**Copycatting smell of death – Deciphering the role of cadaveric scent components used by detection dogs to locate human remains**

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**Abstract**

Human remains detection dogs (HRDD) are commonly used by law enforcement agencies to search for cadavers. Biological material is typically used as a training stimulus, also called aids, to train dogs to recognize the smell of cadavers. While HRDD training approaches have received extensive attention, information remains limited on the olfactory cues used to train them. Here, we aimed to decipher the chemical basis of detection dog olfaction. Five specific objectives were explored to precise whether the composition or the concentration of the training aids drives the HRDDs responses. We recorded the behavioral responses of four HRDDs exposed to different cadaveric-like smells. We found that HRDDs recognized a simplified synthetic aid composed of cadaveric compounds. The lowest concentration at which HRDDs continued to perceive the cadaveric smell was determined. HRDDs were not impacted by slight modifications to the chemical composition of a blend of odors that they have been trained with. HRDDs associated sulfur and nitrogen compounds as human cadaver. Our findings highlight a lack of specificity of HRDDs to cadaveric compounds, which could lead to error of detection. Moreover, all dogs did not positively respond to the same blends, despite being trained with the same aids and procedure. However, we confirmed that dogs could be trained with a simplified blend of molecules. The chemical composition of a training aid has therefore high consequences on the performance of the trained animal, and this conclusion opens additional questions regarding olfaction-based detection animals.

**KEYWORDS**

cadaveric odor, human remains detection dogs, detection dogs training, olfaction, detection dogs perception, forensic science

**Highlights**

* Human remains detection dogs recognize a simplified synthetic aid composed of cadaveric compounds.
* The lowest concentration at which HRDDs perceive cadaveric smell was determined to reach 100ng.
* HRDDs are not impacted by modifications to the composition of a blend they have been trained with.
* HRDDs associate sulfur and nitrogen compounds as human cadaver.

Introduction

In 1985, the German author Patrick Süskind wrote a novel featuring a young man obsessed by the idea of creating a perfume to bewitch humans. He murdered young women and extracted the smell from their cadavers (1). Who would have thought that, 40-years later, Süskind’s idea would be put in action to constitute perfumes mimicking the smell of death and used to train human remains detection dogs (HRDDs)?

HRDDs are commonly used by law enforcement agencies to search for cadavers, body parts, or fluids during criminal investigations, or after a suspicious disappearance (2–4). HRDD handlers rely on operant conditioning to train their dogs, by positively rewarding a desired behavior (sitting or barking) when their dog approaches the olfactory stimulus (5,6). This stimulus is called a training aid, and typically releases a smell mimicking that of a cadaver. This smell can be natural (*e.g.,* cadaveric fluids, tomb soil) (5) or synthetic (*e.g.,* Pseudo corpse® Merck) (Deldalle and Gaunet, 2014). The chemical composition of these odors has been extensively studied for both natural (3,9–11) and synthetic aids (7). The latter poorly mimic the smell of a cadaver, as they are not composed of cadaveric molecules. Consequently, they are not used by HRDD handlers. Instead, handlers preferentially use a wide variety of natural aids, including teeth, hair, and gauze soaked with cadaveric fluids from humans and other mammals (5).

To date, natural aids remain the most reliable odorant source to train HRDDs (3,12,13). Unexpectedly, exploratory assays implemented by our group showed that experienced HRDDs exhibited a positive behavioral response to both natural and synthetic training aids (Pseudo Corpse formulation one and two, Merck, unpublished data), despite no prior training with the molecules in the blend. These assays were conducted following the protocol described in Martin et al, 2022 (14) and two of the four tested HRDDs positively recognized the Pseudo corpse in the arena. These observations indicate that the performance of HRDDs might be more related to the chemical structure of the training aid, rather than to its components. This hypothesis was raised for explosive detection dogs that failed to recognize their training aid when a single odor was mixed with other odors (15). While HRDD training approaches have received extensive attention, knowledge remains limited on the relationship between the composition of training aids and HRDDs efficiency. In fact, the olfactory stimuli inducing a positive response in HRDDs have yet to be identified (5).

