/40$)], dithiolanes [1,3-dithiolane (1,3-DT) ($n = 18/19$)], thietanes [trimethylene sulfide (TS) ($n = 17/22$)] and 2-propylthietane (2-PT; $n = 30/30$), which is secreted by the anal glands of a mouse predator, the stoat (*Mustela erminea*) (Fig. 2*H*). Thus, volatile danger cues, which share similar chemical structures released

either by predators (kairomones) or by a conspecific (APs), activate GG neurons.

Olfactory Subsystem Specificity of Chemical Danger Cues. Most of these GG ligands have, for humans, a strong and aversive smell. In the mouse, odorants as well as pheromones are often recognized by more than one olfactory subsystem (51) (Fig. 3*A*). To verify if this applied for SBT, TMT, and 2-PT, we perfused these substances on the neurons of the different subsystems of the mouse—the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), in addition to neurons of the GG (Fig. 3 *B*–*E*). We found that SBT, TMT, and 2-PT could be detected by MOE and VNO neurons but that the GG comprised the highest proportion of responsive neurons. Indeed, over 70% of the GG neurons responded to these alert cues, suggesting that this olfactory subsystem might play a role not only in the detection of these odorant molecules but also, more importantly, in the perception of the danger quality of these substances (Fig. 3*B*).

Danger Quality of a Chemical Cue Is Deciphered by a Functional Grueneberg Ganglion. The axonal projection bundles of GG neurons reach the olfactory bulb of the brain in a phosphodiesterase (PDE2A)-positive necklace glomerular region (NG) (29) (Fig. S7 *A* and *B*). We found, by c-Fos labeling, that mice exposed to SBT or to the predator cue TMT (10^{-3} or 10^{-7} M) have their NG-associated mitral/tufted and granule cells activated (Fig. S7 *C*–*E*). Thus, these chemical cues might transfer their danger signal to the brain via GG axonal afferences. These GG projection bundles can be sectioned (axotomy), inducing the complete degeneration of the ganglion and the generation of axotomized (Axo) mice (21, 25). Typical freezing reactions as well as a decrease in walking distance have been observed previously in the presence of danger cues (APs and predator kairomones) (10, 20, 21, 50). To test the behavioral relevance of the specific GG recognition of chemical danger cues, we ran a set of experiments scoring the freezing time, the walking distance, and the risk assessment episodes performed by mice with or without a functional GG (control versus Axo mice) (Fig. 4*A* and Fig. S8).

We found that the candidate alarm pheromone, SBT, as well as the predator kairomones, TMT and 2-PT, induced stereotypical fear reactions in control mice (Fig. 4*A*). SBT can therefore be considered as a mouse alarm pheromone. After GG axonal lesions (Axo mice) (Fig. 4*B*), the general odorant detection of the mice was not affected as no difference was observed in their ability to localize a hidden Oreo cookie (21) (Fig. 4*C*). Moreover, in these Axo mice, the presence of SBT, TMT, or 2-PT induced a high score of risk assessment episodes, confirming that the general detection of these odorants via the MOE and the VNO was indeed conserved (Fig. 3*B*). On the other hand, the fear-induced behaviors were replaced by exploratory activity (Fig. 4*A*). A functional GG is therefore necessary to perceive the danger-signaling properties of these chemically related substances.

Discussion

The olfactory system provides sensory information about the chemical composition of the external world. It fulfills a variety of tasks including locating and evaluating food, reproduction, social interactions, and danger avoidance (51). To escape predation, rodents are able to sense olfactory cues of very diverse chemical structures that are emitted by their predators. Related probably to this structural diversity and to the fundamental role of their detection for survival, these cues are recognized by morphologically and molecularly distinct subsystems within the nasal cavity. This multiple olfactory detection ability represents an evolutionary advantage for rodents. The volatile 2-phenylethylamine, a component of carnivore urine, is, for example, recognized in the mouse by the trace amine-associated receptor TAAR4 expressed in the neurons of the MOE (45). On the other hand, the non-volatile major urinary protein family from predators is recognized by the neurons of the VNO (44). Here, we show that the class of volatile heterocyclic sulfur- or nitrogen-containing compounds generated by meat digestion is specifically recognized by the neurons of the Grueneberg ganglion. This result supports previously published observations reporting that TMT detection still occurred in mice where the main VNO-signaling element, the transient receptor potential channels type 2 (TRPC2) (44), or the key MOE canonical component, the cyclic nucleotide-gated channels A2 (CNGA2) (52), have been genetically deleted. Moreover, TMT detection was also reported to be unaffected by a zinc sulfate treatment inducing a transient anosmia (53).

