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Spontaneous orienting of untrained companion dogs naïve to human epilepsy toward odor samples from an unfamiliar human in a controlled non-social paradigm: a proof-of-concept

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Abstract

Background. Evidence for spontaneous seizure detection by dogs remains limited. This study examined whether ictal odor cues elicit behavioral responses in untrained companion dogs.

Objective. To test whether dogs naïve to human epilepsy show spontaneous discrimination of ictal versus interictal odor.

Methods. Thirty dogs, without prior seizure exposure, freely investigated three odor stations (ictal, interictal, blank) using sweat samples from a single unfamiliar donor in controlled, non-social conditions.

Results. Dogs were more likely to investigate the ictal odor first than expected by chance (Monte Carlo p=0.029), indicating an early orienting bias. No differences emerged in sustained engagement. Structured exploratory patterns occurred only in odor conditions (ictal p=0.001; interictal p=0.002), not in the control (p=0.715).

Conclusions. Ictal odor may carry salience sufficient to influence initial attention in naïve dogs. However, findings are based on a single donor and require replication with multiple individuals to assess generalizability.

Key words

- canine
- seizure alert dogs
- human epilepsy
- volatile organic compounds
- olfaction

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent epileptic seizures and affects approximately 1% of the human population worldwide. According to data from the Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy, nearly 500,000 individuals in Italy are diagnosed with epilepsy, with approximately 30,000 new cases reported annually. Patients with epilepsy face a significantly increased risk of mortality, psychiatric and somatic comorbidities, and adverse effects related to antiseizure medications [1].

Epileptic seizures are typically short-lasting (generally lasting less than 2 minutes) and early diagnosis and appropriate treatment can achieve effective seizure control in a large proportion of cases. However, approximately 30% of patients are drug-resistant, representing a substantial therapeutic challenge [2]. Moreover, epileptic seizures typically occur unpredictably [2] and may remain unrecognized or go unnoticed by patients, either because they occur without overt behavioral manifestations [3, 4] as in the case of focal nonconvulsive seizures, or because postictal impairment of awareness and memory prevents accurate recall [5]. This results in unreliable seizure reporting [6, 7], with studies showing that patients may fail to document more than 50% of their seizures [8]. Such underreporting poses major challenges for diagnosis, prognosis, and the evaluation of treatment efficacy. In clinical practice and research settings, patients or caregivers are in fact commonly asked to maintain seizure diaries, which serve as outcome measures, to inform risk assessment and support seizure forecasting. Accurate seizure documentation is therefore essential for sound clinical and scientific practice [2].

In this context, there is a growing need for complementary systems capable of enhancing patients' and caregivers' awareness of seizure episodes, particularly in cases characterized by impaired recognition. Cur-

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rently available technologies are largely based on implanted electroencephalographic (EEG) recording devices combined with predictive algorithms [9]. Although promising, these approaches are invasive, carry non-negligible risks, and their accuracy and feasibility for routine clinical use remain under debate. Moreover, some patients may be reluctant to undergo implantation procedures [10]. Other commercially available systems, such as accelerometers, motion sensors, or multimodal detectors, are primarily designed to identify convulsive movements and alert caregivers; however, they do not provide advance warning to the individual experiencing the seizure [11]. Animal-assisted detection strategies, including trained or spontaneously responsive dogs, have attracted increasing scientific interest as a potential non-invasive complementary approach. This interest stems from the well-documented effectiveness of dogs' exceptional olfactory abilities in a wide range of applied contexts, including tracking, drug and explosive detection, finding human victims of disasters and searching for human remains [12-15]. Within the medical field, macrosmatic species – particularly dogs, but also rodents – have been investigated as diagnostic or screening tools for a range of human conditions, most notably in cancer detection (e.g., lung, breast, prostate, and skin cancers), as well as in infectious diseases such as COVID-19 and tuberculosis [16-20].

Since 1999, dogs have been trained either to respond once a seizure has begun (seizure response dogs, SRDs), or to display premonitory behaviors before the individual becomes aware of an impending seizure (seizure alert dogs, SADs) [21]. However, with the exception of one prospective study [22], which monitored seizure frequency over a 48-week period in ten patients referred to a seizure alert dog service and reported a significant reduction in seizure frequency during and after the dog training period, this acquired ability of dogs has so far been evaluated scientifically primarily through guardian- or trainer-reported questionnaires or retrospective analyses [9, 21-24]. While these studies suggest potential clinical benefits associated with trained seizure alert dogs, they did not experimentally test dogs' performance accuracy and reliability. Importantly, seizure-related responding/alerting behaviors have also been reported in untrained pet dogs [24-26]. However, these reports remain largely anecdotal or, again, based on guardian- and trainer-reported surveys conducted in domestic environments, which do not provide experimental evidence of spontaneous seizure-detection abilities under controlled conditions.

Another aspect that remains poorly understood is the sensory modality underlying seizure detection in dogs. Dogs may rely on visual cues, such as subtle behavioral or postural changes, olfactory cues, or a combination of both, emitted by patients prior to seizure onset or during the ictal phase. Dogs' ability to detect human diseases through olfaction is well established [18-20], owing to their olfactory sensitivity, which is orders of magnitude greater than that of humans, and their capacity to detect volatile organic compounds (VOCs) associated with condition-specific metabolic alterations.

