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MASTER THESIS

A behavioural approach to research the possibility of a heat sense in dog (Canis lupus familiaris)

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Abstract

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Pre-existing material show that the nose temperatures of carnivorian species are clearly below the nose temperatures of typical grazers. The reason for this difference is yet unknown. Analyses of histological sections have revealed a dense innervation of the nose skin, an indication of a sensory function. A heat sense would benefit from a low temperature in the nose, in contrast to other senses. The heat sense could be used for initiating a hunt when wind direction is unfortunate, or for surveillance of territory. The hypothesis of a heat sense present in the noses of carnivorians were researched through behavioural choice tasks with dogs. One long distance and one short distance set-up were used. Results show however that performances were at chance level and that no learning took place. Considerable methodological deficiencies are identified and suggestions of alternative approaches with reduced complexity are given. An additional study of the surface temperature of mammals was also performed to map the relevant temperature spectrum for a heat sense, but due to unreliable measurements the study was ended prematurely and suggestions are given of future improvements.

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Introduction

Furthest out on most mammals' snouts we find a peculiar structure, the rhinarium. It is well determined from the rest of the snout, being hairless and wet. Its function is, on the contrary, not determined at all. Two important traits diverse the rhinaria amongst the mammals, temperature and moisture. This thesis will concentrate on carnivorans' rhinaria that are cold and wet. Through behavioural experiments with dogs, the presence of a heat sense in the rhinaria of carnivorans will be explored as a possible explanation for their wet and cold noses.

The cold nose

A rhinarium is defined as the tissue with a special surface structure that surrounds the nostrils of a mammal. It extends from the nostrils out on the muzzle to a varying extent in different species. The surface is mostly completely hairless, patterned in many cases by small areas varying in shape and size, separated by shallow grooves [1]. Most mammals have it, the suborder haplorhini, water living mammals, and horses and reindeer excluded. For the ease of terminology, the outer tip of a protruding snout will here consistently be called rhinarium regardless of hair growth or wetness.

The properties of rhinaria differ between animal groups. According to work done by Gläser (2014, personal communication) a rough division can be made according to temperature between carnivorans' rhinaria and others'. All grazers have warm and wet rhinaria when awake and alert, while carnivorans' are cold and wet. Some species, there amongst pig, form a separate group by having rhinaria warm and dry.

The cold nose is found in all big families part of the Carnivora order, the clade Pinnipedia excluded due to its water living habit, and nowhere else in other parts of the phylogenetic tree. This indicates that the cold nose is a trait derived from the time before carnivorans split up in basal groups, approximately 58-65 ma [2][3]. The fact that the cold nose has not been sifted out in the course of evolution during these millions of years is a hint towards it having some sort of important functioning. It is also not an acquired trait, as our research group (unpublished observations, 2013) has shown that dog puppies have cold noses already from birth.

Previous nose work

Previous work conducted by our research group has been concentrated on dogs, for several reasons. Firstly, their cold noses were the spark to open up this whole new field of research. Secondly, they are easily accessible. Last but not least, their docility, sociability, and willingness to cooperate make them optimal study participants. Dogs have cold noses only when active and alert; a sleeping dog has a nose temperature around 35 °C and the surface is no longer wet. Their nose temperature is thus not stable, in contrast to body temperature. As seen in the picture series (fig. 1) the temperature changes rapidly when the dog wakes up. In 12 minutes

the nose temperature decreases 13.6 $^{\circ}$ C. Intense physical or mental activity (stress) also cause nose temperature to rise.

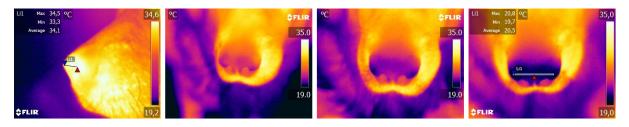


FIGURE 1: A dog waking up. Nose temperature decreases with 13.6 $^{\circ}\mathrm{C}$ in 12 minutes.

Not only mental state affects nose temperature, ambient temperature is also a strong predictor, see fig. 2. Thermographic studies (unpublished observations, 2014 [4]) have shown arctic dogs to have nose temperatures just above 0 °C, which is well below 10-15 °C, a temperature referred to as the cold pain limit for various mammals (see [5] for review). That leaves us with a temperature span for dogs' noses between 1-35 °C, depending on mental state and ambient temperature.

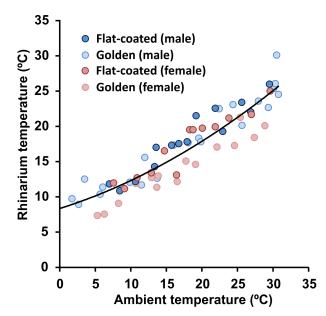


FIGURE 2: Dog nose temperature in relation to ambient temperature.

The distinct reduction in nose temperature when a dog wakes up indicates that the cold nose is a result of active cooling. Passive processes as evaporation of the liquid film on the nose surface and air flow around the nose is not enough to explain such rapid change. A rete mirabile, a net shaped arteriole aggregation, found in the lower part of the nose (Kröger, personal communication 2015) indicates the presence of a heat exchange mechanism, similar to the system for brain cooling [6]. Dogs may thus have two patterns of possible blood flow in their noses. Either the blood comes from the heat exchanger where the blood has been cooled by a nearby vein coming from the nasal passages, or the blood is shunted past the heat exchanger and warms the nose to body temperature.

Sensory possibilities

An active cooling mechanism requires energy, indicating the cold nose to have a function in order be worth the energy expenditure. Staining of the nose skin tissue has revealed a dense innervation (unpublished observations, 2014 [4]), which point towards sensory function. Olfaction or a tactile function may come first to mind, but in both cases low temperature is disadvantageous. For olfaction there are less movements of molecules and chemical reactions take longer time, making a cold nose unsuitable for tracking. Low tissue temperatures make peripheral blood vessels contract and sensory bodies do not transmit signals well, which give rise to a feeling of numbness [7]. On the contrary, a sensory function that would benefit from low temperature is a heat sense.

Heat sense

A cold nose would be suitable for detecting heat radiation since the temperature contrast between the sender (heat source) and the receiver (cold nose) is increased. Also at normal body temperature, heat from the nose would act as noise interfering with the true heat signal. By cooling the nose, the noise to signal ratio would be reduced.

A couple of infra-red sensitive sensory systems are known to exist in nature. The most studied is the pit organs present in some snake families: Crotalinae (pit vipers), certain species of Boidae (Boas) and Pythonidae (Pythons). Pit vipers have two pits above their nostrils directed forward (facial pits), whilst several pits lining the upper jaw (labial pits) is most common in boas and pythons (fig. 3)[8].

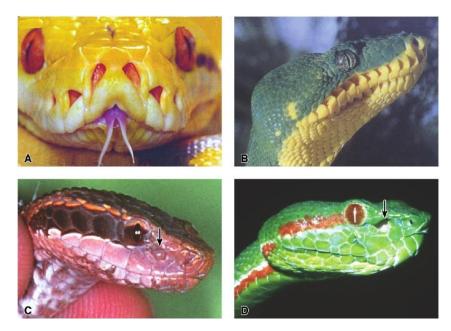


FIGURE 3: Four snake species with two kinds of pit organs. A) Python molurus, albino (Pythonidae). B) Corallus caninus (Boidae). C) Gloydius blomhoffii (Crotalinae, terrestrial).
D) Trimeresurus stejnegeri (Crotalinae, arboreal). A) and B) show labial pits, whereas C) and D) show facial pits. [9]

react [10].

