UNIVERSITÀ DEGLI STUDI DI PADOVA
Dipartimento Biomedicina Comparata e Alimentazione
Department of Comparative Biomedicine and Food Science

Corso di Laurea/First Cycle Degree (B.Sc.)
in Animal Care

Development of a methodology for the assessment of dog reactions to auditory stimuli with positive valence

Relatore/Supervisor
Prof. Paolo Mongillo
Co-supervisor
Cécile Chantal Catherine Guérineau

Laureanda/Submitted by
Elisa Tanghetti
Matricola n./Student n.
1222090

ANNO ACCADEMICO/ACADEMIC YEAR 2021/2022

INDEX

1. ABSTRACT

Emotions can be described as relatively intense, rapid, affective responses to external stimuli causing the body to react. It is assumed that auditory stimuli can elicit emotional reactions in dogs.

There are some indicators useful for the identification of the arousal levels, including some physiological and behavioural parameters, but these remain often ambiguous for the identification of the valence of emotions.

In view of its applications, most research on the topic dealt with negative emotions – such as fear, anxiety, and distress – evoked by loud noises as fireworks, gunshots, thunderstorms.

Much less has been done on the characterization of positive emotional reactions to sounds. One constrain that has hindered research in this area, is that there are no accepted methods for the identification of dogs' reactions to sounds with positive emotional valence.

This thesis deals with the process of standardizing a procedure that would reliably allow to observe and characterise dogs' reactions to sounds with presumptive positive emotional valence. In view of the lack of methods, several different approaches has been attempted to this aim, evaluating their ability to provide a reliable, unambiguous and objective way of assessing dogs' reactions to the sounds.

The first was the Openfield Test, a procedure in which the dog was free to express any behaviour in an unconstrained environment with presentation of different acoustical stimuli. The results showed no relevant behaviours indicators of a positive emotional state (no significant behavioural difference between reactions to positive vs. neutral auditory stimuli).

In the attempt to circumvent the problems that arose with the first procedure, a second test was devised: the Maze Test. It was focused on the identification of a more objective information: the dog preference towards a sound. The test required the dog to choose between two arms (one silent and the other producing the sound) to reach a bowl with food. It was used to validate the appropriateness and the valence of the auditory stimuli used to evoke emotional reactions. The results showed no significant preference towards any arm by the dogs, rather a random choice or a strong bias possibly due to a learning process related to the movement pattern required to complete the test.

The last procedure attempted was the Two Side Room Test, in which the dog was not forced to perform any predefined task. A room was divided in two halves and only one side was producing the sound when the dog entered it. The lack of predetermined spatial paths, and the absence of food, allowed to isolate effects of preference, without the interference of spatial and motivational biases. The percentage of time spent by each dog in each side has been calculated and used to

identify through this new parameter the preference of the dog for a sound rather than silence. The statistical analysis of the data collected showed a significant result, meaning that this method is reliable when highlighting dog preference. Implementing this procedure with behavioural analysis through Observer it could be possible to identify common behavioural patterns indicators of reaction to positive auditory stimuli. This test has never been used to investigate emotions related to positive auditory stimuli, and it is a promising procedure that could be farther adapted for future research.

2. INTRODUCTION

2.1 What is an emotion

2.1.1 Emotional status definition

Emotion is a construct that everyone knows and can recognize but for which there is not one obvious definition (Bradley,2000). Emotions can be described as relatively intense, rapid, affective responses to an external stimulus, causing a specific physiological change (Boissy, 2007) and a behavioural reaction (at least a tendency for an organized action). In humans it is known that emotions encompass a conscious experience, which can be pleasant or unpleasant.

In an emotional situation the body reacts, the heart flutters, pounds, and drops, palms sweat, muscles tense and relax, faces flush, smile, and frown (Bradley, 2000). These examples of physical and behavioural changes are experienced subjectively and can be used to differentiate one emotion from another.

2.1.2 Classification of emotions

Current research provides compelling evidence that at least some animals likely feel a full range of emotions, including fear, joy, happiness, jealousy, rage, anger, pleasure, sadness, and grief (Izard, 2009).

