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Evidence for canine olfactory detection of melanoma

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Abstract

Evidence of chemical markers for melanoma in blood and urine suggests that volatile chemicals might be released from melanoma cells (on the skin surface) in amounts sufficient to allow early diagnosis. When tested using methods normally used in canine olfactory detection of drugs and explosives, two dogs demonstrated reliable localization of melanoma tissue samples hidden on the skin of healthy volunteers. One dog (A) then “confirmed” clinically suspected (and subsequently biopsy-proven) diagnoses of melanoma in five patients. In a sixth patient, this dog “reported” melanoma at a skin location for which initial pathological examination was negative, despite clinical suspicion. More thorough histopathological examination in this individual then confirmed melanoma in a fraction of the cells. In a seventh patient, in whom neither dog nor dermatologist provided a definitive response, melanoma was detected by histopathological examination. Dog B searched four of these seven patients; in each case, responses agreed with those of dog A. These findings warrant further study of the conditions under which detection of melanoma might be enhanced by the biological or non-biological detection of volatile chemicals emanating from skin lesions.

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1. Introduction

Compelling, if circumstantial, evidence exists that the applied use of the canine olfactory system significantly enhances the decision-making capabilities of law enforcement, military,

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transportation security and customs officials. There is some consensus that individuals (e.g. escaped prisoners), various threats to life and crime-related chemical residues can be recognized and localized based on the dog's ability to recognize chemical mixtures and track them to their sources (Gazit and Terkel, 2003; Kauhanen et al., 2002; Williams and Johnston, 2002). Though we are aware of no quantitative data comparing the abilities of humans and dogs in terms of olfactory analysis of chemical mixtures, few would doubt the superiority of the latter. There is also evidence that medical personnel associate different odor qualities with particular diseases or conditions (Lukas et al., 1977; Smith et al., 1982). Collectively, these observations suggest that the canine's olfactory prowess could be of considerable value in medical diagnosis. For example, the ability of well-trained dogs to detect the presence of one or more chemical markers of disease would be of great value in the development of diagnostic tests.

Despite its potential, little exploration of this area has occurred. That melanoma may be a reasonable place to initiate such an investigation was suggested by a brief note written more than a decade ago (Williams and Pembroke, 1989). A patient was described whose dog persisted in exploring a spot on the patient's leg that was subsequently identified as melanoma. Given the evidence for chemical markers for melanoma in body fluids (e.g., Wakamatsu and Ito, 1990; Kelley et al., 1998; Wakamatsu et al., 2002), one can speculate that the dog may have detected one or more markers emanating from the skin lesion's surface.

Annually in the US, estimates of new cases of melanoma and deaths from this form of skin cancer are 47,000 and 8000, respectively. At present, the likelihood of a melanoma being detected varies with such factors as the stage and type of melanoma and level of training of the physician. Efforts to diagnose this form of skin cancer rely almost entirely on aided or (usually) unaided visual inspection by the physician. Given the prevalence, consequences and current difficulties with diagnosis of melanoma, the present work explored the possibility that there might be chemical markers of melanoma. To maximize the likelihood of detecting any markers that might be present, we selected a biological detector likely to be superior to current non-biological approaches: the canine olfactory system. Two questions were posed. First, could dogs that were already highly trained to perform in field scent discrimination trials be trained to locate melanoma tissue samples by smell? Second, can dogs employ odor cues to localize melanoma in patients? Our study did not seek to determine if there might be chemical markers unique to melanoma or whether it could be discriminated from others skin cancers.

2. Methods

2.1. Animals and husbandry

Two dogs were used. Dog A was a 4-year-old male Standard Schnauzer that achieved the American Kennel Club (AKC), titles of CH (champion), utility dog-excellent (UDX), and obedience trial champion (OTCH). This dog served 2.5 years in the Tallahassee police department as a working dog, was certified by the State of Florida as a bomb detection dog and served in research efforts by the US Department of Defense. Dog B was a 6-year-old

female Golden Retriever that had achieved AKC titles of CH, UDX, OTCH and master hunter (MH).