Here, we aimed to decipher the chemical basis of detection dog olfaction. Specifically, we ask how the chemical composition of a simplified blend mimicking the odor of decaying remains impact the responses of HRDDs. To accomplish this, we explored five objectives: (i) whether HRDDs can recognize a synthetic blend made of few cadaveric components; (ii) the role of the absolute concentration of chemicals making up the cue; (iii) how the chemical composition of an odorant allows HRDDs to associate it with a human cadaver; (iv) essential chemical functions (sulfur, nitrogen, aromatic) allowing HRDDs to associate the odorant cue with a human cadaver; and (v) how distracting odors impact the ability of HRDDs to associate a cadaveric odorant cue with a human cadaver. These questions would open a new field of investigation dedicated to the impact of the chemical composition of training aids on the perception of HRDDs, in addition to provide guidances which could be directly applied by the handlers.

**Materials** and **Methods**

*Human remains detection dogs*

Four HRDDs, all male springer spaniels, belonging to the canine support direction of the Belgian federal police (DACH) participated to this study. The dogs had at least one year of expertise in human remains detection (range: 1–5 years). All dogs were trained during their whole career using natural training aids made of gauze or paperclips impregnated with vertebrate cadaveric fluids.

*Chemicals*

The molecules selected for use were based on an extensive review of the literature on the volatilome of human cadavers (16). Only chemicals that are released during the entire decomposition process were selected. Five representatives of the three major chemical families were identified: sulfur compounds (dimethyl-disulfide and diethyl-disulfide), nitrogen compounds (indole and pyridine), and aromatic compounds (p-xylene). Diethyl-disulfide and pyridine are supposed to be human specific (17,18). DMDS and indole were chosen as they are the most common sulfur and nitrogen containing compounds released during the decomposition of vertebrate remains (19–24). The absolute and relative concentrations of each compound were arbitrarily decided based on the published literature (Table 1). This blend (later referred to as ‘’initial blend”) was stored at -20 °C. It was used to answer Questions 1 and 2. We aimed to test the ability of HRDDs ability to associate this simplified blend with the smell of a cadaver.

*Distractive odor*

In some of the assays, distractive odors were included in the behavioral arena (described below). Four distractive odors were prepared: coffee (20g of ground coffee), basil (20g of freshly cut plant), chocolate (20g of crushed dark chocolate), and a synthetic indoor perfume. They were introduced in separate glass bottles with 20 sterile gauzes. Bottles were then sealed and left at room temperature for 24 hours. A single gauze of each distractive odor was then hidden in the arena before the beginning of the assay.

*Q1 – Are HRDDs able to recognize a synthetic blend of few cadaveric components?*

The initial blend was tested on three of the four HRDDs at the headquarters of the police brigade (Neerhespen, Belgium). In a separate room, sterile gauze was impregnated with 50 microliters of the initial blend, and was then hidden in a homemade wall (Figure 1). The gauze was changed for each search, as well as its position in the wall. A period of 15 min was applied before each test to ventilate the room. We recorded the ability of each HRDD to indicate the correct location of the impregnated gauze to their handler.

The same exercise was performed outdoors in a wood with the same three dogs (Figure 1). The initial blend (1 ml) was applied on a rock. During the session, one dog was placed 50 m away from the rock, and was asked to perform a search under real conditions. We recorded the ability of each HRDD to provide their handler with a positive response when facing the correctly marked rock. The behavioral response of each HRDD was recorded once.

*Q2 – What is the role of the absolute concentration of the chemicals making up the cue?*

Here, the initial blend was repeatedly diluted by a factor of 10 (10-1, 10-2, 10-3, 10-4, 10-5, 10-6, 10-7). The ability of all four HRDD to respond positively to each diluted blend, presented at random, was evaluated in a behavioral arena similar to that previously described by Martin et al. (2022). In brief, the arena consisted of an 8 x 8 m room containing 25 cinderblocks in a semi-circle (d = 6 m). An empty plastic cup was placed in each cinderblock. Before and after the behavioral assays, when no chemical cues were present, the handler allowed each dog to search the arena, to confirm that the arena did not induce any response. To avoid the “clever Hans effect” (25), handlers were not informed about the location of the target odor. After the handler left the room, the experimenter introduced a piece of gauze impregnated with the initial blend at one of the tested concentrations, inside one of the plastic cup placed in cinderblocks. In addition to the blend, four distractive odors were placed in other plastic cups, to avoid dogs for false positive response induced by the only presence of a new and concentrated odor. The handler was then invited to enter the arena again with their HRDD and the test was initiated. The handler was allowed to stay close to their dog to give commands, but was not allowed to guide the dog by pointing out the cinderblock. To signal the end of the trial, the handler told the experimenter whether a target cue was present or absent in the arena, and to provide the position of the cue, if applicable. If the position was correct, the handler was invited to stop the search and was allowed to reward his dog. If an incorrect answer was given, the search was stopped without rewarding the dog. The behavioral response of each HRDD was recorded once