- **Primary and secondary emotions**

Primary emotions, considered to be basic inborn emotions, include generalized rapid, reflex-like (“automatic” or hard-wired) fear and fight-or-flight responses generated by evolutionary brain old systems upon the sensing of an ecologically valid stimulus. Animals can perform a primary fear response such as avoiding an object, but they do not have to recognize the object generating this reaction (Izard, 2009).

Basic emotions include fear, sadness, rage, surprise, disgust and happiness.

Secondary emotions involve higher brain centers in the cerebral cortex. Although most emotional responses appear to be generated unconsciously, consciousness allows an individual to make connections between feelings and action and allows for variability and flexibility in behaviour (Izard, 2009).

Primary emotions help organize and motivate rapid (and often more-or-less automatic) actions that are critical for adaptive responses to immediate challenges to survival or wellbeing. In the case of secondary emotions, the neural systems and mental processes are involved, perception, and cognition interact continually and dynamically in generating and monitoring thought and action.

These dynamic interactions (which range from momentary processes to traits or trait-like phenomena) can generate innumerable emotion-specific experiences (e.g. anger schemas) that have the same core feeling state but different perceptual tendencies (biases), thoughts, and action plans (Izard, 2009).

- **The valence – arousal plane**

Emotion can be classified using two-dimensional plane commonly called the valence-arousal plane. The arousal dimension may vary from “not-aroused” to “excited” while the valence dimension can be negative or positive. For example, happiness has a positive valence, disgust has a negative valence, sadness has a low arousal, while surprise evokes high arousal.

High arousal with positive valence is associated with happy emotion, high arousal with a negative valence usually is associated with angry emotions, low arousal with negative valence represents a sad emotion and low arousal with positive valence associates with calm emotion (Bulagang, 2020).

Positive emotions can be categorized as happiness or surprise, while negative emotions can be associated with sadness, anger, fear, and disgust.

- **Positive emotions**

Positive-emotional states associated with an animal’s well-being are usually much more subtle, often less expressive and difficult to reliably assess and distinguish from negative emotional states, which are much more intense and therefore more easily observed and studied (Boissy, 2007).

Happiness can be defined as an emotional state that is characterized as a longer-lasting, steady, persistent, and yet less intense positive-emotional experience (Boissy, 2007). Animal happiness might include everyday experiences of pleasure, opportunities to interact with their environment, conspecifics, caretakers, and having the freedom to achieve one’s own goals (Yeates, 2008).

Pleasure is a passive experience, evoked by an anticipated or received reward, which affects learning, approach behaviour, and decision making, as well as possibly contributing to a longer-lasting state of happiness (Boissy,2007).

2.1.3 Physiological and behavioural aspects associated with expression of different emotions

Emotion is defined as an innate response to an event or situation (internal or external) that comprises behavioural, physiological, subjective (the feeling) and cognitive (subsequent decision-making) components. Emotions are the result of complex processing, by the nervous system, of sensory information gathered from within and outside the animal’s body. The capture and processing of this sensory information are influenced by the biology of the animal species, as well as by individual factors such as the animal’s genetic predispositions, life stage, sex, previous experience, learning and memory. The emotion or emotions resulting from these processes of mental evaluation are thus unique to the individual (Izard, 2009).

The behavioural, physiological and, in some cases, cognitive components of an emotional response can be scientifically evaluated using observable indicators (Beasouleil, 2016) described below.

- **Physiological aspects**

There is a close relationship between emotions and physiology, more particularly the autonomic nervous system and the hypothalamic-pituitary-adrenal axis. Heart rate, heart rate variability, surface temperature, oxytocin, vasopressin, cortisol and alpha-amylase have been implemented and showed potential usefulness in indirect and non-invasive assessment of emotion in dogs (Beasouleil,2016).

- **Behavioural aspects**

Behavioural signals play an important role in intra- and inter- specific emotional communication. Expressing emotions through facial and body movements has been documented in several studies (Csoltova, 2020).

Although there exists a high within-individual variability in behaviour expression, behaviours such as increased activity, repetitive movements, auto-grooming, lowered body posture, lip licking, panting, yawning, crouching, shaking, vocalization (barking, whining), scratching, paw lifting, paw sweating, increased salivation, blinking of eyes have been identified as stress indicating behaviours in dogs (Boissy,2007).

On the other hand, increased physical activity, attentive behavior, tail wagging, lip licking, vocalization, and shake off have been also associated with positive-emotional states in dogs (Yeates, 2008).