Maa *et al.* [27] conducted a larger and more rigorous prospective laboratory study to investigate the olfactory detection of seizure-associated VOCs in humans by professionally trained service dogs under blinded conditions, reporting high discrimination accuracy between ictal and interictal odors (approximately 94% sensitivity and over 96% specificity). In a previous work, Catala *et al.* [28] trained dogs to perform a predefined behavior in response to seizure-associated odors from a small cohort (five patients), demonstrating high discrimination accuracy (a sensitivity of 87% and a specificity of 98%). These findings support the validity of seizure-related VOCs as a potential biomarker detectable by trained dogs. However, when dogs are deliberately trained to detect and respond to these odor cues, as in these studies, their responses reflect a learned process rather than a spontaneous sensory reaction, making it difficult to disentangle intrinsic olfactory sensitivity from the effects of reinforcement, attentional focus, and repeated exposure. Essentially, these studies did not clarify whether olfaction represents a primary sensory modality spontaneously used by dogs, or whether it becomes dominant as a result of training and odor-specific learning. In an attempt to clarify this aspect, Powell *et al.* [29] examined the behavioral responses of naïve pet dogs exposed to seizure-associated sweat samples collected from unknown individuals with epilepsy. The odors were delivered via a remote system positioned directly beneath the seated guardian's thighs, thereby creating the impression that the scent originated from the dog's own guardian. Dogs displayed increased attention and proximity-seeking behaviors in response to seizure samples. However, the odor stimuli were presented within an established dog–owner relationship, meaning that the observed responses occurred in a strongly relational context; therefore, the potential influence of familiarity, attachment, prior experience, or contextual learning on spontaneous olfactory sensitivity cannot be excluded. Consequently, it remains unclear whether seizure-associated odors elicit a spontaneous behavioral response in untrained dogs, independent of learning, social context, or prior experience. More broadly, the precise contribution of canine olfaction to seizure detection remains undercharacterized.

The present study reports results from the first experimental phase of a broader research project aimed at addressing this question, focusing on spontaneous exploratory behavior in untrained dogs with no prior exposure to epilepsy and no experience with medical detection tasks. Sweat odor samples were collected from an unfamiliar person and presented to dogs within a socially neutral experimental paradigm. By removing potential confounds related to training, experience, familiarity, and the dog-guardian relationship, this approach was designed to directly assess whether odors present during the ictal state may be intrinsically salient to dogs and elicit spontaneous orienting responses prior to any form of learning. Given the exploratory nature of this proof-of-concept design, the study aimed to provide initial evidence to inform subsequent steps toward the identification of potentially generalizable seizure-related odor signatures. A deeper understanding of the contribution of olfactory strategies engaged by dogs in seizure

detection will refine current knowledge of the underlying physio-ethological mechanisms involved. Such knowledge could be leveraged to better support episode recognition and reporting through these valuable tools, particularly in vulnerable individuals whose seizure onset or ictal phase goes unrecognized or unnoticed, either because episodes lack overt behavioral manifestations or because conscious awareness is impaired.

MATERIALS AND METHODS

This study was part of a larger research project on seizure alert dogs that received approval from the Ethics Committee (Ref. no. CE_116/21, 23.11.21) and the Animal Welfare Committee (OPBA_67_2024) of the University of Milan and was conducted in compliance with national and EU legislation and institutional guidelines. Written informed consent was obtained from all human participants after they received detailed information about the procedures; however, dog guardians were not informed about the specific aim of the study. In the case of minor participants, written informed consent was provided by a legal guardian. Participation was entirely voluntary, both with respect to providing sweat samples and to allowing dogs to take part in the activities.

Participants (patients)

Eligible patients for the general project were individuals of either gender, older than 6 years, with a diagnosis of focal epilepsy. They could be either hospitalized, admitted to neurological units for prolonged presurgical video-electroencephalographic (video-EEG) monitoring aimed at characterizing the electroclinical features of seizures in view of potential surgical treatment, or non-hospitalized patients. In the present study, we selected sweat samples from a non-hospitalized 6-year-old female volunteer receiving care at Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy. Consistent with the exploratory proof-of-concept design of the present study, samples from a single patient were used to provide a standardized odor stimulus across all dogs. This approach minimized inter-individual variability in odor profile, thereby enabling a more controlled comparison of ictal versus interictal conditions across dogs.

Sample collection and handling

Sweat was selected as the biological matrix, in line with previous epilepsy-related studies [27-29], because VOCs released during epileptic seizures readily diffuse into sweat and because its collection is entirely non-invasive. Sweat samples were collected by a familiar caregiver wearing nitrile gloves. Sterile gauze pads (10×10 cm) were used and applied with a double pass to the skin of the neck and/or forehead.

Sweat samples were collected at two different time points, with three samples obtained from the patient at each sampling occasion: 1) an interictal baseline condition, during seizure-free intervals i.e., at least 6 hours before or after a seizure to exclude potential pre-ictal or post-ictal influences, in line with Catala *et al.* [28] and Elger *et al.* [2] an ictal condition, during focal seizures or during the convulsive phase of secondarily generalized focal seizures. After collection, each gauze pad was subsequently cut into four sections, yielding 12 subsamples per patient for each sampling phase, which were placed into hermetically sealed polymeric tubes (Securitainer tube 29×63 mm, Nolato, Netherlands), bearing the subject's ID. Samples were transferred within 45 minutes to a monitored refrigerator at 4 °C, and subsequently transported under refrigerated conditions (+4 to +8 °C) to the Animal Physioethology Laboratory of the dog testing center, Department of Veterinary Medicine (DIVAS), University of Milan, Lodi (Italy). Samples were accompanied by detailed documentation specifying sampling conditions and timing and were stored at -18 °C until testing. Previous research has shown that freezing preserves human body odor samples without altering how they are perceived [30].