2.2. Training and testing procedures

2.2.1. Retrieval tube and area searches

Dog A was trained first and its training was more lengthy. First, retrieval tubes were used to associate the odor of skin cancer cells with praise from the trainer-handler. These were pieces of PVC tubing (~ 27 mm OD and ~ 23 cm long) that were perforated, to allow release of volatile chemicals, and permanently capped at one end. The removable cap allowed the placement of a sample consisting of a mixture of basal, squamous and melanoma tissues. A local research dermatologist prepared this sample, denoted as BSM. Residual tissues from histopathological examination were combined, wrapped in gauze and frozen at -80°C . Just prior to each 20 min training session, the BSM sample was thawed and placed in the retrieval tube with forceps. Each trial consisted of the tube being placed briefly in front of the dog's nose and then thrown in front of the dog. The dog's task was to retrieve the tube. Roughly 100 such trials were conducted for dog A over a period of several weeks. This was followed, for dog A, by approximately 100 area search trials in which the dog's task was to search a grassy area of roughly 400 m^2 and retrieve, from among a set of PVC retrieval tubes, the one containing the BSM tissue sample. Only eleven such area search trials were conducted with dog B.

2.2.2. Box tests

Based on strong evidence that both dogs could locate the BSM tissue samples in area searches, a directed search test was devised. The purpose of this test was to assess the dog's ability to identify the odor of melanoma in the presence of distractor stimuli likely to be encountered in a medical setting. The tissue used for this was a portion of a large ($\sim 6\text{ cm}^2$) recurrent melanoma removed from the back of a patient who provided informed consent for the use of this tissue sample for research purposes. As with the BSM sample, this tissue was wrapped in gauze and frozen at -80°C . Fig. 1 depicts the wooden apparatus (box) constructed for this mode of testing. For a given trial, the melanoma sample was thawed and placed in one of the compartments, with the location being varied over the course of trials. On some trials, all of the remaining nine compartments were empty. On others, from one to nine of the remaining compartments held various distractor stimuli (e.g., adhesive bandages, gauze, latex gloves, rolls of tape). The person placing the stimuli wore

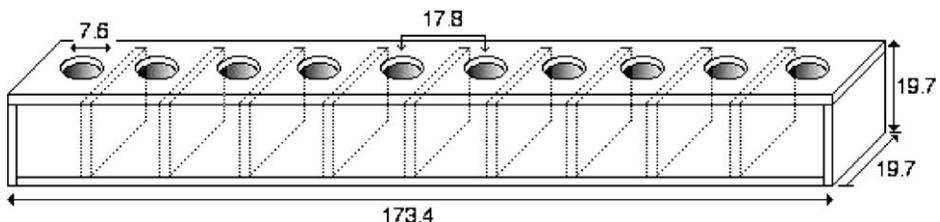


Fig. 1. Apparatus used for box testing. Dimensions are in centimeter.

latex gloves, positioned all distractors before the melanoma tissue sample and discarded the gloves once the box was set up for a given trial. For all box trials, the melanoma target was present at one of the ten locations.

After an interval of several minutes, the dog was led to sniff each of the ten compartments. For dog A, a correct response was defined as sitting next to the compartment containing the melanoma sample and pawing at the opening to this compartment. For dog B, a correct response was sitting next to the melanoma-containing compartment and mouthing the opening to this compartment. The handler was blind as to melanoma tissue sample location. No time limit was imposed on the dog-handler team. Both handlers were given the latitude to encourage the dog to recheck any location an unlimited number of times. Only the handler made the decision as to when to end a given trial and which location was selected. The box apparatus was scrubbed at the end of each day, using a 10% bleach solution. On any given day, no more than six box trials were conducted. A conservative approach was used to evaluate responses, in that the number of empty compartments was subtracted from ten to yield the total number of possible responses. For example, for a trial in which one compartment contained the melanoma and four contained distractor stimuli, five possible outcomes were assumed for statistical analysis.