2.1.4 Value of emotional reactions for survival and evolutionary aspects

One of the crucial roles of emotion from an evolutionary perspective is to facilitate behavioural and physiological adaptation to a changing environment. The essential survival function of emotions is to seek out rewards and resources while avoiding harm and punishment. The reward is associated with experiencing positive-emotion, while the consequence of reward omission or punishment is a negative emotional experience (Rolls, 1990). This shows that emotional response system is founded on the appetitive and defensive motivational systems.

From a social point of view, enhanced processing gains of emotional cues may help individuals to quickly initiate adequate approach or avoidance behaviour and therefore increases the chance of survival or well-being.

2.2 Emotional reaction to sounds

2.2.1 Types of emotions elicited by sounds

We know that sounds can elicit a full range of emotional responses in human listeners (Bradley, 2000); while in animals, and specifically in dogs, accurate studies are needed to assess it.

Existing studies show how some sounds can cause fear, anxiety and stress in dogs, as well as other can induce relaxation and may be useful to increase their welfare (Boissy, 2007).

2.2.2 Characteristics of sounds associated with specific emotions

There is a restricted group of sounds for which research has been developed in order to assess which emotions can be elicited by them in dogs. **Music** effects on dogs have been researched and investigated in terms of activity, vocalizations, body movement, body shaking and heart rate. It resulted that dogs respond to classical music by sleeping and resting more, reducing vocalizations and increasing time sitting or lying down, which is consistent with human studies, that suggest music can reduce agitation, promote sleep, improve mood and lower anxiety. Heavy metal music, in contrast, appears to increase behaviours that suggest agitation and stress in both dogs and humans (Kogan, 2012). Human voice, specifically the one coming from an **audiobook**, has been showed to cause the calmer behavior amongst other auditory stimuli as classical, pop and relaxation music. This is because praising words, combined with praising intonation, activate reward regions in dog brains (Lindig, 2020). **Thunderstorms, fireworks, gunshots,** and all the other sudden loud noises coming from unpredictable events are one of the most triggers for fearful behaviours in dog (Grigg, 2021). **Regular and irregular household noises** (microwaves, vacuum cleaner, smoke alarms,…) elicit fear and anxiety behaviours in dogs, too (Grigg, 2021).

2.2.3 Neurophysiological mechanisms underlying reactions to sounds

On the neural level the amygdala has long been identified as a key structure of emotional detection both in animals and humans. The **amygdala** is a small, oval-shaped structure in the brain that plays an important role in identification of emotional stimuli and the following arousal, including emotions such as fear, anger and anxiety. Via the thalamus, the amygdala receives inputs from all the senses: there is empirical evidence that amygdala processes auditory cues. The thalamus also sends signals to the autonomic nervous system, resulting in physical reactions such as muscle tension, shaking, and sweating.

The **insula**, another brain structure located inside the temporal lobe, plays an important role interpreting positive or negative stimuli and regulating the following arousal. It participates in making the distinction between positive and negative stimuli: positive feelings are associated with activity in the rostral areas of the insula and negative feelings with the more caudal areas.

Amygdala and insula are coordinated, with Amygdala initiating arousal (especially in response to negative) and insula helping to discriminate between positive and negative stimuli (Wynne, 2020).

2.2.4 Other factors influencing reactions to sound

The effect of an auditory stimulation of a nonhuman animal varies greatly with the type of sound, the ethology of the species, the personality and the learning history of individual animals. Also individual preferences should be considered (e.g. some may prefer silence to music) as well as previous experiences, which may have conditioned positive or negative associations with a particular type of sound and will influence the response of that particular animal (Wynne, 2020).

Animals may become habituated to a sound, and therefore any reaction may decrease over time.

2.2.5 Emotional characteristics of sound shared across species

It is important to consider how each species has evolved to encounter auditory stimuli: comparative acoustics have shown that the anatomy of the head, the distance between the ears and the shape and mobility of the pinnae influence the effective acoustic perception. Even among a single species, such as the *Canis lupus familiaris*, physical differences among breeds, as individuals' size, head shape, the shape and the mobility of the pinnae but the learning history can create variation that would affect perception and reactions to auditory stimuli (Wynne, 2020).