Dogs

All dogs involved in the study were privately owned companion dogs aging at least 1 year old, recruited from friends and acquaintances, with no reported prior experience in scent detection, olfactory discrimination tasks, or formal training involving odor cues. Importantly, all dogs were naïve to the odor of the seizure, meaning that they had no known prior exposure to epileptic seizures, either in humans or other dogs. This criterion was adopted to ensure that any observed behavioral responses would reflect spontaneous reactions to the presented odor stimuli rather than prior learning or experience. Dogs had to be free from overt signs or existing diagnoses of distress and/or pain during the experimental session. Additionally, dogs needed to show interest in new situations and objects, as novel objects were involved in the sniffing task they would undergo if they were enrolled. Thirty-four companion dogs aged 1-14 years (median age=5.5 years), including 11 males (one neutered) and 23 females (19 spayed) were recruited for this study through word of mouth at the National Association Assistance Dogs Il Collare d'Oro and at the Department of Veterinary Medicine and Animal Sciences, University of Milan. Overall, the sample comprised 10 mixed-breed dogs and 24 purebred dogs representing 14 breeds, including Labrador Retriever, Australian Cattle Dog, Belgian Shepherd (Tervuren), Border Collie, Jack Russell Terrier, Wirehaired Pointing Griffon (Korthals), English Setter, Wirehaired Dachshund, Maremma Hound, Czechoslovakian Wolfdog, Chihuahua, Lagotto Romagnolo, Dachshund, and Airedale Terrier. Dogs were accompanied by their guardians throughout all experimental phases (see the following section on Experimental setup and testing), and their behavior during the sniffing phase was video-recorded for subsequent analysis. The final sample size met and exceeded the minimum required for the study, as determined by an a priori power analysis conducted in R (pwr package) and WebPower. The analysis assumed a medium-to-large effect size (f=0.40), a significance level of α=0.05, and a desired statistical power of 0.80, yielding a minimum required sample of 25 dogs.

Experimental setup and testing

Behavioral testing of dogs was conducted at the Animal Physioethology Laboratory of the Department of

Veterinary Medicine, University of Milan, Lodi, Italy. Three odor stations were arranged on the floor in a semicircular configuration, equidistant (130 cm apart) and positioned 300 cm from a central starting point, where the guardian was seated and released the dog at the beginning of each trial. Each station consisted of a plastic pot mounted on a fixed base (49x35 cm) containing the samples and covered with a perforated metal plate (15x15 cm), allowing diffusion of volatile compounds while preventing direct contact with the biological sample. After testing the first ten dogs, we observed that 40% of them (4/10) did not approach the experimental setup during the sniffing phase and remained in proximity to their guardian, despite having freely explored the room during the preceding acclimatization phase (see below for details). The only procedural difference between the acclimatization and sniffing phases was the presence of the sniffing stations on the floor. To facilitate spontaneous approach to the stations, an identical empty food bowl (16 cm diameter) was placed next to each pot at a fixed distance of approximately 2 cm *(Figure 1a, b)*. Following this adjustment, failure to approach the experimental setup was no longer observed in subsequent dogs.

The three stations contained: A) a polymeric tube filled with a sweat sample collected during an ictal phase, B) a polymeric tube filled with a sweat sample collected during an interictal baseline phase from the same individual, and C) a polymeric tube filled with a clean gauze pad serving as a control. Each dog was tested once and constituted a single independent observational unit in the experimental design. Each trial comprised two sequentially structured phases separated by a 5-minute break: an acclimatization phase and a sniffing phase.

Acclimatization phase (10 min): before the sniffing phase, the guardian and the dog entered the laboratory room and were welcomed by two researchers (MA and FP), who provided instructions and then left the room. In particular, the guardian was instructed to sit on a chair at the starting point, release the dog to explore freely, and remain seated facing away from the interior of the room, including the area where the odor stations would later be placed. During this time, the guardian completed documentation provided by the researchers, to prevent any visual or verbal interaction with the dog. This procedure closely mirrored the sniffing phase, with the sole difference being the absence of the odor stations positioned on the floor in the center of the room.

Break (5 min): at the end of the acclimatization phase, only one researcher (MA) re-entered the room. The guardian was then asked to leash the dog and temporarily leave the laboratory to allow the experimental setup to be arranged. To minimize position-related interference, the left-center-right position of each odor station was pseudorandomized across trials using a true random number generator (http://www.random.org), such that all possible presentation sequences (e.g., ABC, BCA, CAB) were counterbalanced across dogs. At the end of each session, the slots were changed, and the sample stations were thoroughly cleaned with a vapor machine (Vaporetto PRO 90 Turbo, Polti, Italy).