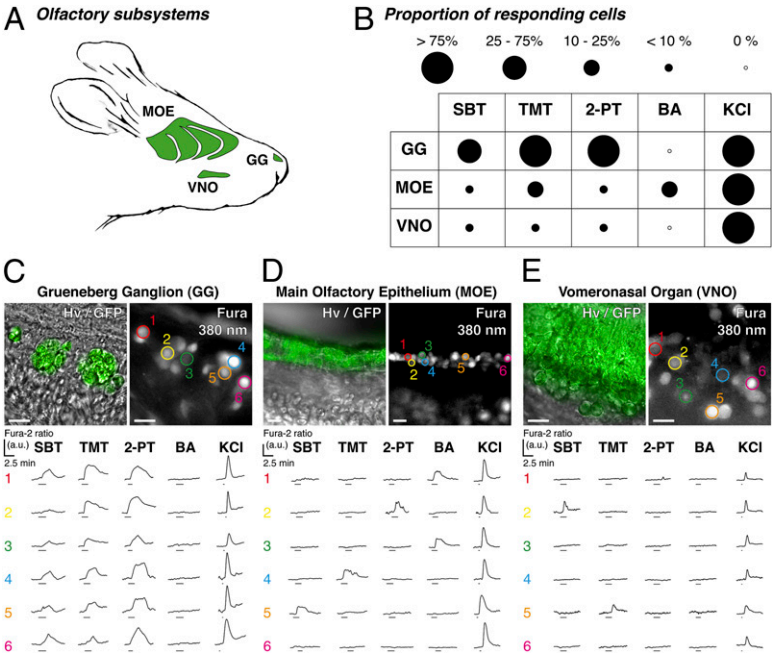

Fig. 3. Chemical danger cues show an olfactory subsystem specificity. (*A*) Schematic representation of a mouse head with the localization of the different olfactory subsystems (main olfactory epithelium, MOE; vomeronasal organ, VNO; and Grueneberg ganglion, GG). (*B*) Comparative table of neuronal activation showing strong olfactory subsystem dependence. SBT, TMT, and 2-PT (10^{-4} M) activated 72%, 97%, and 100% of the tested GG neurons, respectively (n = 114). The same cues activated 7%, 12%, and 4% of MOE neurons, respectively (n = 124) and 5%, 2%, and 1% of VNO neurons, respectively (n = 153). The aversive odorant BA (10^{-4} M), used here as a control, induced calcium transients in 15% of MOE neurons (n = 124) but did not activate GG neurons (n = 19) or VNO neurons (n = 78). Decreasing the concentration of SBT perfused to 10^{-6} M still activated 2% of MOE neurons and 57% of GG neurons. (*C*) Six representative GG neurons (colored and numbered circles), observed after the uptake of Fura-2 AM, were tested for activation. The different danger cues (SBT, TMT, and 2-PT) activated the majority of GG neurons tested whereas BA did not. Similar investigations as in *C* were performed on MOE (*D*) and VNO (*E*). Fura-2 ratio is indicated with arbitrary units (a.u.). Perfusion times are indicated by horizontal bars. (Scale bars, 20 μm.)

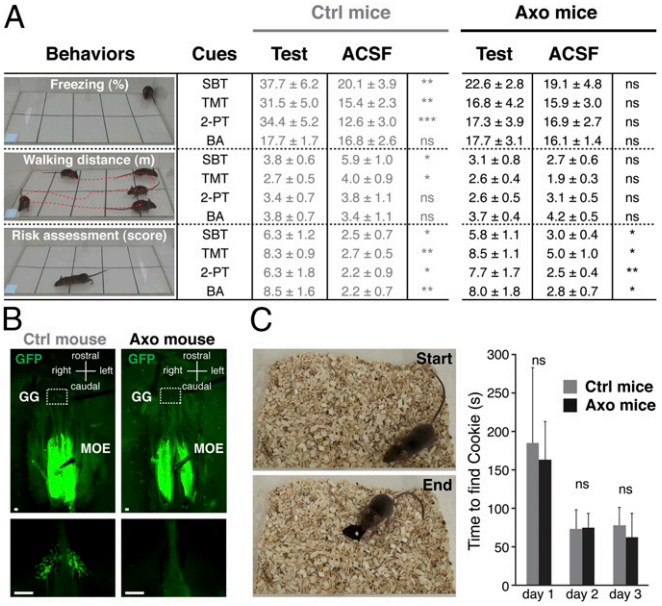

Fig. 4. Detection of chemical danger cues depends on a functional GG. (*A*) Behavioral experiments involving control (Ctrl; $n = 6$) and axotomized (Axo; $n = 6$) mice exposed successively for 5 min to a chemical cue (SBT, TMT, 2-PT, BA) and to ACSF deposited on a blotting paper (blue square). The percentage of freezing time (%) and the walking distance (m) show that SBT as well as TMT and 2-PT, but not BA, induced a stereotyped fear behavior abolished after axotomy. On the other hand, the scoring of risk assessment episodes was not affected by axotomy. (*B*) Mice phenotyping. Ventral whole-mount of the nasal cavities of a Ctrl (*Left*) and of an Axo (*Right*) OMP-GFP mouse (fluorescent view). The GG (*Lower*: white rectangles enlarged) and MOE regions are shown. (*C*) The general olfactory function was not affected by axotomy. No difference was observed between the two phenotypes in the latency to find a buried Oreo cookie in the bedding over 3 consecutive days. Ctrl mice (gray bars) and Axo mice (black bars). Mean ± SEM of $n = 6$, $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant. (Scale bars, 0.25 mm.)