*Q3 – How does the chemical composition of an odorant cue affect the ability of HRDDs to associate it with a human cadaver?*

The two previous experiments demonstrated the ability of HRDDs to respond to a simplified and diluted initial blend (Table 1). Here, we evaluated how each compound contributed to detection by HRDDs. We modified the initial blend by diluting it by a factor of 10-3, and out of the mix one odor was removed each time, so four odors were always present (Table 2). We then exposed the HRDDs to these partial blends using the method described for Question 2, and recorded their ability to detect them. The behavioral response of each HRDD was recorded once

*Q4 –* *Which chemical functions (sulfur, nitrogen, aromatic) are essential for HRDDs to associate the odorant cue with a human cadaver?*

Here, the initial blend was adjusted in two ways. The same behavioral arena was used as in Q2 and 3. HRDDs were first exposed to “removed blends,” in which the compounds belonging to the same chemical family were excluded from the initial blend. Then, HRDDs were exposed to copycat blends, in which non-cadaveric compounds belonging to the same chemical family were added (Table 2). The behavioral response of each HRDD was recorded once

*Q5 –* *How does the presence of distracting odors impact the ability of HRDDs to associate a cadaveric odorant cue with a human cadaver?*

Here, we formulated distracting floral blend, composed of four plant volatiles (limonene, β-caryophylene, α-phelandrene, and α-pinene) mixed to the same proportions. First, we validated that the floral blend did not induce a positive behavioral response in the HRDDs. Then, each compound from the initial blend was added to it, one at a time (Table 2). The behavioral response of each HRDD was recorded once.

*Behavioral assay sequence*

To avoid any learning from the dogs during the assays, twelve months separated the first exposure to the cadaveric blend (all assays from Q1) from the assays associated with the other questions (Q2-Q5). During that period, no dog was exposed to the blend. All assays performed to answer Q2 to Q5 were randomly organized. Each dogs performed 10 behavioural asays per day of experiment. Finally each day of experiment were separated by a period of one month. This procedure was followed to reduce dogs learning process between tests sessions.

**Results**

*Q1 – Are HRDDs able to recognize a synthetic blend of few cadaveric components?*

Two out of the three detection dogs recognized the initial blend, and marked it as they would a cadaver. The third dog expressed an interest in the smell by sniffing the gauze for 4 sec, but did not mark it as a cadaver. In the outdoor test, the same two dogs responded positively to the odor, as in the indoor test. The third dog showed no interest in the marked rock.

*Q2 – What is the role of the absolute concentration of the chemicals making up the cue?*

All four HRDDs responded to the four highest concentrations of the initial blend (undiluted, and 10-1, 10-2, 10-3 dilutions). Three of the four HRDDs positively detected and respond to the 10-4 and 10-5 dilutions. The 10-5 dilution corresponded to 112.3 ng molecules on the gauze (47.00 ng DMDS, 22.51 ng pyridine, 22.1 ng p-xylene, 14.60 ng indole, 6.11 ng DEDS). No HRDD was able to detect the 10-6 dilution of the initial blend.

*Q3 – How does the chemical composition of an odorant cue affect the ability of HRDDs to associate it with a human cadaver?*

Removing DEDS, pyridine, or p-xylene from the simplified blend did not prevent any dog from responding correctly. Removing DMDS or indole prevented one of the four dogs from responding positively.