Sniffing phase (2 minutes): the guardian and the dog re-entered the room, while the researchers exited and remained in an adjacent room for the duration of the phase. The guardian sat at the starting point, unleashed the dog, and turned away from the experimental area while completing additional documentation. The dog was thus free to explore the room and the experimental setup without guidance, commands, or reinforcement

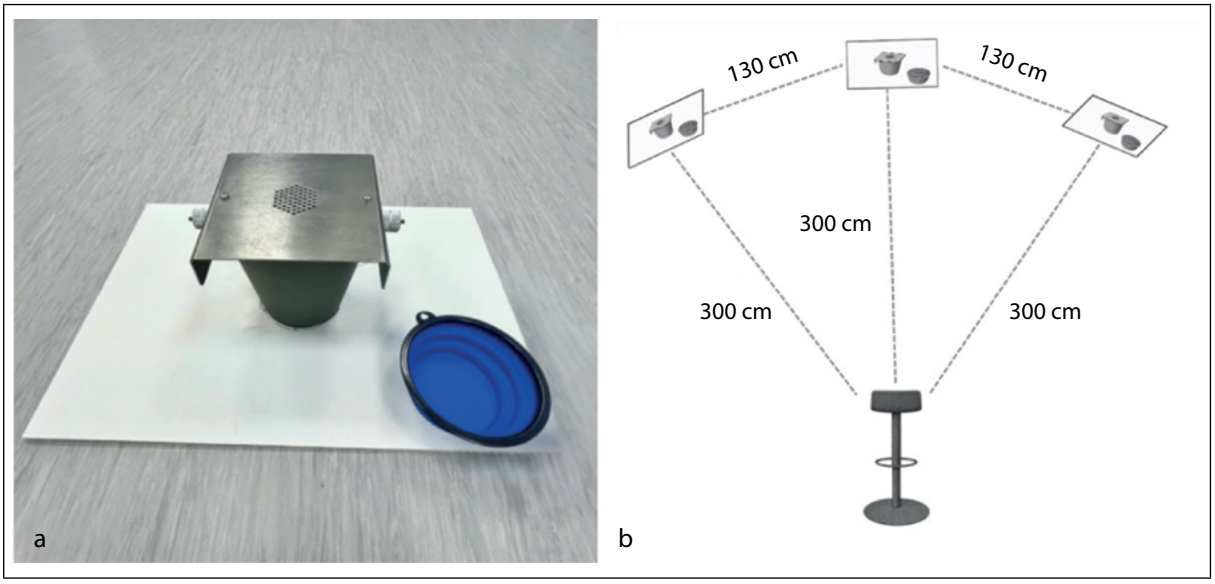

Figure 1
a) Detail of the experimental odor station; b) Experimental testing room and spatial arrangement of the odor stations. Three odor stations were arranged on the floor in a semicircular configuration. Stations were spaced 130 cm apart from each other and positioned 300 cm from a central frontal starting point, marked by the guardian's chair. From this position, the dog was released and allowed to explore the experimental setup freely during the sniffing phase.

(Figure 2). Dog behavior was monitored remotely by a single researcher (FP), who had not arranged the setup, using two wall-mounted digital video cameras (GoPro HERO7, Italy), operated from the adjacent room. The combined recordings provided complete visual coverage of the dogs, ensuring continuous monitoring of exploratory behavior and enabling the detection of any relevant signs of severe distress or anxiety that would have led to immediate interruption of the trial, while also allowing accurate coding of the predefined behavioral parameters during subsequent video analysis. In addition, guardians were explicitly informed that they could stop the test at any time if they had concerns regarding their dog's well-being or safety. After 2 minutes, both researchers re-entered the room and formally terminated the trial. The experiment was conducted under double-blind conditions as the spatial position of ictal, interictal, and control samples was unknown to the guardian-dog dyads and to the monitoring researcher (FP), while the researcher responsible for arranging the odor stations was neither present in the testing room nor had any visual access to the room during the sniffing phase, including remote access via the video monitoring system.

Video coding

The video-recorded sniffing phase was analyzed using PotPlayer (v. 1.7). To minimize the risk of unconscious confirmation bias, the analysis was conducted without access to information regarding the sequence of odor samples presented [31]. Behavioral responses were quantified using the following variables, which captured different temporal and functional aspects of olfactory exploration:

1. sniffing duration, defined as the total time (in seconds) spent sniffing each station during the 2-minute sniffing phase and treated as a continuous variable in subsequent analyses. Longer sniffing was considered an index of sustained attention to the corresponding olfactory stimulus, reflecting the extent to which an odor maintained the dog's engagement over time. Evidence of prolonged engagement with the ictal odor would be indicated by longer sniffing duration at that station compared to the interictal and control stations (A_duration > B_duration and C_duration);
2. sniffing frequency, defined as the number of distinct sniffing episodes directed toward each station during the 2-minute sniffing phase and treated as a continuous variable in subsequent analyses. This measure was used as an index of repeated engagement with the odor source, reflecting the dog's tendency to return to and re-explore a given stimulus. A higher level of repeated engagement with the ictal odor would be suggested by a higher sniffing frequency at the ictal station relative to the other conditions (A_freq >B_freq and C_freq);
3. initial orienting behavior, assessed by recording the first station investigated after release. This measure was used as an index of immediate attentional bias toward the olfactory stimuli. For inferential purposes, a dichotomous variable (A_first) was derived to indicate whether the ictal station was approached first (yes/no). In addition, to analyze how dogs distributed their attention across stimuli over time, the order of olfactory investigation was recorded for each station (A, B, and C) as the position in the exploration sequence (first, second, or third), reflecting the order in which stimuli were selected during spontaneous exploration. Stations that were not investigated during the trial were coded as "not sniffed";
4. approach-related measures, which included a) non-investigation and b) latency to first sniff. Non-investigation was defined as the absence of sniffing at a given station during the sniffing phase and quantified as the proportion of dogs that did not investigate each station. Latency to first sniff was defined as the time elapsed (in seconds) from the moment the dog was released to the first sniffing interaction with the first station approached. This parameter was analyzed as a continuous variable to assess quantitative differences in exploratory onset across odor conditions. In addition, to facilitate the characterization of distinct behavioral profiles of initiation, latency to first sniff was dichotomized using the sample median (short *vs* long latency) for specific analyses. This categorization allowed the identification of qualitatively different patterns of initial engagement, potentially reflecting differences in stimulus salience or approach-related processes, that could not be captured solely by comparisons of central tendency.