The facial pit organs are more effective in infra-red radiation detection than the labial pits. A membrane densely packed with infra-red sensitive terminal nerve masses is located separately from remaining tissue, surrounded by insulating layers of air. This feature is lacking in labial pits, which makes the amount of heat conducted from other tissues higher in this type of pit. That reduces the accuracy of the heat sense, since it has been found that the infra-red radiation most probably heats the surface of the membrane making it possible for thermoreceptors to

Labial pits also are shallower and not so densely innervated as facial. (For review, see [8]) The depth of facial pits has an important function in forming a pattern of the infra-red input. Radiation from a certain angle, can only hit the sensitive membrane in each pit at a certain position. As in simpler eyes (pin hole design), the facial pit organs have openings narrower than the membrane surface below. When the target (or snake) move, the area hit by the radiation is shifted. This makes it possible to map the direction and the outline of the stimulus [9].

When the heat sense is active, a mental map of the environment is created, such as in vision. This aids the snake in hunting, making it possible to detect prey 60 cm away (maximum distance for pit vipers, shorter distances apply to other species). Finding the best spot to bite is also aided by the heat sense, since infra-red sensitive receptors are present also inside the mouth in pit vipers [8].

An "inactive" heat sense still has a basal rate neural firing frequency, that can be shifted up when warm stimuli are present and down when cold stimuli are present. Recording things colder than the environment is thought to be useful when hunting lizards and other ectothermic prey [9]. Python pit organs are however most sensitive in the wavelength spectrum between 8 and $12 \ \mu m$, which corresponds to the radiation emitted by mammals [11].

Leaving the snakes, the one mammal currently known to posses a heat sense is the common vampire bat (*Desmodus rotundus*). They also have pit organs facing forwards located in their nose region, which enables them to direct their bites to areas with blood vessels lying close to the prey skin surface [8]. The pit organs are thought to work much in the same way as described for snakes above. A characteristic of the vampire bats' heat sense, that is of high relevance to our dog research, is that dense connective tissue insulate the pit organ region making it 9 °C colder than surrounding tissue in the face [12]. An unpublished observation in Campbell 2002, [8] should also be noted, claiming a similar temperature difference present in the nasal region of the ball python (*Python regius*).

Other known examples of heat senses in nature are confined to the insect class. The beetle *Melanophila acuminata* seeks forest fires to lay its eggs in the burned trees. They respond to infra-red radiation of a shorter wavelength than snakes, 2-4 μ m, which corresponds to the radiation coming from forest fires. Their pit organs are positioned to the legs, and the bottom of the pits are covered with sensilla. The transduction mechanism is thought to be that a hollow chamber in the sensilla expands in response to infra-red radiation, and this in turn deflects a structure connected to sensory nerves [8].

Several different species of butterflies also have a heat sense to protect their wings from damage from the sun [8]. This is however not used for detecting infra-red radiation in order to gather information about shape, size and direction of the source emitting the radiation. The mechanisms responsible for this kind of heat detection are therefore omitted.

Physics

For a heat sense to function, there must be a stimulus. Heat sense do not refer to sensitivity to conducted heat, which we often refer to as "heat". Conducted heat is vibrations in a material spread to another material when in contact. In a heat sense, the absorption of photons from the infra-red spectrum (approximately 0.75 to 1000 μ m [13]) is relevant.

Human perception of temperature arises from photons hitting our skin and transferring their energy, thereby increasing the skin temperature. This temperature (or more often, change in temperature) is registered by thermo-/coldreceptors whose neural discharge is determined by temperature. The temperature input is then related to core body temperature to create our perception of warm and cold [14]. In snakes it has been found that the transduction mechanism probably is the same, and not an ability to detect infra-red radiation directly, as in the phototransduction in the eye for visible light [10]. A crucial difference between snake and human sensitivity is that human receptors lie embedded in the layers of the skin, while snakes' receptors are exposed to the outside, only covered by a thin membrane [8].

All materials with a temperature above absolute zero emit infra-red radiation. (The information stated in the following paragraphs are to be found in FLIR Handbook [15]) A material that absorbs all incoming radiation in all wavelengths (that is, nothing passes through and nothing is reflected) and emits the same amount, is called a black body. The amount of emitted radiation in each wavelength is determined by temperature. The higher the temperature, the more of the radiation emitted have shorter wavelengths.

A perfect black body has an emittance of 1. Emittance is a measure of the total amount radiation absorbed by an item set in proportion to the total amount radiation absorbed by a perfect black body. Emissivity is the same thing as emittance, but a generalised property of a material. Perfect black bodies are not common in reality, most things have an emittance somewhere between 1 and 0. It should be noted that emittance does not tell the distribution of emitted radiation over the wavelength spectrum, except that it always is confined to the spectrum covered by the perfect black body.

These are the general terms needed to roughly explain the functioning of a heat camera. The lenses are made of a material transparent to infra-red radiation, that also is able to refract it in order to form a picture. Inside the heat camera are a number of sensors, sensitive to a limited part of the wavelength spectrum. A metal or semiconductive material are hit by photons and through a link to a larger reference mass, the difference between the two components can be measured every time a photon hits. The number of hits are then counted during the time the shutter is open. A high photon count correlates with high intensity of radiation, that is high How the corresponding mechanism would look like in dogs is unknown. Sure is that the dog nose has an emissivity almost equal to one in the far infra-red spectrum, which is not the case in the visual spectrum (unpublished observations, 2014 [4]). The question is then: Why absorb all incoming infra-red radiation when your nose is cold, if not to asses the difference?

Application area

A heat sense could be useful in several aspects of a carnivoran's life. It could be used when puppies try to find their mother's teats and their other senses are undeveloped. The belly area is warmer because of hair loss, and big blood vessels lying close to the skin surface. For adults a heat sense could ease the detection and tracing of prey when wind direction is unfortunate. More likely is though, that it would have its application in surveillance. If a carnivorian is out hunting, it can move around the targeted prey and catch its scent. However, in order to detect threats against the territory or young in terms of predators or con-specifics, the threat chooses the angle of attack, and it has to be detected irrespective of wind direction. This study has been planned with this latter application in mind.

Questions and hypothesises

The aim of the study is to answer the following questions:

-Can we trigger dogs to use their supposed heat sense in experimental set-ups?

-Are they able to separate warm and cold items, from long or short distances?

-What temperature span is relevant for a carnivorian? What is the surface temperatures of animals they would need to detect?

-How does the surface temperature vary with ambient temperature?

-Does the temperature vary in the same way for bigger and smaller animals? It should be more important to a small animal to keep a low surface temperature, since they have a big area in relation to their volume. A high surface temperature would mean a big heat loss, an energy expenditure hard to compensate for a small animal.

Method

Riding hall testing

General explanation

In these test settings the aim was to determine whether dogs can distinguish warm from cold items at a distance of 10 meters. In the first task the items consisted of warm and cold water cans placed in boxes, and in the other task a person was hidden behind a screen.

Participants

7 dogs of different breeds (1 golden retriever, 1 Rottweiler and 5 Labradors), sex (3 females and 4 males) and age (spanning between 4 months- 8 years) were used. All of the Labradors had an ongoing hunting training, the other dogs had a training relevant for an every-day purpose.

Environment and time

The testing was localised to an unheated riding hall, size approximately $20m \times 40m$ with a footing consisting of wood chips. Training occurred once a week from beginning of February until end of April, generating a data set of approximately 60-70 runs per dog and a total of 409 runs.