Dogs and wolves

Dogs can hear high-pitched sounds thanks to their predatory heritage: their ancestors, the wolves, prey on small rodents as mice, so the ability to hear the tiny animals' squeaks is important for survival. Dogs also have a high ability to detect tiny differences between frequencies (Tune, 2020).

2.2.6 Species-specific characteristic that can elicit emotional reactions

High frequency sounds are believed to be more salient for many species (i.e. attracting greater attention and prompting greater alertness) than lower frequency sounds.

Dogs can hear sounds as high as 47,000 to 65,000 Hz.

Comparing dogs to humans, dogs' sensitivity to high frequency sounds is greater, whereas at lower frequencies it does not differ much. The amplification process of the ear may be stronger in dogs, thus, at high frequencies dogs can detect much softer sounds than we can (Tune, 2020). This is relevant whenever a dog is introduced to a human environment, and it's an important base for dog reactions investigation.

Despite these differences, it has been demonstrated that voice areas exist in dogs and that they show a similar pattern to voice areas in humans. Sensitivity to vocal emotional valence cues engages similarly located auditory regions in dogs and humans (Andics, 2014).

Emotional contagion in dogs has been investigated recently and a study shows that dogs behave differently after hearing non-emotional sounds of their environment compared to emotional sounds of humans and conspecifics: dogs expressed more behavioural indicators for arousal and negatively valence states after hearing negative emotional sounds, indicating a pattern of state-matching (Huber, 2017).

2.3 How are emotions expressed in dogs and methods to assess them

In dogs, emotions can be detected observing and measuring changes in behavioural and physiological parameters.

Physiological parameters:

- **Heart Rate**: it has been utilized as an effective way for assessing the sympathetic branch of the Autonomic Nervous System (ANS) activation in both animal and human studies. It is a reliable indicator of arousal, but not of emotional valence: besides emotions, it can be affected by different factors, as physical activity (Boissy, 2007).

- **Heart-Rate Variability (HRV):** it has been shown to be an effective tool to measure the sympathetic and parasympathetic balance of the ANS. Positive-emotion-eliciting stimuli, such as food, human-dog interaction, and listening to music, all resulted in changes of HRV parameters in dogs (Boissy, 2007).

Assessing canine emotional states by implementing HRV indices has been gaining research popularity in recent years. It has been proposed that HRV parameters might be sensitive indicators of emotional valence and tend to be less affected by physical activity compared to heart rate (Boissy, 2007).

- **Superficial Temperature:** both positive and negative emotions lead to physiological changes associated with alterations of blood flow, leading to surface temperature fluctuations (Boissy,2007).

In dogs, there has been a growing interest to investigate negative emotion-induced surface heat increase associated mainly with stress, fear-based aggression, and separation anxiety, both in clinical and home. It is considered a useful to assess arousal intensity (Csoltova, 2017), but there has been some debate about whether it can distinguish emotional valence.

- **Oxytocin**: is a neuropeptide and biomarker that is associated with a positive-emotional state in dogs (Boissy, 2007).

- **Vasopressin:** is a neuropeptide closely related to oxytocin. In dogs, vasopressin increase has been associated with acute stress responses and was also reported in dogs with separation anxiety.

- **Cortisol:** salivary cortisol levels in dogs has become a well-established method for measuring stress response and its impact on dogs' well-being in several settings. Differences in the intensity and nature of a given stressor may impact endocrine responses, thus affecting cortisol release (de Souza, 2018).

- **Alpha-Amylase:** measuring decreased levels of alpha-amylase, a crucial salivary enzyme, is another non-invasive approach to test sympathetic nervous system deactivation. Salivary alpha-amylase increase in response to both acute and chronic stressors has been documented in different animal species, including dogs.

Behavioral parameters:

- **Vocalization:** dogs use a wide range of different context-specific subunits of barks and mixed sounds as a means for communication of emotional arousal and both positive and negative emotional states: barking encompasses a wide range of both positive and negative emotional states, while, on the other hand, whines are more associated with negative emotions.

- **Activity:** it's a form of exploration or information gathering and might be a relevant indicator of the animal's well-being (Boissy, 2007).