In addition to exploration-related measures, potential indicators of distress were quantified. Specifically, the frequency of scratching, yawning, and panting was recorded for each dog during the experimental session.

Finally, to further characterize dogs' exploratory strategies, the subsample of dogs (n=24) tested under conditions in which both the pot and the bowl were consistently present at all three stations was selected for additional analyses. Specifically, an Object variable was introduced to characterize orienting strategies within each approached station. The Object variable was coded categorically to indicate whether dogs investigated the pot only (P), the bowl only (B), both objects with the pot contacted first (PB), and both objects with

Figure 2
Dog freely exploring one of the odor stations during the sniffing phase.

Table 1
Distribution of sniffing order across stations in all dogs (n=30)

	First n (%)	Second n (%)	Third n (%)	Not sniffed n (%)
Station A	14 (46.7)	4 (13.3)	4 (13.3)	8 (26.7)
Station B	9 (30.0)	12 (40.0)	2 (6.7)	7 (23.3)
Station C	7 (23.3)	8 (26.7)	7 (23.3)	8 (26.7)

Station A: ictal; station B: interictal; station C: empty (control). Percentages are calculated within station (row percentages) for each station.

the bowl contacted first (BP). To verify that these dogs were representative of the full sample, all previously defined behavioral measures were re-evaluated in this subsample.

Statistical analyses

Of the 34 dogs initially involved, four failed to interact with the odor stations during the sniffing phase, remaining at the starting point near the guardian, and were therefore excluded from the analyses. Unless otherwise specified, these were conducted on both the full sample (n=30) and on the subsample of dogs (n=24) tested under conditions in which both the pot and the bowl were consistently present at all three stations.

To exclude potential procedural biases, the distribution of presentation sequences (ABC, ACB, BAC, BCA, CAB, CBA) was examined using chi-square goodness-of-fit tests. In this analysis, as well as in all subsequent applications of chi-square tests throughout the study, Monte Carlo significance estimates were applied when expected cell counts were small (≤5) to ensure accurate p-value estimation. In addition, adjusted standardized residuals were inspected to identify specific categories contributing to significant deviations from expected frequencies. To control for spatial biases, the effect of the ictal station's spatial position (left, center, right) on dogs' initial orienting behavior was examined based on its location within the presentation sequence for each trial. The association between the spatial position of station A and the likelihood of being approached first was evaluated using chi-square tests of independence. Preliminary inspection of the data revealed that latency variables were positively skewed across stations. Given the non-normal distribution of the data and the limited sample size, non-parametric statistical tests were used for all inferential analyses.

Sniffing duration and sniffing frequency were treated as continuous variables and compared across odor conditions (ictal, interictal, control) using Friedman tests. The relationship between sniffing duration and sniffing frequency was examined using Spearman's rank correlation coefficient, computed separately for each odor condition in the full sample. The strength of correlation was classified as absent (0.00-0.09), weak (0.10-0.29), moderate (0.30-0.49), or strong (0.50.1.00) [32]. The correlation analyses were exploratory and aimed at characterizing the structure of olfactory exploration rather than testing multiple independent hypotheses. Therefore, no correction for multiple comparisons was applied.

Initial orienting behavior (first station investigated) was analyzed using chi-square goodness-of-fit tests to evaluate deviations from a uniform distribution across stations. Sniffing order (first, second, third) was analyzed for each station using chi-square goodness-of-fit tests against a uniform distribution. Differences in non-investigation across stations were analyzed using Cochran's Q test. Latency to first sniff was analyzed as a continuous variable using Kruskal–Wallis tests according to the identity of the first station approached. To specifically examine latency patterns associated with the ictal odor, additional analyses were restricted to trials in which the ictal station was chosen first. In addition, the variable was dichotomized using the sample median as a conservative cut-off, defining short (≤median) and long (>median) latencies. Differences in the distribution of short and long latencies across first-choice stations were assessed using chi-square tests with Monte Carlo estimation of exact p-values. The Pot-Bowl (PB) variable was examined in the 24-dog subsample using chi-square goodness-of-fit tests. All statistical analyses were performed using IBM SPSS Statistics (version 30), with statistical significance set at p≤0.05.

RESULTS

In the total sample (n=30), sniffing order, reported as frequencies and percentages in *Table 1*, differed significantly across stations. Specifically, the distribution of sniffing order for station A significantly deviated from a random distribution (χ^2=8.93; Monte Carlo p=0.029, *Figure 3*). Inspection of residuals indicated that this effect was primarily driven by an overrepresentation of first investigations at the ictal station (14 observed *vs* 7.5 expected; residual =+6.5), accompanied by underrepresentation in the second (4 *vs* 7.5; residual =–3.5) and third positions (4 *vs* 7.5; residual =–3.5), while