*Q4 –* *Which chemical functions (sulfur, nitrogen, aromatic) are essential for HRDDs to associate the odorant cue with a human cadaver?*

The lowest rate of positive responses was observed when sulfur compounds were removed from the mixture (2 out of 4 dogs responded positively). However, when these compounds were replaced by a non-cadaveric sulfur compound, the rate of response returned to 100%. Removing nitrogen compounds did not affect the ability of dogs to respond positively. Replacing these compounds with a non-cadaveric nitrogen compound led to three out of four positive responses. The removal of the aromatic compound from the blend did not impact the behavioral response of HRDDs, nor its replacement with a non-cadaveric aromatic compound. The rate of response remained 100% when all compounds from the initial blend were replaced with non-cadaveric compounds belonging to the same chemical families.

*Q5 –* *How does the presence of distracting odors impact the ability of HRDDs to associate a cadaveric odorant cue with a human cadaver?*

 None of the four HRDDs responded to the plant distractor blend. When DMDS or DMDS + DEDS was added to this blend, all dogs responded positively. The addition of DEDS or indole or pyridine + indole to the plant distractor blend resulted in three of the four dogs responding (the same dogs responded to the two latter blends). Two of the four dogs responded to the plant distractor blend supplemented with p-xylene. Just one dog responded to the plant distractor blend with pyridine.

**Discussion**

This study evaluated how the chemical composition of training aids affects the behavioural response of HRDD. While there are many reports on the chemical composition of training aids (including vertebrate cadavers), information remains limited on detection dog response (2,5,12,14,26–32). The chemical basis of HRDDs odor recognition remains largely unquantified (12).

The dogs used in the present study were chosen because of their experience in necro-searches in Belgium (33). The sequence of the different blends was randomly assigned for each dogs in several days, at one month of interval, to avoid any learning of the blend through the experiments. The results of the Q1 test showed that HRDDs originally trained with a natural aids (containing several hundred compounds; (3), are able to detect a simplified cadaveric blend composed of just five cadaveric molecules. It is unlikely that cadaver dogs are able to detect hundreds of individual compounds released by natural aids. For instance, previous studies showed that explosive detection dogs that trained daily with a mixture of compounds later respond to certain individual chemicals (34). However, this previous study investigated a mixture of unnamed explosives, for which the chemical composition was not provided.

The present study reaffirmed that canine bio-detectors are very sensitive (12,35,36). For example, dogs were shown to be more sensitive at detecting Covid-19 than PCR tests (37), differentiating virus-infected cells from healthy ones (35). HRDDs are able to detect the cadaveric residues on a piece of cloth, even after several washes (36). However, the current study provided the first limit of detection for cadaveric material. We showed that around 100 ng of cadaveric compounds could still elicit a positive response in experienced HRDDs.

Our results showed that the chemical composition of the blend did not impact the ability of HRDDs to identify a cadaveric scent. Even though the synthetic blend purchased from Sigma Aldrich did not contain a single cadaveric compound, it did elicit a response from most tested HRDDs. Because these dogs were trained using highly variable cadaveric aids, they probably learned to respond to a wide range of chemicals, belonging to a limited number of chemical families, allowing them to recognize several compounds within a blend (34). The odors released by decaying remains are indeed highly variable (38–42). Two of the four dogs did not recognize the blend when sulfur compounds were removed. Out of these sulfur compounds, DMDS was the key compound for cadaver recognition by HRDDs, as it was the only compound that induced 100% response when mixed with plant-derived distractive odors. Sulfur compounds, including DMDS, are commonly released during the entire decomposition process of vertebrate tissues (16,20,38,40,43,44). DMDS are by-products of sulfur based amino acids, as methionine, degradation (45,46).

DMDS is a major compound that is released during the entire decaying process, and might explain why HRDDs are particularly responsive to this chemical (40). Nitrogenic chemicals seem to have a secondary role in detection. For instance, indole is the only nitrogen compound detected through the entire decomposition process (43,47). Like DMDS, indole is a typical cadaveric odor that originates from the decomposition of various aromatic amino acids, including phenylalanine, tyrosine and tryptophane (22,47,48). Dogs focus on by-products released during protein degradation, probably because the natural training aids used by their handlers were mostly composed of proteins (*e.g.,* muscle, flesh, blood). Aromatic compounds seem to provide negligeable cues for cadaver recognition by HRDDs. P-xylene is released throughout the entire decomposition process, but in much smaller amounts than the other compounds of the blend (10,44). In addition, p-xylene is an ubiquitous atmospheric compound, limiting its reliability as a cadaver cue (32).