- **Lip Licking:** observed higher frequencies of oral behaviour in a form of lip/nose licking and/or tongue flicking have previously been proposed to indicate acute stress in a social context (Csoltova, 2017). Nose licking has been also observed in frustration-provoking situations when access to a food reward was denied to dogs. On the other hand, other research findings suggest that lip licking could be associated with positive emotion, performed in response to verbal and physical human interactions as seeing the owner after separation (Boissy, 2007).

- **Tail Wagging:** a higher frequency of tail wagging recorded in social settings has been proposed as an indicator of emotion reflecting positive valence and contact-seeking behavior.

- **Play Behaviour:** play behaviour is a pleasurable experience and thus a possible indicator of a positive-emotional state. Play behaviour includes a complex set of different motor activities and play patterns. However, there have been some debates regarding whether play behaviour is a reliable indicator of positive welfare, since the play occurs under less favorable environmental conditions as well (Boissy, 2007).

Besides the presence of numerous parameters useful to understand the arousal levels, they remain often ambiguous for the identification of the valence levels, thus, cannot be used alone to detect dog emotions. Actually, there is not a consensus method to detect positive emotional reactions of dogs to sounds.

3. MATERIALS AND METHODS

The aim of the activity described in this thesis was the development of a methodology for the assessment of dog reactions to auditory stimuli with a potentially positive valence. To this aim, several candidate procedures were assessed, evaluating their ability to evoke distinctive behavioural responses to auditory stimuli with presumptive different emotional valence. Procedures that were deemed as not appropriate were discarded and eventually replaced with novel procedures, which were developed by taking into accounts the drawbacks and limitations arisen from the non suitable ones. The procedures will be hereafter described in the chronological order they were performed in lab, to highlight these limitations and the process that led to the identification of the most suitable procedure. All testing took place in one room of the Laboratory of Applied Ethology of the University of Padua (Legnaro, PD).

3.1 Openfield Test

This procedure aimed to observe the dog behaviour, freely expressed in response to auditory stimuli. Dogs were exposed to the stimuli in a room without any constrain on their behaviour. A toy was provided to allow the dog to play, if she/he wanted to do so.

3.1.1 Subjects

For this experiment five dogs were recruited. Recruitment criteria included dogs between 1 and 7 (included) years old without any hearing impairment.

The hearing condition of the dog was first assessed by asking to the owner whether the dog had any overt impairment, then verified in the lab by an experimenter, who produced a little noise (rubbing hands) near the right then the left ear of the dog. If the dogs reacted to this sound for each ear, the test could be done, otherwise it would be ended there.

3.1.2 Equipment and setting

The room for the Openfield (OF) Test was a 4.8 m x 4.7 m area, which walls were covered by curtains to avoid sounds reverberation and with windows and blinds fully closed to avoid outside lights and sounds. The room was provided with artificial lights only. The OF area was empty except some solid objects: a chair for the operator and 1 plastic Kong® which were positioned as shown in Fig.1.

The experiment was recorded through different cameras, positioned as shown in Fig.1. The use of multiple cameras allowed the recording of the entire OF area, keeping a good view on the behaviour expressed by the dog. Furthermore, the orientation of a moving camera was remotely controlled by an experimenter, in order to follow every detailed movement of the dog.

Adjacent to the OF Area there was another room where 2 speakers and a tripod camera were positioned. The sound produced by the speakers was hearable all around the OF Area because the 2 rooms were connected by a door (Sound Door). At one meter away from the Sound Door the sounds produced by the speakers had an intensity around 70-75 dB.

In the OF area some relevant zones were identified and marked: the Sound zone close to the Sound Door, the Exit door zone and the Human zone, around the chair where the operator was sitting.