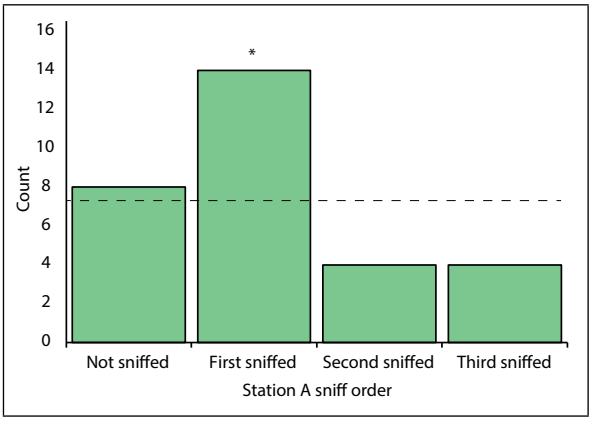

Figure 3
Distribution of sniffing order for station A. The dashed line represents the expected frequency under a uniform distribution (n=7.5 per category). *p≤0.05 based on adjusted standardized residuals.

non-investigation showed only a minimal deviation (8 *vs* 7.5; residual =+0.5). For station B, the distribution of sniffing order did not reach statistical significance (χ^2=7.07; Monte Carlo p=0.070). However, residual inspection suggested a tendency toward second-position investigations (12 *vs* 7.5; residual =+4.5). For station C, sniffing order was fully consistent with a random distribution (χ^2=0.13; Monte Carlo *p*=1.000), with small residuals across categories (range −0.5 to +0.5), indicating no positional bias. No significant differences were observed in the proportion of dogs that failed to investigate the three stations (Cochran's Q test: Q=0.12; Monte Carlo p=1.000). The number of dogs that did not investigate each station was comparable (ictal: 8/30; interictal: 7/30; empty: 8/30), indicating that the samples obtained during the ictal condition were not selectively avoided.

The chi-square goodness-of-fit test showed no significant deviation from a uniform distribution of presentation sequences (χ^2=2.40, p=0.791), thus excluding potential sequence biases. The likelihood of the ictal station being approached first was not influenced by its spatial position (left, center, or right). Specifically, no significant association was observed between the position of station A and first investigation (χ^2=1.69; Monte Carlo p=0.468).

In the total sample of dogs (n=30), no significant differences were observed for any other behavioral variables between the ictal and interictal or control conditions. Descriptive and inferential statistics for continuous variables (sniffing duration, sniffing frequency, and latency to first sniff) are reported in *Table 2* as medians and interquartile ranges (IQR; 25th-75th percentiles), with distributional properties further summarized by skewness and kurtosis. Sniffing duration and sniffing frequency were strongly and positively correlated within each odor condition (ictal A: Spearman's ρ=0.83, p=0.001; interictal B: ρ=0.81, p=0.001; control C: ρ=0.79, p=0.001), indicating a consistent association between sustained and repeated engagement across odor conditions. Latency to first sniff was positively skewed across all stations, particularly for the interictal (B) and control (C) stations, with higher skewness and kurtosis values indicating the presence of extreme latency values.

When latency to first sniff was examined dichotomized using the sample median (10.5 sec), the overall association between first choice and latency category did not reach statistical significance (Pearson's χ^2=4.35; Monte Carlo p=0.165). Descriptively, however, distinct exploratory profiles emerged across conditions *(Table 3)*.

Across the full sample (n=30), no behavioral signs of distress, fear, or anxiety were observed. Stations that were not investigated were simply ignored and were not associated with any observable signs of discomfort or withdrawal.

In the subsample of dogs that were presented with both the pot and the bowl at all three stations during the sniffing task (n=24), the PB variable revealed a non-random distribution of sniffing choices across container types *(Table S1 available online as Supplementary material)*. For both the ictal (A) and interictal (B) stations, the distribution of PB categories significantly deviated from a uniform distribution (A: p=0.001; B: p=0.010), indicating the adoption of a structured sniffing strategy within odor-containing stations.

Inspection of residuals indicated that, for station A, the deviation from uniformity was primarily driven by a marked overrepresentation of the BP category (12 observed *vs* 4.5 expected; residual =+7.5), accompanied by underrepresentation of the B category (1 *vs* 4.5; residual =−3.5) and the PB category (2 *vs* 4.5; residual = −2.5). A comparable pattern was observed for station B, where the BP category was again overrepresented (11 *vs* 4.8; residual =+6.3), while both the P (2 *vs* 4.8; residual =−2.8) and B (2 *vs* 4.8; residual =−2.8) categories were underrepresented. In contrast, no significant deviation from uniformity was observed for the control station (p=0.715). Residuals for station C remained small across categories (range −1.7 to +1.3), indicating the absence of a structured exploratory pattern, with the pot never being sniffed.

Table 2
Descriptive statistics of interest-related variables in all dogs (n=30)

Variable	Median	IQR (25-75)	Skewness	Kurtosis	P
Duration (sec)					Friedman test
Station A	2	0.0-2.0	0.633	−0.175	
Station B	2	0.5-3.0	0.239	−0.341	0.811
Station C	1	0.0-3.0	1.295	2.121	
Frequency (n)					Friedman test
Station A	1	0.0-1.0	-0.422	0.042	
Station B	1	1.0-1.0	-0.192	0.459	0.713
Station C	1	0.0-1.0	-0.298	−0.295	
Latency to first sniff (sec)					Kruskal-Wallis test
Station A	12	4.0-21.0	1.158	0.628	
Station B	5	3.0-10.0	2.892	8.516	0.16
Station C	37	8.5-59.0	1.161	1.300	

Station A: ictal; station B: interictal; station C: empty (control); IQR: interquartile range.