2.2.3. *Testing with tissue samples “planted” on healthy volunteers*

The performance of both dogs in box testing provided support for the idea that canine olfactory detection of melanoma might be feasible in actual patients. This phase of testing also indicated that items normally present in clinical settings did not generate odors easily confused with that of melanoma. Therefore, the ability of the dogs to locate melanoma tissue samples “planted” on healthy volunteers was assessed. Each volunteer bathed, using unscented soap, and then changed into either a swimsuit or a T-shirt and sweatpants. Five blind test trials were conducted with each dog. On each, the melanoma target was at one location and either nine or ten distractor stimuli were present. These trials were interspersed with, respectively for dogs A and B, 64 and 68 non-blind training trials and 26 and 17 blind blank trials. For all three types of trials, the individual placing the bandages wore a fresh pair of latex gloves for each trial. Fig. 2 shows the apparatus used in these tests, in which the volunteer lay on his or her back. For blind blank trials, varying numbers of empty bandages were attached to different parts of the body. For the remaining two trial types, the melanoma tissue sample was thawed, taped inside an adhesive bandage and then attached, using a second adhesive bandage, to some location on the skin surface. Melanoma location was varied from trial to trial. From 3 to 34 distractor samples were prepared by wrapping gauze inside adhesive bandages; these were placed on various locations on the body. Once the preparation of the volunteer was complete, the individual that had placed the target and distractor bandages climbed stairs to a position on a balcony overlooking the floor location where training and testing took place. Except with the training trials, the handler was ignorant on each trial as to whether a melanoma target was present.

Several minutes after the bandages were positioned for a given trial, the trainer-handler led the dog into the room. The trainer-handler pointed toward each bandage so that the dog would sniff at each one at least once. The dog was led repeatedly to each bandage until either an alert was observed or the trainer-handler surmised that no alert was forthcoming. No time limit was imposed and the handler was allowed the same degree of latitude as with

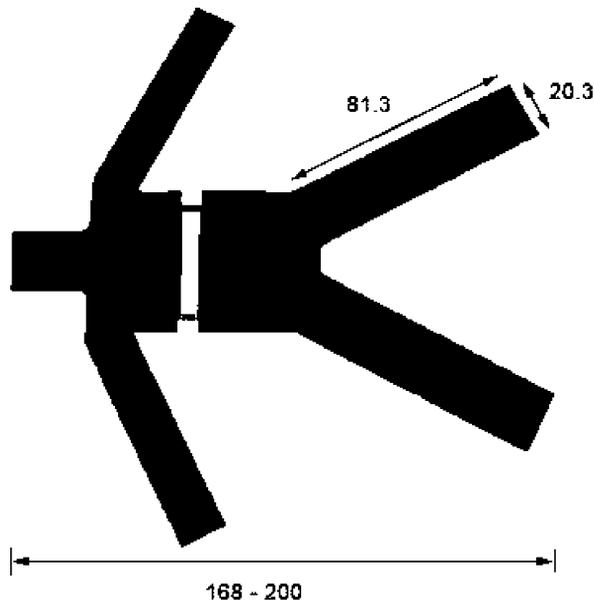


Fig. 2. Platform on which healthy volunteers and patients were positioned for searches. Dimensions are in centimeter.

box trials. For both the dogs, the correct responses were the same as those in earlier phases of testing. Dog A was required to sit and then paw at the correct bandage while dog B was required to sit and then touch the correct bandage with its lips and/or tongue.

2.2.4. Actual patient searches

Given the success with the previous phases of testing, the potential for the olfactory detection of melanoma to be used in actual patients was directly evaluated. It should perhaps be noted that the outcome of this final phase was much less certain than may appear on first thought. Although strong box and healthy volunteer performance by the dogs was consistent with the notion that localization in patients would be possible, various alternative views are plausible. For example, the dogs could have been responding to cues present in any excised human skin tissue or to chemicals released from only the underside of the melanoma tumor (versus the side exposed to the external environment). Either possibility would have left dogs unable to localize this form of skin cancer in actual patients.

Patient volunteers were seven individuals for whom examination by a local research dermatologist had provided some clinical suspicion that a single skin location might contain a melanoma. In each case, the patient provided informed consent for one or both dog-handler teams to conduct a search immediately after the clinical examination. Patients were asked to bathe, just before the dog search, using unscented soap. For each trial, the individual placing the bandages wore latex gloves, covered the suspect skin location last and then discarded the gloves. The suspect spot was covered by an adhesive bandage after from 7 to 29 additional locations were covered with identical adhesive bandages. In some cases these bandages were visible and in others (details in [Table 1](#)) they were covered by thin,