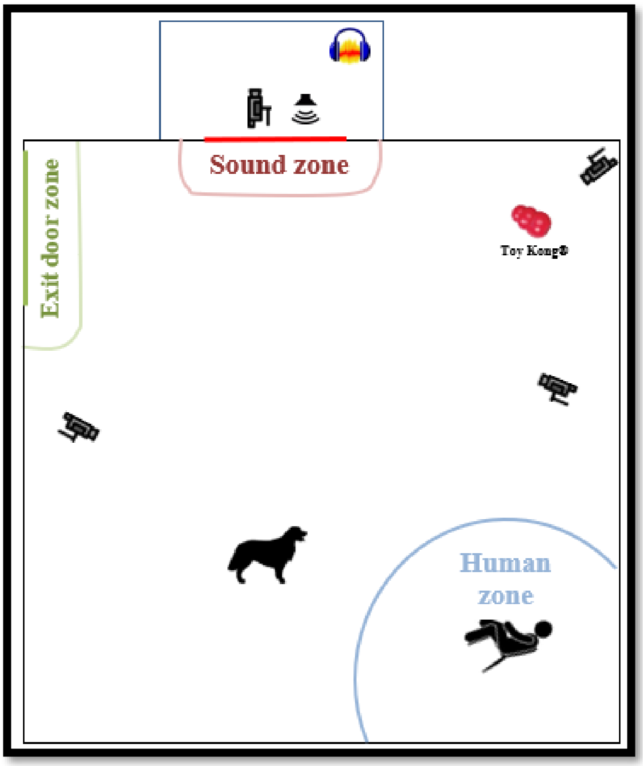

Figure 1: configuration of the OF area with position of the cameras, the doors, the toy and the different zones.

3.1.3 Sound stimuli

Audacity software was used to create and produce the audio file. Sounds were collected with a microphone in real situations, or extracted from Youtube videos.

The typical scheme of a sound compilation included 3 minutes of silence – 0,5 minute of sound – 2 minutes of silence – 0,5 minute of sound – 2 minutes of silence – 0,5 minute of sound – 2 minutes of silence.

In any given compilation, the same sound was used for all the 'sound' intervals. The types of sounds proposed were:

- Neutral – an artificial sound with frequency = 4 000 Hz and amplitude = 0,5. Abiotic sounds hearable by dogs have already been considered neutral or non-emotional stimuli in other studies (Annika Huber, 2017);
- Positive: dog vocalizations while welcoming the owner, greeting the owner or playing with the owner (from the Lab); or puppy vocalizing being with mum (Youtube);
- Positive: classical music (Beethoven or Bach).

3.1.4 Procedure

The test needed the cooperation of 2 experimenters.

The first (dog operator) stayed the whole test inside the OF area with the dog, sitting on the chair. This operator was unfamiliar to the dog, and was chosen among the lab staff so to be of the same sex of the owner. The operator had to spend a little time with the dog before the beginning of the experiment for the dog to habituate to the operator him/herself. A little play/petting session was done, and/or a little walk according of how the dog reacted to the "stranger". This introductory session lasted until the dog seemed sufficiently at ease with the operator. At that point, the actual test began.

The second experimenter (sound operator) was hidden behind the Sound Door (fig. 1), triggering the audio file on the computer.

The floor was fully washed before and in between the experiments, to reduce bias in dog behaviours due to foreign odors or remains.

Before beginning the test the dog operator entered the room without any personal device or objects which may catch the attention of the dog, took the dog on leash, positioned himself on the chair and unleashed the dog.

During the test the operator read or pretended to read a book, and has never initiated eye, gestural or vocal contact with the dog, and did not react to acoustic stimuli.

If the dog came to solicit the person closely (e.g. touched with paw or muzzle the person or jumped on him/her) the dog was petted for approximately 5 seconds. If the dog had the front paws on the person, it was pushed gently on the ground. If the dog re-solicited, the operator started this behavioural scheme again.

The procedure lasted as long as the stimuli compilation, so 10.5 minutes.

Each dog underwent 3 sessions of the OF, in each of which a different pattern of sound compilation was used.

3.1.5 Data collection

For the data collection and extraction the software Observer (Nolduls, The Netherlands) was used; the behaviour was collected with a continuous focal animal sampling method according to a specific ethogram, not fully reported here for lack of space. Data were collected about the time spent by dogs in different areas within the room, their movement and orientation (to the sound, human, exit, elsewhere) and its emotional status, defined as reported in Table 1.

Table 1: Partial ethogram of behaviours coded with their detailed definition. Only behaviours related to the dog's emotional status are reported.