Can and person task set-up

All dogs except the Rottweiler and one Labrador were originally trained on a set up hereafter called "can task". The Rottweiler and the Labrador were trained on a task here after called "person task". In both tasks a starting point was indicated 10 m from the targets. Each run, one target was cold and the other warm, varying between left and right side. Stimulus side was regulated by following a pseudo-random list consisting of 6 or 8 choices allowing no more than 2 consecutive repetitions of the same side, with an equal total number of left and right positions.

A t-shaped fence split the lane from 4.3 m on towards the targets and blocked the dogs from easily running behind the set-up. Two fans were positioned just behind the starting point in line with the targets, creating a wind speed of 1m/s at the targets. This reassured that no olfactory directional information from the targets could be used by the dogs. A screen opaque to both visual and infra-red radiation was put up before the starting point. This served as a waiting place for the dog and handler while the stimulus side was changed in the set-up. The role of dog handler, test leader and camera man/observer alternated between the persons present. For full overview of the set-up, see fig. 4.

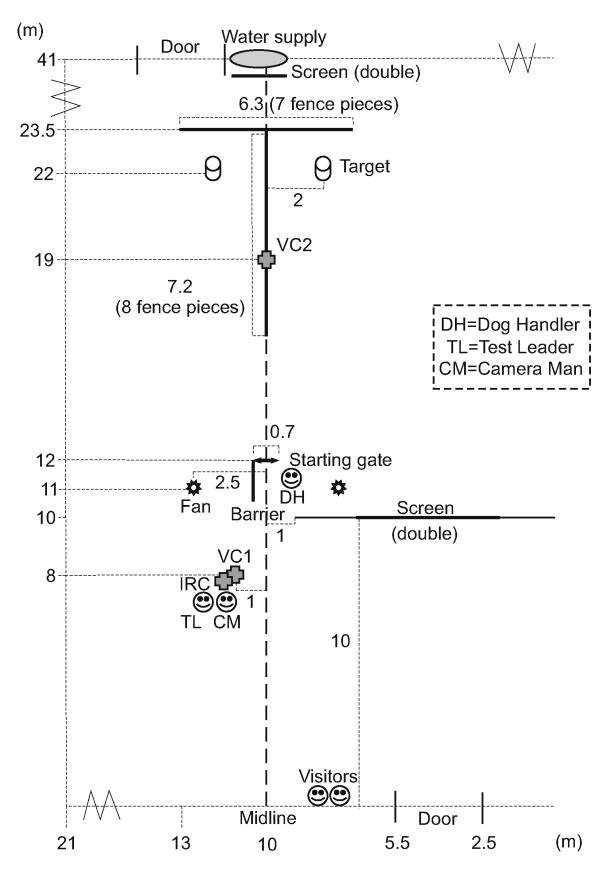


FIGURE 4: Top view of riding hall set up. VC1 and VC2 are denotations for the video cameras used for documentation. IRC shows the position of the infra-red camera.

Can and person task procedure

From the waiting position behind the screen, the dog and its handler walked up to the indicated starting point after a signal from the test leader. On the starting point the dog, sitting or standing, decided whether to run left or right with no signals available from the handler, who did not know which side was warm. If the dog did not leave the starting point on its own initiative, the handler urged it to run off towards the targets.

A reward was always provided only at the warm side. If the dog chose the wrong side, two options were available; either to call the dog back for another try, or to let it run back and around the fence to fetch the reward at the correct side. Both alternatives were used during the experiment period, though in varying degree to match the behaviour of each dog. After a run, the dog was called back and led behind the waiting screen. During that time the stimulus side was/was not changed according to the random list. When no change in stimulus side was addressed in the can task, the test leader went up to the targets and made noises as if they were turned, to exclude the possibility of solving the task by hearing.

Each training session consisted of 8 or 10 runs, where the 2 first were defined as test runs where the handler new which side was correct. This gave the handler the possibility of directing his/her dog to the correct side. The test runs were not included in the registered result. From beginning of March an upper limit for nose temperature was introduced. When a dog before or during a session had a nose temperature 5 °C above expected temperature in relation to ambient, according to a temperature curve elaborated in prior work (fig. 2), the dog was either to take a break to lower its nose temperature or the session was ended incomplete. This was however not applied coherently due to practical reasons.

Targets in can task

In the can task, the targets consisted of 20 l plastic black cans filled with warm water, giving the cans a temperature of approximately 28 °C. These were placed in separate boxes hidden behind screens blocking all visual information, still letting infra-red radiation through. Until end of March the screens consisted of thin white tarpaulin, which reduced the heat signal coming through with approximately 5 °C. This was replaced by white single layer silage plastic, reducing the heat signal with approximately 1 °C. The boxes were insulated on all sides except one, making it possible to alternate warm and cold stimulus by turning the boxes behind the screens. The resulting warm area was 30*30 cm. Reward consisted of treats or a retriever dummy that was located just in front of the screens behind a plank, invisible to the dogs.

Targets in person task

In the person task the stimulus was a sitting person behind white plastic screen, nothing was hidden behind the other. As with the smaller screens, the screen material was changed from tarpaulin to silage plastic as described above. The target person was strictly informed to stay as silent as possible during the trials. When finding the person, the dog was rewarded with tug of war play or treats.

Processing of data

Each trial generated either a correct or a wrong choice. The percentage of correctness was calculated for each dog, for each session. If a dog reached a result of 80% or more, three times in a row, its behaviour was recorded as stable. It had thus reached our learning criterion. 80% is a commonly used limit in animal training, based on the hands-on experience of animal trainers [16]. The learning criterion was prioritised compared to other measures of performance quality.

The following statistic procedures was used in processing the data. All tests were executed in SPSS (23), and the calculations needed for Fishers method were made in Microsoft Excel. A confidence interval of 95 % was applied to all procedures and chance level refers to a probability of 0.5.

-Binomial tests were performed for each dog separately, to reveal whether their performance differed significantly from chance level or not. The data generated from the runs are dichotomous and a constant probability of correct choice in each run is assumed. That means that the number of correct choices will be binomially distributed for a specific number of runs [17].

-Fishers method (Eq. 1) was used to combine the p-values from the binomial tests, thereby assessing whether the dogs as a group performed better than chance level. By using this method, a combining of p-values could be made without violating the requirement of independent measures [18, pp.795-796]. This had not been the case if a binomial test had been conducted directly on the full data set, since the same dogs would appear several times, i.e. dependent measures.

$$X_{2k}^2 = -2\sum_{i=1}^k \ln(p_i) \text{ where } X_{2k}^2 \sim \chi_{2k}^2$$
(1)

-Logistic regressions were made for each dog separately in order to determine whether the dogs' performances over the testing period improved or impaired. Logistic regressions were also made to assess whether the order of runs in the series had an impact on their performance. The regression performed had to be logistic due to the dichotomous nature of the data, as described above [19].

-The method of generalized estimating equations was used to acquire the same information as the logistic regressions, but for the group as a whole. By using this method, the repeated measures of dog individuals' performance in series on different occasions were accounted for [20].

-Generalized linear model with the category "Dog individual" as fixed factor was used to compare the dogs to each other, to find out whether there was a difference in their respective performance trend over the testing period. This model was also used to assess whether the dogs' performances were affected by the order of runs to different extents [21].

Indication training

From mid April the training was changed for most dogs, one was kept on the original can task. The new goal was to stepwise shape the dogs' behaviour into having them independently indicate a warm object amongst cold ones.