Table 1

Dog performance on box and healthy volunteer trials

Dog	Number of trials	Proportion of trials with correct response	Probability of observed performance based on chance alone
Box trials			
A	16	1.0	$10^{-8.99}$
B	11	1.0	$10^{-7.26}$
Healthy volunteer trials			
A	5	1.0	$10^{-5.04}$
B	5	1.0	$10^{-5.12}$

close fitting cotton clothing. Once the patient was in place on the platform (Fig. 2), the trainer-handler led the dog into the room. All conditions of testing, definitions of correct responding and treatment of data were the same as for healthy volunteer testing. For each patient, handlers were told only that the individual might have a melanoma. As with the healthy volunteer tests, the individual that had placed the bandages observed the trial from a balcony. Dog A searched all seven patients and dog B searched four of these patients.

2.3. Data analysis

Although we recorded performance for non-blind trials and for trials on which no melanoma was present, those trials on which melanoma was present and the handler was blind as to placement of this sample are of greatest value for addressing the question we posed: “Can dogs discriminate, by odor, melanoma from surrounding healthy skin?” With our experimental design was one in the dog-handler team was presented with several stimuli, all at once, on each trial. The number of possible choices was considered to be equal to the total number of stimuli presented. Responses were categorized as either correct (melanoma localized) or incorrect (dog either indicates on a distractor stimulus or fails to indicate at all). A single response was recorded for each trial rather than for each stimulus presented on a given trial; there was no definitive response by the dog to communicate that a given sample was not melanoma. The likelihood of the dog selecting the melanoma by chance alone was taken as $1/X$ where X is defined as number of distractors + 1 (since one melanoma was present). Similarly the likelihood of an incorrect response was taken as $(X - 1)/X$: the number of distractors divided by total number of stimuli. These are often referred to as Bernoulli probabilities (Rosner, 2000). To calculate the likelihood that the performance by a given team on a set of box, healthy volunteer or patient trials was due to chance alone, we employed the multiplication law (Rosner, 2000) and calculated the product of all Bernoulli probabilities for each combination of dog-handler team and test type. Probabilities less than 0.01 were considered as significantly different from chance.

3. Results

Performance during retrieval training was not recorded. After performance with retrieval tubes was judged by the trainer-handler to be near perfect, box trials were begun. Table 1

Table 2
Dog performance on patient searches

Patient and test information	Response of dogs	Pathology
66-year-old female		
Lesion biopsied but not removed 4 years prior to dog test	A – Melanoma localized	Biopsy 11 days after dog test
21 adhesive bandages before test, one of which covered suspect area (~30 mm across) on left shoulder	B – Melanoma localized	Lentigo maligna Clark's level - I Breslow thickness - 0
53-year-old male		
30 adhesive bandages, one of which covered suspect area (~17 mm across) on back of right shoulder	A – Melanoma localized B – No search done	Punch biopsy 7 days prior to dog test Superficial spreading malignant melanoma Clark's level - II Breslow thickness - 0.38 mm
54-year-old male		
14 adhesive bandages, one of which covered suspect area (~30 mm across) on right lateral lower back, which was different from surrounding skin in terms of texture but not pigmentation	A – Melanoma localized B – Melanoma localized	Punch biopsy 6 days before dog test Initial pathology failed to indicate melanoma but subsequent step section of entire specimen of excised tissue showed malignant melanoma Clark's level - II Breslow thickness - 0.41 mm
46-year-old male		
Visual inspection consistent with Spitz nevus, junctional nevus or superficial spreading malignant melanoma	A – Melanoma not localized	Biopsy immediately after dog test
8 adhesive bandages before test, one of which covered suspect area (~4 mm across) on abdomen	B – Melanoma not localized	Superficial spreading malignant melanoma Clark's level - II Breslow thickness - 0.32 mm
80-year-old female		
12 adhesive bandages, one of which covered suspect area (~30 mm across) on left lateral triceps	A – Melanoma localized	Punch biopsy 9 days before dog test
(Patient had clearly noticeable body odor, attributed to incontinence, at time of dog test)	B – No search done	Malignant melanoma in situ Clark's level - I Breslow thickness - 0

Table 2 (Continued)