The results of our study showed that HRDDs do not target specific cadaver compounds. When all cadaveric compounds were replaced with non-cadaveric compounds from the same chemical family, HRDDs still positively recognized the blend mimicking a cadaver odor. This generalization ability of HRDDs was previously identified (49) : animals trained to respond to a specific molecule are likely to respond to all compounds sharing similar chemical structure. However, the length of the carbon chain appears to contribute to the mechanism of generalization (Lazarowski et al., 2020). This could be a problem as dogs could positively recognize untargeted compounds, which could (at least partly) explain the lack of detection exhibited by some dogs during the experiment. Moreover, due to individual variability in HRDD response, more than one dog should be used in searches.

In conclusion, using a simplified aid that mimics the odor of a decaying corpse could help HRDDs to recognize all compounds from the blend, and ensure that all dogs respond to the same compounds. How HRDDs perceive cadaveric compounds requires further investigation, along with the mechanism of the odor learning process during HRDD training.

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TABLE 1 Formulation of the initial blend made of cadaveric odorant cues.

|  |  |
| --- | --- |
| **Compound** | **Concentration (M)** |
| Dimethyl-disulfide (DMDS) | 1 |
| Pyridine | 0.57 |
| p-xylene  | 0.42 |
| Indole  | 0.25 |
| Diethyl-disulfide (DEDS) | 0.1 |

TABLE 2 Composition of solutions used to answer Q3 to Q5.

|  |  |  |
| --- | --- | --- |
| **Blends** | **Compounds** | **Research question** |
| **Removed blends**Blend excluding DMDS | DEDS, pyridine, indole, p-xylene | Q3 |
| Blend excluding DEDS  | DMDS, pyridine, indole, p-xylene | Q3 |
| Blend excluding pyridine  | DMDS, DEDS, indole, p-xylene | Q3 |
| Blend excluding indole  | DMDS, DEDS, pyridine, p-xylene | Q3 |
| Blend excluding p-xylene | DMDS, DEDS, pyridine, indole,  | Q3/Q4 |
| Blend excluding DMDS and DEDS  | pyridine, indole, p-xylene | Q4 |
| Blend excluding pyridine and indole  | DMDS, DEDS, p-xylene | Q4 |
| **Copycat blends**Blend sulfur replaced  | Β-mercaptoethanol, pyridine, indole, p-xylene | Q4 |
| Blend nitrogen replaced  | DMDS, DEDS, 4-benzoxyaniline, p-xylene | Q4 |
| Blend aromatic replaced  | DMDS, DEDS, pyridine, indole, cyclopropylbenzene | Q4 |
| Blend all replace | Β-mercaptoethanol, 4-benzoxyaniline, cyclopropylbenzene | Q4 |
| **Plant distractor + cadaveric compound**Plant scent including DMDS | Limonène, β-Caryophyllène, α-Phellandrène, α-Pinène, DMDS  | Q5 |
| Plant scent including DEDS  | Limonène, β-Caryophyllène, α-Phellandrène, α-Pinène, DEDS | Q5 |
| Plant scent including DMDS and DEDS  | Limonène, β-Caryophyllène, α-Phellandrène, α-Pinène, DMDS, DEDS | Q5 |
| Plant scent including pyridine  | Limonène, β-Caryophyllène, α-Phellandrène, α-Pinène, pyridine | Q5 |
| Plant scent including indole  | Limonène, β-Caryophyllène, α-Phellandrène, α-Pinène, indole | Q5 |
| Plant scent including pyridine and indole  | Limonène, β-Caryophyllène, α-Phellandrène, α-Pinène, pyridine and indole  | Q5 |
| Plant scent including p-xylene | Limonène, β-Caryophyllène, α-Phellandrène, α-Pinène, p-xylene | Q5 |

Dimethyl-disulfide (DMDS), Diethyl-disulfide (DEDS)

**Figure Legends**

FIGURE 1 Indoors and outdoors test of initial blend recognition by human remains detection dogs.