In the first training step the distance from the starting point to the can targets was shortened to five meters. A remote controlled treat dispenser (Premier, Treat & Train remote dog training system) was placed about one meter in front of the starting point. No other reward was present. On signal from the test leader, the dog and its handler walked up to the starting point where the dog was seated. Whenever the dog turned its head towards the warm can, the test leader pressed the remote control; a beep was heard and a treat was dispensed. The dog either responded to the beep, stood up and collected its reward, or it awaited permission before it went. It was then seated at the starting point once again and the procedure was repeated until the dog deliberately turned its attention to the warm object. The handler and the dog went behind the waiting screen whilst stimulus side was shifted. The training continued this way until the dog started to look distracted. Over time, the number of repetitions on each side before shifting stimulus side became fewer, starting on 5-6 repetitions in the beginning to end up following a random list (created under same conditions as described above).

In the second step treat dispensers were placed just in front of the cans. When the dog indicated the warm can the test leader pressed the remote control and the dog was allowed to collect its reward by running the five meters to the treat dispenser. Increasing indication time was required for the dog to get its reward. By this point the experimental period was coming to an end, thus suggestions on further development of this training method is given in the discussion section. Since this is a training method and the dogs have not been put to any tests since the training was introduced, no evaluation of potential improvement in performance have been made.

Training room experiments

General explanation

In this task the dog should distinguish hot from cold items at a distance of 60 cm by pressing pedals on a machine. In contrast to the riding hall set-ups, this set-up aimed for short range decisions under controlled circumstances.

Participants

One male Bernese mountain dog, 4 years of age, participated. He has former experimental experience of heat sense training and has performed well in similar tasks (Gläser, personal communication). The set-up used was familiar to him, but a correct response behaviour in this particular task had not been established in former training.

Environment and time

All training and testing took place in two small rooms. In the first room, the temperature was set to 18 °C and the area was divided into two compartments connected through a door. In the second room the temperature was 20 °C and no sectioning of the area had been made. Training occurred once a week from mid March until the end of May.

Procedure and apparatus

For this training, two different apparatuses have been used, however both with similar functionality.

The automatic apparatus was a wooden box (1 m^3) with two circular openings (diameter 12 cm) in the front wall (fig. 5). It contained a heat element set to 35 °C (Bartscher[®] Warming tray, 60*20 cm, 800 W) painted with black, water based paint, a white polystyrene plate (4 cm thick) as insulation and a preprogrammed rotation mechanism connected to the polystyrene plate. Two pedals were placed 30 cm apart on the lower edge of the machine. Connected to the pedals was the remote control of a treat machine (Premier, Treat & Train remote dog training system); pressing the right pedal automatically generated a beep and a reward, whereas pressing the wrong pedal only generated a buzz.

The polystyrene plate used as insulation was placed between the heat element and the two openings in the front wall. In contrast to the front wall, only one 12 cm opening had been cut in the polystyrene plate. This allowed heat radiation to come through on only one side, given that the opening in the polystyrene plate was aligned with one of the openings in the front wall.

In order to eliminate visual information, the two openings in the front wall was covered with black plastics opaque to infra-red radiation. To completely block all residual heat signal between every trial, the plastic-covered openings were covered by a wooden shutter plate that was hoisted up and down automatically.

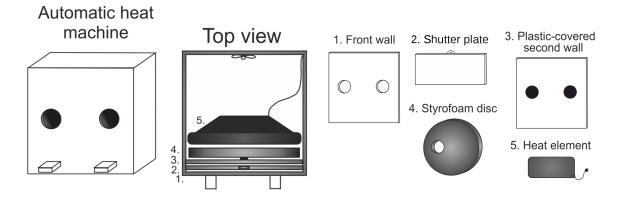


FIGURE 5: Frontal view of the automatic heat machine, top view and labelled components.

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The rotation mechanism controlled the position of the polystyrene plate opening, to either align it with the left or the right wall opening. The rotation schedule was pseudo-randomised; 30 trials with equal number of lefts and rights, balanced so that each half of the list contained as many lefts and rights as the other. No more than three consecutive lefts or rights were allowed, to reduce the risk of having dogs developing a side preference whilst still in training.

After 3 weeks of training, the materials in the machine were exchanged for the same ones used in the riding hall experiments for consistency. The heat element was replaced by two 20 l black, plastic water cans and the black plastic covering the front openings was replaced by two layers of silage plastic. The polystyrene disc was painted black to reduce visual contrast between the disc and the water cans behind.

A starting point was marked on the floor with two boxes and tape markings, 1.2 m from the machine. The dog was asked to lie down between these markings when starting every trial. A lying position was to prefer due to the dog's preference of not sitting straight. A skewed sitting could result in a perception angle towards the heat machine, a possible source of side preference. When the dog had reached its starting position, the test leader pressed a remote control to hoist the shutter plate up. When the dog seemed to have taken the options in, the test leader encouraged it to go forwards to the machine and press a pedal. A choice must have been made within 10 seconds, or the plate was automatically hoisted down again, and the trial was registered as "no choice".

Depending on whether the trials were testing or training, the test leader gave different cues and commands. In testing trials the only signals given in starting position were the command "warm" and if the dog did not respond, also repetitions of the command in an encouraging way, in combination with a light push. However, in this stage of testing, the test leader always new which side was warm, thus unintended signals cannot be ruled out. In training trials additional guidance was given to a varying extent, from leading the dog to the correct side, turn its attention to the warm hole and encourage it to press the pedal, to small gestured pointing.

When the correct pedal was chosen, reward consisted of vocal praise from test leader and treats falling from a treat machine placed on top of the heat machine. When the wrong pedal was chosen, no treat was delivered and the dog was called back for another try.

Irrespective of training or testing trials, the session was interspersed with other small tasks unrelated to heat, to keep the dog from getting bored. The session was ended when the dog either refused to do any more trials or when a number of thirty trials was reached. Regardless of situation, a last additional trial was always made into a correct choice in order to finish the session off in a positive way.

Changes in set-up

After 5 weeks of training, the set-up was changed into the one the dog was previously trained on, since no progress was seen and the dog had developed unwanted pattern strategies. This machine was manual, stimulus side was shifted by the test leader. Two identical 900 ml plastic bottles, one filled with warm (45 °C) water and the other one with cold (20 °C) water served as stimuli. The bottles were placed in polystyrene boxes, cut open in front and above. These were put on an elevated board, with a wooden wall behind and a dividing wall in between. Attached to the bottom board were two pedals 40 cm apart. Sticking out from the machine was an extension of the dividing wall, detachable and available in different lengths. For these trials a 60 cm long extension wall was used.

Stimulus side was regulated according to the same prerequisites as described in the former setup. Reward was distributed by having treats hidden behind the correct pedal, falling out when the pedal was pressed. The starting point was at the back compartment of the room, 2 m from the machine. A fan directed forwards made sure no directional olfactory information reached the dog. Procedure corresponded to the one used with the automatic machine, apart from here, the dog was waiting behind a door that had to be opened by the test leader before getting the command "warm".

Processing of data

The data processing procedure applies to trials done on both machine types. For each testing session, percentage of correct choices was calculated. Sessions where the number of trials did not reach 30 was also included in the data set. A learning criterion of performing 80% three times in a row, applied also to this task. The data set is too small and fractioned for a statistical analysis to be meaningful.