The potential pathway(s) implicated in the detection of chemical danger cues by GG neurons remain(s) to be identified. Putative signaling elements have already been described (21, 31, 54–59), suggesting that GG neurons might use different pathways from those present in MOE or VNO neurons. Some proteins identified have a temporal pattern of expression and/or a restricted localization implying the presence of neuronal subpopulations in the GG. From our c-Fos labeling of the olfactory bulb, we can speculate that the GG neurons activated by alarm pheromones comprise the substantial subpopulation of PDE2A-expressing neurons. Interestingly, these PDE2A-positive neurons also express in their primary cilia the particulate guanylyl cyclase pGC-G and the CNGA3 ion channels (54, 57). These two membrane-bound proteins could play a role in danger cue detection. Further experiments will still be necessary to sort out and understand the GG transduction machinery, which might also involve different ions such as calcium or sodium.

The chemical danger cues activate a hard-wired circuitry to trigger innate fear behaviors (50, 60–62). Previous reports have shown that GG neurons send their axons to glomeruli in the dorsal zone of the olfactory bulb (24–26, 28, 29), a region also shown by genetic deletion experiments to trigger innate fear behaviors such as freezing (50). Freezing is one of the hiding strategies of the prey (behavioral crypsis) that can be considered as an innate survival behavior (1). Another form of antipredator adaptation also involves olfactory emissions (chemical crypsis) (3). We might here speculate that, in the presence of a predator, the production of alarm pheromones, such as SBT (63), not only signals danger to conspecifics but also induces freezing and might also modify the prey scent, which could fool the predator by mimicry of its own scent (2, 3).

In mammals, more than one chemical molecule probably acts as an alarm pheromone to communicate danger (64). For identifying mouse alarm pheromones, we chose a SPME fiber because of its high sensitivity to volatile chemicals. Unfortunately, probably not all volatile cues were trapped by this fiber due to their particular physico-chemical affinities. During our chemical procedure, we also found compounds not referenced in the chemical libraries. They deserve further investigations to verify whether they share similar chemical features and contribute to the danger message.

From our chemical, physiological, and behavioral screenings, SBT came out as a mouse alarm pheromone. It was produced under different alarm conditions by both female and male mice of different ages. It acted as a danger signal for mice of both genders, inducing intracellular calcium increases in APs sensing GG neurons. SBT activated the associated mitral/tufted and granule cells in the olfactory bulb. Its exposure led to an increase in plasma corticosterone and to the subsequent display of innate fear-related behaviors. SBT has been previously shown to induce intermale aggression in mice in association with the pheromone DHB (37). This behavior was observed only when the synthetic SBT was added to urine. SBT could therefore be released in the environment as a volatile chemical and as a compound linked to the mouse major urinary proteins (Mups). We showed that the volatile SBT signals a dangerous situation to a conspecific by initiating responses in GG neurons without association with mouse Mups whereas the Mups-linked SBT gives information as a long-lasting message in the urine for sex-related indications that will be detected by VNO neurons (65, 66).

The ability to detect the short-lived molecules encoding danger is essential for species survival. Molecules possessing the same chemical signature (heterocyclic sulfur or nitrogen-containing compounds) are emitted by predators (kairomones) and by stressed conspecifics (alarm pheromones); they induce similar innate fear reactions. In summary, our study shows that the chemical structure of danger cues refers to their specific coding quality and is deciphered by the Grueneberg ganglion neurons. It points to a double role for this olfactory subsystem in intraspecies as well as interspecies communication of danger. Our results provide further insight into how organisms chemically communicate and detect the presence of a danger to improve the general overall fitness of the species.

Materials and Methods

A detailed description of materials and methods is given in *SI Materials and Methods*. Briefly, male and female C57BL/6J and OMP-GFP gene-targeted mice were used for the experiments. SPME and GC/MS methods were used to collect and analyze volatile compounds. For calcium imaging, acute olfactory tissue slices were generated with a vibratome in cold oxygenated ACSF. Fura-2AM was used as a calcium indicator to record cellular activations after chemostimulation. For behavioral sessions, the risk assessment, the freezing duration, and the walking distance were evaluated as well as the general olfactory function of the mice. For statistical analyses (mean ± SEM), unilateral Student's unpaired/paired *t* tests were performed for comparisons. Significance levels are indicated as follows: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

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