Table 3
Latency to first sniff relative to the sample median (10.5 sec)

Station	n	Short latency n (%)	Long latency n (%)	Latency pattern
A	14	8 (57.1)	6 (42.9)	Mixed (both rapid and delayed initiation)
B	9	7 (77.8)	2 (22.2)	Predominantly short (rapid initiation)
C	7	2 (28.6)	5 (71.4)	Predominantly long (delayed initiation)

Station A: ictal; station B: interictal; station C: empty (control); Total sample size (n=30).

Results for all other behavioral variables in this subsample of dogs (n=24) were consistent with those observed in the full sample (*see Supplementary materials available online for details on statistical analyses)*. Importantly, also in this subset, the distribution of presentation sequences did not significantly deviate from a uniform distribution (χ^2=1.00; Monte Carlo p=0.984), indicating the absence of systematic biases in the order of presentation of the odor stations under standardized testing conditions. The probability of the ictal station being sniffed first did not differ as a function of its spatial position (χ^2=0.59; Monte Carlo p=0.878). It is worth noting that, for the distribution of sniffing order, differences in statistical significance emerged for stations A and B, with station A significant in the full sample but not in the subsample (χ^2=5.67; Monte Carlo p=0.144), and the reverse pattern observed for station B (subsample: χ^2=8.33; Monte Carlo p=0.044). These differences likely reflect sampling-related variability associated with the reduced sample size, which can affect the stability of p-values in discrete data, without altering the underlying pattern of behavior, which remained consistent with that observed in the full sample. Indeed, inspection of residuals indicated a tendency for station A to be sniffed first more often than expected (11 *vs* 6; residual =+5.0). For station B, the sniffing-order distribution significantly deviated from chance, primarily driven by an overrepresentation of second-position investigations (10 *vs* 6; residual =+4.0) and an underrepresentation of third-position investigations (2 *vs* 6; residual =−4.0). For station C, similarly to the full sample, sniffing order was consistent with a random distribution (χ^2=1.33; Monte Carlo *p*=0.753), with residuals remaining small across categories (range −2.0 to +2.0), confirming the absence of a structured positional bias.

DISCUSSION

In the present study, we sought to determine whether untrained companion dogs, naïve to epileptic seizures, show spontaneous differential responses to odor samples collected during the ictal state compared with interictal samples and blank controls under controlled experimental conditions, within a socially neutral laboratory paradigm. The aim was to isolate the olfactory component from seizure-related learning, familiarity, and socially mediated reinforcement. In this initial experimental phase, all dogs were presented with odor samples originating from a single individual in order to provide a standardized stimulus across trials.

Across behavioral measures, odor associated with seizure samples selectively influenced initial orienting behavior. Dogs were significantly more likely to investigate the ictal station first, whereas no differences emerged in sniffing duration, sniffing frequency, non-investigation, or latency to first sniff. Thus, this odor did not increase sustained exploration, repeated investigation, or approach speed. Instead, it selectively biased the prioritization of the first behavioral choice during the free-exploration phase of the task. This profile is consistent with stimulus salience expressed at the level of early behavioral prioritization, rather than prolonged or repeated exploratory engagement, suggesting that dogs prioritize the ictal station without increasing exploratory processing relative to the other conditions. In other words, the ictal samples appear to influence initial station selection at the onset of exploration, without modulating sustained exploratory investment. Some explanations may account for the observed patterns, which likely reflect different facets of stimulus salience and may coexist. First, the observed effect may reflect perceptual salience. If odor cues present in samples collected during the ictal state contain distinctive VOCs, they may be rapidly detectable and sufficiently discriminable upon initial sampling. In this case, extended investigation would not be necessary for perceptual differentiation, and exploration would not increase beyond the initial approach. Under this account, prioritization reflects efficient sensory discrimination rather than enhanced motivational engagement. Second, the effect may reflect biological salience, whereby odor cues present in samples collected during the ictal state signal a physiologically altered state that is behaviorally relevant even in the absence of prior learning. Stations containing ictal samples may thus be preferentially selected because it conveys information about an atypical biological condition. Importantly, biological salience does not necessarily imply strong motivational valence, a distinction widely discussed in incentive-salience models of reward processing [33, 34]. Within this framework, a stimulus may act as a potent attentional attractor despite lacking, or even before acquiring, clear appetitive or aversive value. If differential appetitive or aversive value were driving behavior, one would expect corresponding modulation of sniffing duration, frequency, or repeated returns, which was not observed. Thus, the present data do not support motivational valence as the primary driver of the observed dynamics. It is worth noting that no behavioral indicators of distress, anxiety, or withdrawal were observed while dogs were exploring the stations or in their immediate proximity, suggesting that the stimuli, including odor cues present in the ictal samples were not aversive within the stan-

dardized experimental context. As described in the Materials and Methods section, in initial trials conducted prior to the introduction of the bowl, a small number of dogs remained at a distance from the apparatus and did not approach the stations. This pattern likely reflected limited spontaneous engagement with the setup rather than a specific response to the odor stimuli. The inclusion of the bowl functioned as an approach facilitator and resolved this issue.

The heterogeneity in latency among dogs that first approached the ictal station further refines the interpretation of biological salience. Although inferential analyses of latency did not reveal statistically significant differences, descriptive patterns showed that the ictal condition was selectively prioritized while being associated with both rapid and delayed initiation, whereas the interictal odor was approached rapidly but typically in second position, suggesting uniform detection without selective prioritization, and the control condition showed delayed initiation and random sampling order, consistent with reduced behavioral relevance. Such a configuration suggests that the stimulus present in ictal samples may be both perceptually distinctive and biologically relevant yet variably interpreted at the individual level. Its prioritization at the selection stage might be consistent with heightened perceptual discriminability and possible activation of novelty-driven orienting mechanisms. At the same time, the coexistence of rapid and delayed latencies indicates that the stimulus may be less predictable or less readily categorizable than baseline (interictal) human odor, thereby engaging differential appraisal processes across individuals.