Mammal surface temperature

General explanation

The shoulder region of differently sized animals were photographed with an infra-red camera. The photos were analysed to extract the surface temperature of each animal. This was done in different ambient temperatures in order to obtain a relationship between a span of ambient temperatures and the corresponding surface temperatures.

Equipment, participants, environment and time

All photos were shot with an infra-red camera FLIR T640 (FLIR Systems, Wilsonville, Oregon, USA). Choice of animals was restrained to those who could be seen as a potential threat or prey to a carnivoran, and furthermore also were available at Skånes Djurpark [22] (list of animals, see table 2). If a species present in the animal park still could not be spotted a blank was noted. Data were collected on three occasions in February and March, thereafter the study was ended. On the first two occasions the photos were taken during midmorning to somewhat past lunch time and on the third occasion they were taken during and after dusk.

Procedure

Photos were not taken in direct sun light, instead periods of overcast was awaited. Included in all pictures was the shoulder area of the animal, primarily chosen due to accessibility. It was visible regardless of the animal's position, if it was sitting, lying or standing. Shortest possible distance to the animal was always aimed for, but it varied considerably between species and between the occasions, from 1 m to more than 30 m. A correction for distance was made when analysing the photos, as well as an approximate measure of the relative air humidity (data for Hörby from SMHI records [23]).

Pictures were analysed in FLIR+ software by making circular measurements over an approximated shoulder area. Only pictures of good quality with shoulder area visible were analysed. Temperatures from the same species were combined into a mean value for each occasion. Ambient temperature was measured with a thermometer several times during each photo session. The data were to be compiled into graphs for each species covering a temperature range between 0-20 $^{\circ}$ C, and also be analysed for a potential temperature difference between big and small animals. Since the study was ended prematurely, due to major methodological problems (see Discussion), no such further analysing was made.

Results

In this section the results from riding hall testing, training room testing and mammal surface temperature will be presented. The two tasks in the riding hall are merged both in calculation and presentation, since they test essentially the same thing.

Riding hall testing

The outcome of the binomial tests showed that no dog chose the warm stimulus more often than the cold (see table 1 under binomial test). Fisher's method showed that this was true also when calculations were made on the whole data set (see table 1 under Fisher's method). The logistic regressions and the method of generalised estimating equations, showed that the dogs did not have a trend in their performance over the testing period, that is, no increase or decrease in correct choices over time. This is the case on the whole sample of dogs (fig. 6a), as well as when considering each dog individually (fig. 6b). (See table 1 under Logistic regression: "Series number" for individual values and Generalized estimating equations: "Series number" for the whole data set.) No dog thus reached the learning criterion of performing 80% correct on three consecutive training sessions.

One concern during testing was that the dogs might get worn down during the session and therefore would perform worse towards the end of it. The logistic regressions and the method of generalised estimating equations showed that no dog was significantly affected by the order of runs (fig. 6d), neither was the group as a whole (see fig. 6c). (See table 1 under Logistic regression: "Number in series" for individual statistical values and Generalized estimating equations, "Number in series" for the whole data set).

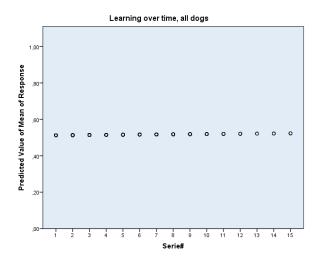
Generalized linear model showed that there was also no difference between the dogs in performance over time (fig. 6b), nor did they differ in how much they were affected by run order in the series (fig. 6d). (See also table 1 under Generalized linear model: "Series number" and "Number in series".) No interaction between "Dog individual" and "Series number"/"Number in Series" was present in the generalized linear model, meaning that the dogs' runs were equally distributed over the series and in run order (see table 1 under Generalized linear model, "Dog"*"Series number" and "Dog"*"Number in series"). The results are thus computed without including interaction in the model.

Qualitative observations state that some dogs were prone to use pattern strategies when deciding which side to choose. Unilateral patterns were common, as well as alternation patterns of every two. The possible use of additional patterns are not ruled out, but are harder to detect. TABLE 1: Compiled statistical output for all seven dogs and their joint performance. Percentage correct is calculated for all runs performed by a dog. The values lie close to 50% which is chance level. This is confirmed by the binomial tests and Fisher's method where no p-value is significant, meaning that the null-hypothesis of no difference between performance and chance level is retained.

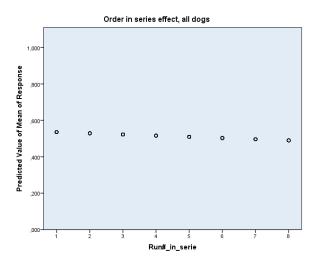
Logistic regression for series number show that no dog had trend in performance over the series, since B-values are close to zero and P-values are insignificant. The generalized estimating equation method gives the same information, but for the dogs' merged performance. The logistic regression made for run number in series indicates that the a dogs' performances are independent of whether a run is in the beginning or in the end of a series, since B-values are close to zero and P-values are insignificant.

Possible differences between the dogs' trends over time and their susceptibility to run order effect are presented under generalized linear model, but no such differences are present. There was also no interaction between the variables "Dog" and "Series number" or between "Dog" and "Run number in series", meaning that the individual dogs' runs were equally distributed over all series and over the runs within the series.

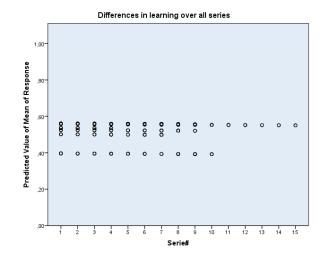
	N	N	Description	Dimensiol to at	Logistic regression		
Test object			Percentage correct (%)	Binomial test (P-value)	Series number (B/P-value)	Run number in series (B/P-value)	
1	74	15	55,4	0,416	-0,038 0,549	-0,026 0,825	
2	44	7	50,0	1,000	0,021 0,884	0,010 0,943	
3	28	4	53,6	0,85	-0,004 0,990	0,267 0,167	
4	68	9	55,9	0,396	0,192 0,065	-0,214 0,063	
5	65	9	52,3	0,804	0,000 0,996	0,015 0,892	
6	71	10	39,4	0,097	-0,092 0,302	-0,054 0,623	
7	59	8	55,9	0,435	0,001 0,994	0,046 0,704	
	Fisher's method Generalized estimating equations					estimating equations	
				(P-value)	Series number Run number in series (B/P-value) (B/P-value)		
All	409	62	51,6	0,709	0,003 0,898	-0,026 0,492	
	Generalized linear model						
	Series number Run number in se (B/P-value) (B/P-value)						
					-0,002 0,958	-0,023 0,615	
Individual differences			(Dog*Series number)	(Dog*Run number in series)			
					P=0,539	P=0,434	



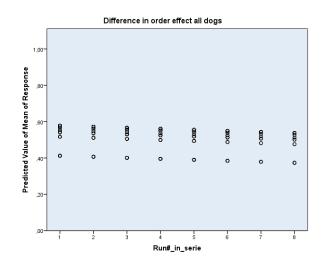
(A) The probability of making the right choice lies close to 50% over all series for all dogs as a group, with no performance trend visible.



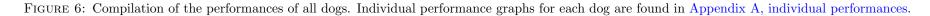
(C) For the group as a whole, no impact of run order in the series can be observed.



(B) Performance trends for all individual dogs. No dog's performance departs significantly from the others, meaning that no dog experienced a change in performance over the series.



(D) Effect of run order for all individual dogs. No dog's effect departs significantly from the others, meaning that it did not matter whether a run was in the beginning or in the end of a series for any of the dogs.