Emotional status (mutually exclusive, exhaustive)	
Behaviour name	Definition
Negative state	FEAR-THREAT-DISTRESS: FEAR: lower than normal posture (legs non completely extended, wider standing area) accompanied by body tension. It can be, but not necessarily, be accompanied by tucked tail, flattened ear, horripilation, trembling, urination, panting. THREAT: curled lips to show teeth accompanied by body tension and possibly by vocalizations (barking or growling). It can be accompanied by FEAR or not. STRESS: body tension accompanied by stress signals such as lips licking, yawning, Movement of the eye/head to avoid the visual contact with the stressor. Possibly accompanied by changes of activity like agitation o freezing.
Alert	Laying, sitting or standing motionless with a clear sensory orientation (ears and head), body tension WITHOUT signs of fear, threat or distress.
Playing (solitary play or play soliciting)	<u>Solitary playing</u>: non-reciprocal motor patterns (pawing, play bow) directed toward an inanimate object without engaging or orienting toward the human. May also include locomotor behaviors (inhibited running, voluntary downs) not directed toward human. <u>Play soliciting</u>: play bow, exaggerated approach, approach/withdrawal, paw intention, leap-leap (all previous behaviors directed to the human) leaving the toy near the human while the dog being oriented to the human and looking at him, gazing human and toy one after another.
Resting/Relaxed	Moving, sitting or lying down with no clear/complete sensory orientation and without tension in relevant body parts (tail, ears, legs).
Exploration	Close visual and/or olfactory inspection of the door, other objects or parts of the room.
Other behavior	Any kind of behavior not described above (if doubtful, put in comments what behavior you are seeing).

3.1.6 **Results**

As evident by figure 2, although no statistical analysis was performed, dogs spent longer time in the Sound Zone and in the Exit zone, when a positive sound was played than during silence or playback of a neutral sound. Moreover, dogs were oriented for longer time towards the source of sound than elsewhere, when a positive or neutral sound was played, than during silence (fig. 3). Relaxed behaviors occupied most of the time with Positive Sound, seemingly more than with Neutral sound or with Silence; exploration and playful behaviors did not seem to differ between the different auditory stimuli (fig. 4).

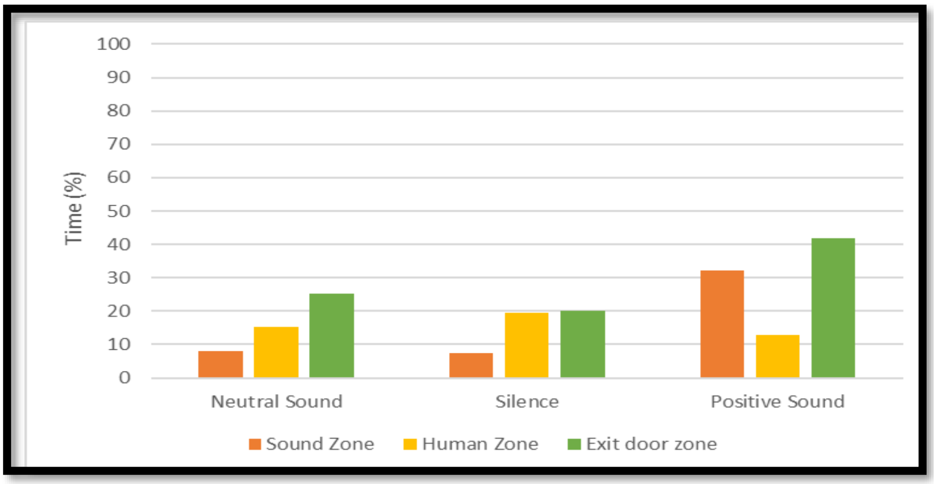

Figure 2: mean percentage of time spent by subjects in the relevant zones (Sound zone, Human zone and Exit door zone) during the different sound stimuli.