Patient and test information	Response of dogs	Pathology
48-year-old male		
Diagnosis 10 years before dog test of suspect area (~20 mm across) on lower left back was junctional nevus with severe nevocellular atypia; no excision was done	A – Melanoma localized	Biopsy immediately after dog test
10 adhesive bandages, one of which covered suspect area	B – No search done	Lentigo maligna Clark's level - I Breslow thickness - 0
68-year-old female		
15 years earlier, patient had a superficial spreading malignant melanoma removed from left forearm	A – Melanoma localized	Biopsy immediately after dog test
10 adhesive bandages, one of which covered suspect area (~8 mm across) on posterior left shoulder	B – Melanoma localized	Superficial spreading malignant melanoma Clark's level - III Breslow thickness - 0.97 mm

Notes: (1) Clinical follow-up of all patients proceeded for at least five years and showed no evidence of metastasis of melanoma to other organs. (2) Visual examination prior to dog test and biopsy showed no indications of micro-ulcerations in any patients.

summarizes performance in the box and healthy volunteer trials. The box data demonstrate that both dogs could be trained to localize the unknown set of chemicals released by melanoma tissue samples removed from patients and repeatedly employed in testing. Both dogs readily generalized from BSM to melanoma as the target stimulus. The healthy volunteer data show that the mix of distractor chemicals given off by the living human body did not noticeably hinder the localization of melanoma tissue hidden in bandages. These data are in agreement with the absence of false positive responding by both dogs in blind blank trials in which only empty bandages were planted on healthy volunteers. As with the box data, perfect performance suggests that these tasks were not difficult for either dog.

Table 2 summarizes patient clinical information, pathological findings, and the results of the canine searches in actual patients. These data indicate that significant generalization occurred from one or both of the prior phases of training and testing to actual patient searches. Dog A localized the known or suspected melanoma in six of the seven patients. Dog B searched four of these patients and failed to localize melanoma in only the one patient in whom dog A's search had failed. The probabilities that the observed levels of performance were due to chance alone were $10^{-7.1}$ and $10^{-3.5}$, respectively, for dogs A and B.

4. Discussion

The present results provide some evidence that there are volatile cues released from melanoma tissue that allow lesion localization by the canine olfactory system. Our work did not address whether the putative cues might allow melanoma to be distinguished, by

the canine olfactory system, from the two other types of skin cancer. Thus, our working hypothesis is that there is, for the dog, an odor of melanoma that is recognizable as different from normal skin. Several shortcomings of our study should be noted and addressed in future work. First, the protocol did not preclude the possibility that the person who applied the bandages to the patients could have cued both trainer-handlers. If this were occurring, however, one would not have expected both dogs to fail with patient 4. Nonetheless, this flaw can, and should, be completely eliminated in future work. Secondly, the number of patients tested was quite small and many types of melanoma were not represented. Finally, one might argue that our approach provided an over-estimate of the potential value of the canine olfactory system since the inclusion of only patients with known or suspected melanoma raised the likelihood that canine searches would be met with success.

If future work supports the notion that volatile chemicals can be used to aid diagnosis of melanoma, it would be useful to determine whether such cues are released from the cancer cells themselves or merely represent the body's defensive responses. It would also be of value to quantify the degree to which chemically scanning the body surface with biological or non-biological systems can, when combined with current medical practice, improve the likelihood of detection of melanoma or other types of skin cancer.

5. Conclusions

While the present findings suggest the potential value of using volatile chemical markers to aid disease diagnosis, our results also demonstrate the need to generate new information on both the chemicals released from the body during disease states and the limits of the canine olfactory system. Progress in these areas will help make it possible to select those diseases for which the greatest diagnostic benefit is predicted. For a given disease, the relative merits of biological (e.g., canine olfactory system) versus non-biological sensing may be determined only through rigorous proficiency testing that incorporates a range of objective endpoints. Recent strides in the use of various instruments to analyze breath chemistry for use in aiding detection of breast and lung cancer (e.g. Di Natale et al., 2003; Phillips et al., 2003) suggest that tests of the canine olfactory ability to detect these cancers in breath may be of value. Coordinated evaluation of the two approaches, including quantification of selectivity and sensitivity, would provide a rational means of allocating research and development efforts in the future. Any progress in these areas, in the context of medical applications, will help address the long-standing need for the development of optimal canine olfactory testing protocols in such applied areas as forensics, explosives and drug detection (Brisbin et al., 2000; Tripp and Walker, 2003).

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