Training room testing

14 out of 26 correct (54%) in the first session with the automatic machine was used as a reference measure. Thereafter all testing sessions were interspersed by trials where the dog was guided to the correct choice, since it had a strong tendency to develop a pattern dependent solving strategy. Both obligate uni-lateral choices and alternation on every second choice were observed. No difference in behaviour or performance was noticed when the materials were changed from heat element and black plastics to plastic water cans and silage plastics.

When the set up was changed to the task the dog was priorly familiar with, the results are slightly more spread. Results span from 27% to 73% correct, but with most sessions close to 50%. Subjective observation report seemingly more confidence in choices in high result sessions, but also that the dog in general repeatedly seemed tired or unmotivated to do the task during work.

Mammal surface temperature

Roe Deer (Capreolus capreolus)

Wild Boar (Sus scrofa)

The following section presents the surface temperature data collected from Skånes Djurpark. All referred measurements are available in table 2. Mean temperature measurements of surface temperature from all animals reach from 6.7 °C to 31.9 °C. These extreme values both come from the same species, the muskox. The corresponding not averaged extreme values are 6.2 °C to remarkable 57 °C. When comparing the two occasions with equivalent ambient temperatures, substantial variance can be seen. The European bison, Przewalski's horse and the roe deer differ approximately 4 °C between the measurements, while the muskox differ with almost 25 °C. As a rough estimation, the data suggest an approximate surface temperature of somewhat above 10 °C for a mammal in an ambient temperature of 5 °C and a surface temperature of somewhat below 10 °C in ambient temperature of -1 °C. This is however not to regard as a difference due to the small sample size and the high variation in the data set.

Date:	2015-02-05	2015-03-04	2015-03-18
Ambient temp.:	-1 °C	5°C	5°C
Arctic Fox (Vulpes lagopus)			8,25
Aurochs (Bos primigenius)	11,30	15,69	14,27
Brown Bear (<i>Ursus arctos)</i>			11,40
Eurasian Elk (<i>Alces alces</i>)	10,58	19,68	
European Bison (<i>Bison bonasus</i>)	8,67	14,85	10,52
European Wild Cat (<i>Felis silvestris silvestris</i>)			11,34
Fallow Deer (<i>Dama dama</i>)	12,25		11,50
Red Fox (Vulpes vulpes)		10,87	10,45
Muskox (Ovibus moschatus)	6,70	31,90	7,50
Przewalski's horse (<i>Equus ferus przewalskii</i>)	10,23	9,00	13,53
Rabbit, domestic (Oryctolagus cuniculus)		7,00	
Rabbit, wild (Oryctolagus cuniculus)			8,60
Raccoon (<i>Procyon lotor</i>)			11,45
Red Deer (Cervus elaphus)	9,33	11,30	
Reindeer (Rangifer tarandus)	10,80		

7,80

11,17

14,68

17,45

10,18

TABLE 2: Mammal surface temperature from three measuring occasions.

Discussion

This section will firstly contain a brief review of all results. Then the surface temperature of mammals will be discussed in greater detail, with suggestions on methodological improvements included. Thereafter training room testing and riding hall testing will be covered together, with main emphasis on possible factors influencing the outcome and suggestions on how to handle these in the future. A concluding section will summarize the remaining questions future studies hopefully will be able to answer.

In the data collected for this thesis, no support for the hypothesis of a heat sense present in the rhinaria of dogs was found. Choice tasks in the riding hall setting generated performances from all dogs, individually and together, at chance level. A positive learning curve was expected, but no dog experienced a trend in its performance over the testing period. No order effect was detected, that is, it did not matter whether a run was in the beginning or in the end of a series. In the training room short distance setting, the results are corresponding. Some sessions have a correct percentage above 50%, but overall, the dog's choices were random.

The temperatures measured from mammals at Skånes Djurpark, show that surface temperature lies around 10 °C both in -1 °C ambient and 5 °C ambient temperature. However, it should be noted that on the two measuring occasions with equivalent ambient temperature, the surface temperature for several animals differ with 4 °C, which must be regarded a considerable difference. Extreme surface temperatures of 57 °C was measured, which itself indicates issues with the method.

Mammal surface temperature, issues and suggestions

The study was interrupted after only three measuring occasions. In the second batch of photos it became obvious that the sun, even though not clearly shining, left residual heat on all objects. Some of the animals' enclosures were in complete shade when the photos were taken on the second occasion, see for example the fox in table 2, hence there is no difference in temperature between the two measurings in 5 °C ambient. This suggests that photos only can be taken in thick overcast, or after sun down, to give comparable temperatures.

Photos on the third occasion were taken after/during nightfall. These temperatures are thus more likely to be representative, but, the approach is associated with yet other difficulties. Park animals are not used to visitors walking around in the park after dark. When someone tries to photograph them, they become vigilant and easily scared. Photos are thus taken at a further distance, sometimes not at all, if a herd decides to flee. A long distance reduces the accuracy of the temperatures measured. The second problem is that animals go to sleep and many of the animals listed have shelters or dens to resort to, which effectively prevents photographing. An issue not related to the animals is the ambient temperature. Temperature during night is low most of the year. According to SMHI [24] data, the average minimum temperatures per month spans from -4 °C in January and February to 11 °C in July and August. That is a rather short span and even though fluctuations from the mean could be substantial, using this method for a larger study requires a certainty of which situations a possible heat sense is used for. Surveillance of young or territory is of great importance during early summer on day time. If a supposable heat sense does indeed have such a function, the relevant ambient temperatures will be higher than 11 °C.

Irrespective of whether photos are taken day or night time, several factors are hard to control. Body, and consequently surface temperature, is dependent on the animal's activity level. Physical activity can be observed first when the researcher arrives at the enclosure, what happened the minutes before is not known. Internal factors such as circadian or estrous rhythms also effect body temperature [25] although to a limited extent. In a study aiming for approximate results these factors can be overlooked, but in elaborate work several replicates of measures would be recommended to neutralise a potential effect.

The method of thermography has several advantages. It is non-invasive and used from a distance, which cause minimal disturbance and stress for the animals, and it is also cheap and easily utilised. A solution to the issues discussed above would be to initiate a long-term study, and to only take photographs during overcast. Since several replicates are needed, and the temperature varies notably even within the park, a continuous temperature scale might be preferable to the discrete steps used for this study. Joining the zoo workers when feeding the animals would help in getting access to animals reluctant to pose.

Heat sense

Although these data do not contain any indication of a heat sense, the matter should not be seen as settled. Our method chosen has shown to be insufficient or even inadequate for getting dogs to use a heat sense, which opens up the possibility for future studies using a better approach.

Training intensity

One major issue, which applies to riding hall as well as to training room testing, is training intensity. Each dog has performed about 60-70 runs over the whole testing period. This is to be compared with a corresponding experiment done with vampire bats [12], where 1200-1400 trials during a total period of two to three months were needed to reach a stable behaviour of 75% respectively 85% correct choices in two individuals. The numbers seem high when regarding that vampire bats do not have the same mental capacity for learning tasks as dogs do, but they might still be an indication of the magnitude of trials needed.

Early training

The vampire bats have a documented functional heat sense [12] with a clear function in finding big blood vessels close to the prey skin surface. Since no such documentation is available for dogs, the approach to test a heat sense with the presumption that it is functional in adult dogs, might be misleading.