Importantly, this variability did not translate into increased sniffing duration or frequency, arguing against strong motivational valence as the driver of the observed dynamics. Taken together, these findings suggest that odor cues present in samples collected during the ictal state operate primarily at the level of stimulus selection and early orienting, potentially driven by perceptual distinctiveness, reduced predictability, and biological rather than motivational relevance. The absence of differential motivational engagement further supports the interpretation that the observed response reflects a spontaneous, pre-associative detection mechanism rather than an experience-dependent, value-driven process. However, because the present study employed odor samples from a single donor, the observed orienting bias cannot be assumed to reflect a seizure-specific olfactory signature shared across individuals. An alternative explanation that cannot be excluded is that dogs responded to stimulus-specific olfactory features present in the samples obtained during the ictal condition from this individual.

Analysis of exploratory strategy within stations (Object variable) revealed structured sampling behavior for both ictal and interictal odors, but not for the blank control. In particular, in odor-containing stations, the distribution differed significantly from chance, with dogs predominantly exhibiting the BP pattern (both objects, bowl first) and showing fewer single-object inspections, especially bowl-only interactions. Because the pattern was comparable across ictal and interictal conditions, it is unlikely to reflect a seizure-specific effect. Instead, the presence of human odor per se seems to organize local investigation once a station has been selected. Because the bowl was identical across conditions, contained no food, and provided no differential reinforcement, its motivational value was constant and unrelated to seizure state or human trace. The concurrent marked underrepresentation of bowl-only (B) inspections suggests that dogs did not treat the bowl as a preferred object per se. Rather, the bowl likely functioned as an initial point of contact that facilitated engagement with the station, from which dogs subsequently proceeded to sample the pot. Thus, seizure-related salience appears to operate at the level of between-station prioritization, whereas within-station sampling structure reflects general exploratory dynamics, with dogs initially engaging the more familiar and positively associated entry point before proceeding to the pot. In contrast, the control station showed no structured distribution, and pot-only inspections were absent, indicating that in the absence of relevant odor the micro-sequence was not consistently expressed. In this respect, the inclusion of the bowl appears methodologically justified: it promoted approach to the apparatus without biasing odor discrimination, allowing between-station prioritization effects to emerge without increasing failure to approach or avoidance of the experimental setup. From a practical standpoint, these findings suggest that incorporating a neutral but positively valenced engagement cue may enhance participation in laboratory detection paradigms without confounding stimulus discrimination. This principle may be important for the design of standardized olfactory testing protocols and for early phases of training in applied detection contexts, where reliable approach to the odor source is required before shaping learned detection behaviors.

To our knowledge, this is the first study specifically designed to characterize the spontaneous olfactory strategy of untrained dogs responding to seizure-associated human odor under controlled, socially neutral conditions. Prior research with untrained dogs has largely identified socially directed behaviors, such as staring, proximity seeking, pawing, or nudging, as indicators of seizure-related responsiveness [26, 35-37]. Although informative, these responses occur within relational contexts and cannot isolate the underlying structure of olfactory exploration. By contrast, the present paradigm quantified multiple temporal dimensions of spontaneous odor investigation (initial orienting, latency, duration, frequency, and within-station sampling strategy) in the absence of dyadic cues, reinforcement histories, or social referencing, enabling early sensory-attentional processes to be examined independently of social interaction. Despite its strengths, this study has some limitations that should be acknowledged. Particularly, the study does not determine the chemical identity or specificity of the volatile compounds involved, nor does it address discrimination between epileptic and non-epileptic seizure events. Moreover, the use of samples from a single individual, while allowing strict control of inter-individual odor variability in this exploratory phase, limits the generalizability of the findings.

Future studies including multiple donors are required to determine whether the observed behavioral prioritization reflects seizure-related physiological cues that are shared across individuals or stimulus-specific odor features.

CONCLUSION

Odor cues present in samples collected during the ictal state may have selectively biased initial orienting in untrained, naïve dogs within a controlled, nonsocial paradigm, without increasing the duration or frequency of exploration. This dissociation between first choice and sustained investigation suggests that odor cues present in samples collected during the ictal state act primarily as early salience-driven signals rather than cues influencing ongoing exploratory processing, indicating intrinsic perceptual and/or biological salience sufficient to bias spontaneous selection in the absence of learning or reinforcement, without evidence of strong motivational valence. By isolating olfactory cues from social interaction and controlling for stable individual odor signatures, primary sensory processing was distinguished from socially mediated alerting. From a mechanistic perspective, the results are consistent with the possibility that ictal-related VOCs engage canine olfaction at an early sensory-attentional level. From a mechanistic perspective, the results are consistent with the possibility that VOCs present in samples collected during the ictal state may contribute to the observed behavioral prioritization by engaging canine olfaction at an early sensory-attentional level. Ongoing research within the same project is extending this approach to a multi-donor design to determine whether such effects truly reflect generalizable seizure-related olfactory cues.

Authors' contributions

FP: Writing – original draft, Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. GC: Writing – review & editing, Methodology, Investigation, Conceptualization. MA: Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the Authors used ChatGPT-5.2 to improve the readability of the manuscript during its preparation. The Authors have reviewed and edited the content and take full responsibility for the final publication.

Conflict of interest statement

The Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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