Puppies are able to cool their noses rapidly, which might have a function in finding their mother's teats (see Introduction). Synaptic pruning (described in [26]) is as most intensive during early life, and means that neural connections not being used are deleted. If a weaned puppy does not use its presumable heat sense any longer, neural connections supporting it could be weakened substantially. The human world our dogs are brought into is full of irrelevant sources of heat: radiators, computers and so on, meaning that even if dogs indeed have a heat sense, they are from young age taught that heat sources are of no relevance to them. Training on warm objects when a dog is young might counteract such an effect.

Reduce complexity

The set-ups used in our different settings are complex to a dog. In the riding hall, the task is not only to choose the warm side. The dog should also: walk beside its handler to the starting point, sit or stand still at the starting point, run off towards a target, fetch an item (for most dogs), bring it back and give it to its handler. Although the dogs are already well familiarised with most of the components, this increases the level of difficulty. If the heat sense functioning is heavily impaired, or dormant, the task should be made as easy as possible.

In order to shape a dogs behaviour, the main thing is to make sure that the dog understands what the task is. That is most often, in modern animal training, accomplished through positive reinforcement. To force a dog to perform a task, or punishing it for not to, is not only morally outrageous, it has also been proven inefficient from a learning point of view [27, pp 72-75, 83-89]. The second thing is to reward the right behaviour, at the right moment. If a dog performs the correct response, but the reward is delayed and the dog does something else when the reward comes, it could misinterpret our intentions. It would learn the task eventually, but a correctly timed reinforcement gives less room for confusion. The dogs often turned their gaze and attention to their handler (which did not respond), a behaviour interpreted as asking for help when fronted with tasks they themselves are unable to solve [28]. Different patterns were observed to be used by the dogs in order to solve the task. The prevalence of such behaviour might also be reduced if the task is made easy enough.

To improve the timing of reward was the main reason to why the indication training was introduced. In learning terms it is an operational conditioning of the warm stimulus, having the beep from the treat machine as a secondary (conditioned) reinforcer, and the treats as a primary reinforcer. To make it even clearer for the dog what properties of the target are important (is it the temperature, or is the task maybe to find the target with a small scratch?) an optimal solution would be a reward appearing directly "from" the heat source when the dog interacts with it, but a practical solution to that has not yet been found.

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To continue the line of suggestions on simplification of the set-up, the environment must also be considered. Before establishing a stable behaviour and a successful training method, an environment with few disturbances insisting on the dog's attention is preferable. The training rooms used in the machine tasks are such environments. Therefore the establishment of the behaviour to choose the warm side in a choice task is suggested to start there. First when a stable behaviour is seen (80 % right) generalizations to other environments, such as riding halls, are recommended.

Motivation

Sometimes the motivation to perform the task wanted lacked. This was most obviously seen in the training room setting, but also in some of the dogs performing the riding hall tasks. There is no straightforward explanation for this, but possible reasons could be: The dog gives up, it can not solve the task, why bother trying?; The reward is not good enough or; The dog finds the task boring. Giving up when faced with an unsolvable task could be managed through simplifications of the task as discussed above. If the dog is not motivated by the reward offered, different kinds of reward should be tried out. When no reward tested is good enough to motivate the dog in question to work, that particular dog might not be the one best suited to participate in the experiments. To find the task boring could be a combination of the two previous reasons, but to have flexibility and different tasks in the training routine could prevent the dog from tiring. Two choice tasks could be alternated with a task walking around in the room rewarding interaction with the warm stimulus whilst ignoring interaction with the cold and playful switch training (switching the warm and cold item(s) in front of the dog, rewarding warm choices).

It should be heavily stressed that these suggestions apply for training, not for testing, since smell for example is not controlled for. A warm plastic can smells more and possibly different from a cold one, which enables the dog to use its sense of olfaction to solve the task. This does not have to be an issue in training though, since a supposable heat sense would work in combination with other senses and not on its own.

Expectancy on work situation

A majority of the participating dogs are working dogs, which means that they come from lines bread on mental characteristics suitable for a certain task. These dogs are hunting Labrador retrievers; they have a high energy level, are capable of concentrating on a task for long periods of time and they like to retrieve items.

One of the assumed prerequisites for a heat sense is the cold nose. As stated in the Introduction section, internal factors as stress level affects the nose temperature. When these dogs are excited to start working, expecting a retrieving task, they are experiencing a positive stress, ergo, their nose temperatures are high. This could make the choice task impossible to solve, if the assumption of a cold nose is correct. Regardless of the dogs' training background it has to be made clear for them that heat training is something set apart from what they usually do.

They should not hunt, they should not track and it is no obedience training. How to create the right mindset for heat training, is a puzzle to solve for each individual dog.

The complexity of nose temperature

Since nose temperature is a rather new research field, much basic data are lacking. The knowledge is limited regarding what determines the nose temperature in different situations, situations more complex than sleep and awakening. Also the variation in nose temperature, both between and within subjects, is largely unexplored (see fig. 2 for current basal measurements). In the sample of dogs participating in the study, some always had warm noses, whereas some keep their nose temperature low. Correlation of nose temperature to different factors as sex, age, breed, training experience and so on, would give valuable information in understanding this intricate area.

This has implications when a cut-off limit for too high nose temperatures is to be set. Is the observed temperature within the normal range, ambient temperature and other factors in the environment regarded? If there is a cut-off limit in nose temperature, above which the heat sense is heavily impaired, is it then relative to an individual's base line nose temperature, or is it absolute, set by physical properties in the detection of infra-red radiation? The latter would infer that dogs always having warm noses are unable to use a heat sense due to physiological characteristics.

The lineage that eventually would lead to carnivora, emerged at the earliest 90 ma years ago during the late Cretaceous [3]. In Asia, where many of the modern mammalian groups (there amongst odd- and even-toed ungulates) are hypothesised to first have appeared, the climate during this period was humid to semi-humid and the temperature was warm temperate to subtropical [29].

Avoiding to draw any decisive conclusions, this complicates the hypothesis of the cold nose being due to a heat sense. A heat sense in a cold nose could have an advantage in reasonably cold weather, where the prey or con-specifics have surface temperatures clearly contrasting to the ambient. Then there is an even bigger contrast between the nose and the animals. If the approximation of when the line leading to Carnivora emerged and also the approximation of climate is correct, why would a heat sense emerge in a climate where the animals of interest have a temperature close to ambient? Even if the predecessors of Carnivora would have been mostly night active, the humidity would have been a problem, since infra-red radiation is heavily absorbed by water in many wavelengths (fig. 7).

There is however an opening to the latter problem, an absorption window. Animals emit infrared radiation in the wavelength spectrum between 8 and 12 μ m [11], and as seen in figure 7, there is a local minimum in vaporised water's absorption at 10 μ m. This means that infrared radiation of wavelengths around 10 μ m can travel further through the air before getting completely absorbed by gaseous water molecules, than infra-red radiation of shorter or longer wavelengths. Snake pit organs are also indeed most sensitive to radiation of wavelengths between 8 and 12 μ m [11]. A similar specialisation could also be the case for carnivorans (no physiological resemblance intended, only functional).

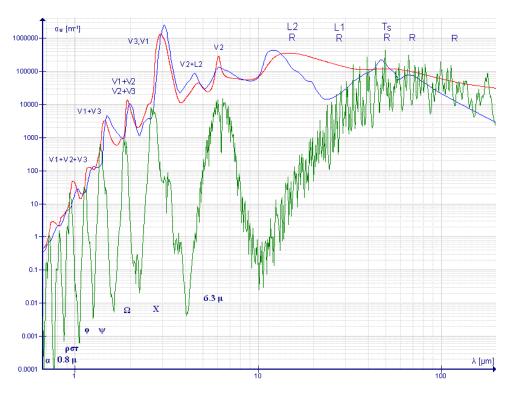


FIGURE 7: Water absorption spectrum. Absorption coefficient for water - liquid (red line), vapor (green) and ice (blue) between 667 nm and 200 m, which covers the red visual range and the most part of the infrared range. [30]

If assumed that the night temperature is low enough for a heat sense to be effective, the question of day active carnivorians in warm climates remains. The African wild dog (*Lycaon pictus*) is (still) resident in Africa and is day active, in contrast to most big carnivores living south of Sahara [31]. It has low nose temperature (Gläser 2014, personal communication) and hunts primarily by sight [32]. Why has it, and other species falling into the same category, not lost their energy consuming cooling mechanism?

Still, some facts remain. The carnivorians noses are cold and they are cooled actively. Energy demanding processes widely represented in the phylogeny, have a function. The rhinaria of carnivorians are densely innervated and the function of both touch and olfaction is impaired in low temperatures. No other hypothesises explaining the function of the cold nose have been presented. That leads me to conclude that the heat sense hypothesis still deserves a thorough analysis, because as long as the supposable mechanisms of perception and transduction of infra-red radiation are completely unknown, no reliable statements of temperature limits and practical functioning can be made.

Conclusions

The hypothesis of a functional heat sense in dogs is not supported by the data collected. Suggestions on methodological improvements in heat training are amongst others: simplification of tasks to only include choice and reward, start training with operant conditioning inside a controlled environment and to start training from early age when possible. Basic information on how nose temperature is regulated and varying with context are missing. Nose temperature data correlated with ambient temperature need to be extended to assess the variation between individual dogs and also between, for example, age groups and different breeds. Measurements of mammal surface temperatures are recommended to only be made during thick overcast. There are also benefits of using a continuous scale of ambient temperature instead of fixed steps.

So many questions...

...and so many answers still to find!

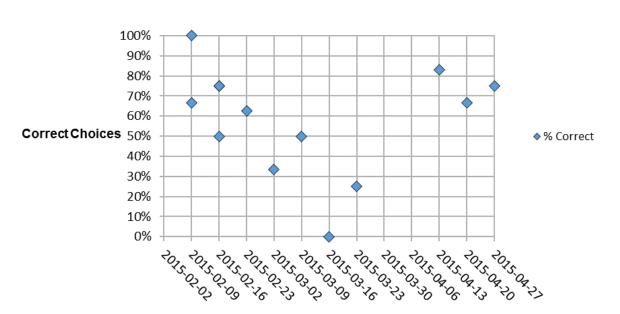
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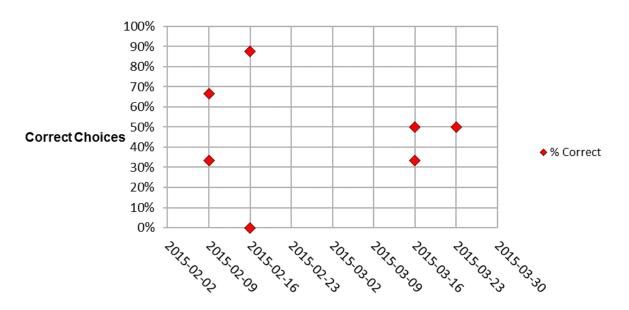
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Appendix A, individual performances



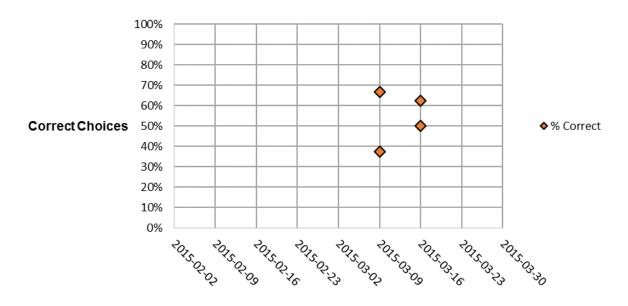
Kevin

(A) Kevin's performance. 74 runs distributed over 15 series.



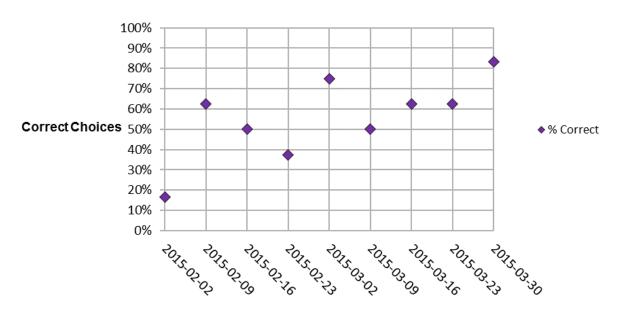
Bonnie

(B) Bonnie's performance. 44 runs distributed over 7 series.



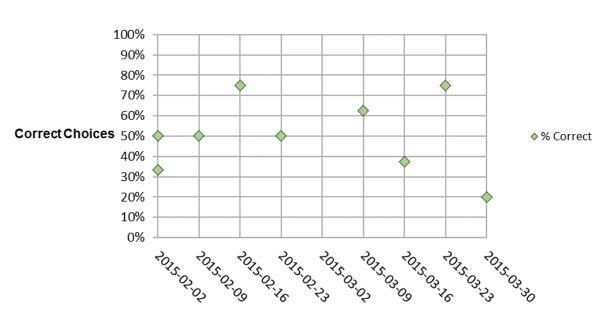
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Molly
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(C) Molly's performance. 28 runs distributed over 4 series.



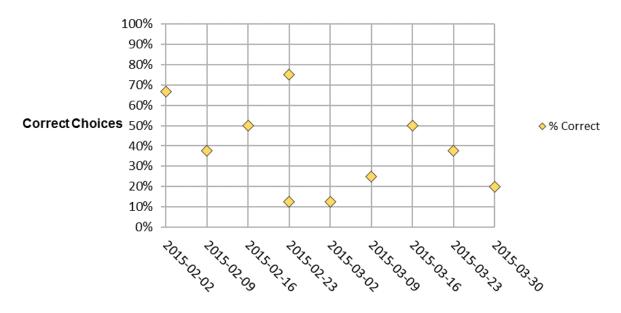
Neal

(D) Neal's performance. 68 runs distributed over 9 series.



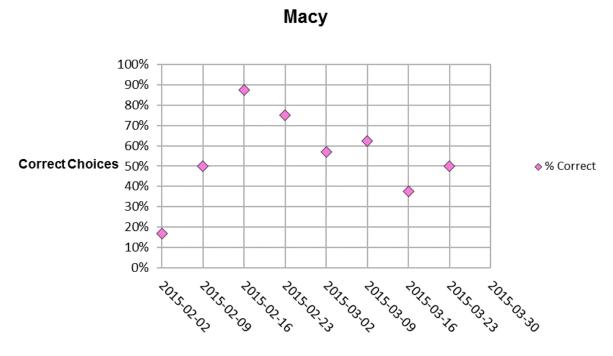
Gary

(E) Gary's performance. 65 runs distributed over 9 series.



Melvin

(F) Melvin's performance. 71 runs distributed over 10 series.



(G) Macy's performance. 59 runs distributed over 8 series.

FIGURE 8: Percent correct choices presented for each dog, for each series performed.