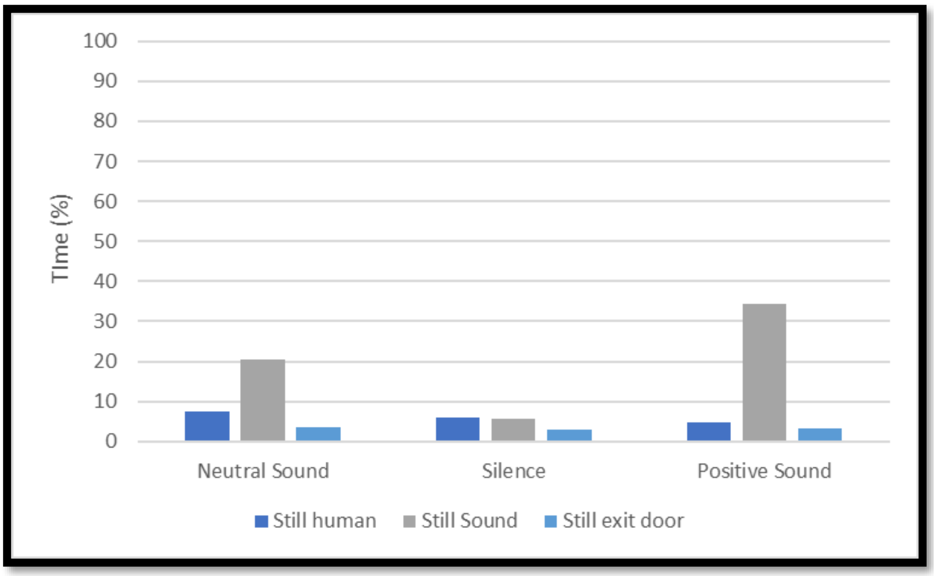

Figure 3: mean percentage of time spent by subjects being oriented towards different test components (human, exit door, sound door) during the different sound stimuli.

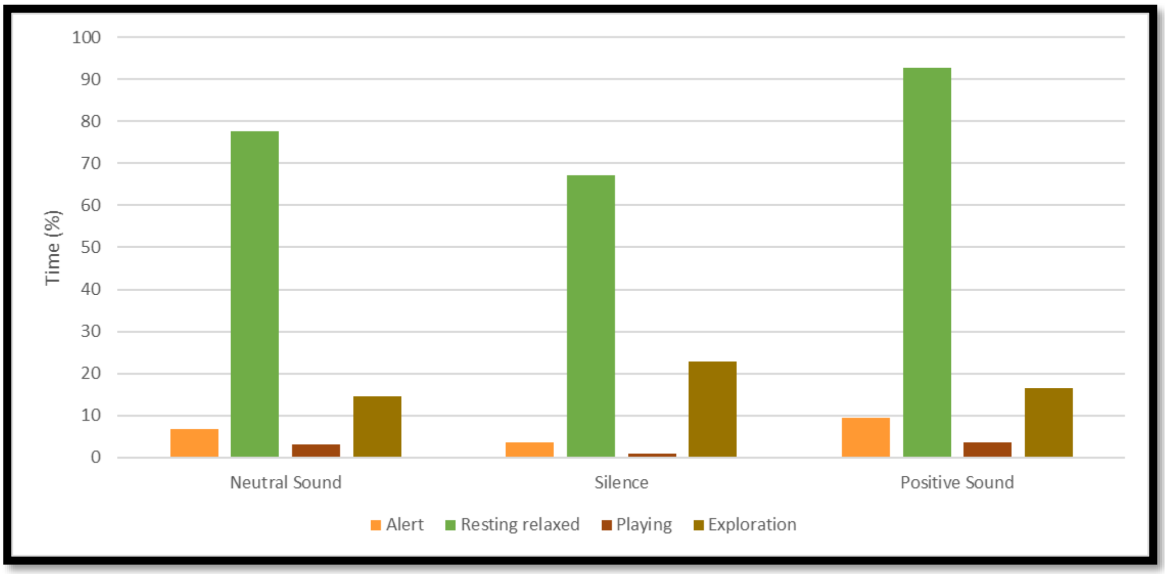

Figure 4: mean percentage of time spent by dogs in different emotional states (alert, resting/relaxed, playing and exploration) during the different sound stimuli.

3.1.7 Discussion

The higher percentage of the time spent by dogs in the Sound Zone and in Exit Zone and their Orientation towards the Sound during Positive Sounds compared to the other auditory stimuli can be explained by a higher interest, attraction towards the sound and desire to reach it. Hence, the descriptive analyses suggests that the Positive Sound triggered the emotional arousal of the subjects. However, there are no relevant behaviours, point events or emotional states (as exploration and playing) that are common indicators of emotional states with positive valence and that differed accordingly to the different auditory stimuli. Time spent in different zones and orientation alone are indicators of emotional arousal, attraction, interest, but not of positively valenced emotions as happiness, joy, pleasure. Furthermore, it can't be declared that a behaviour commonly expressed during positive emotional states is an indicator of it, since it may occur also during harsh conditions (ex. playful behaviours) or its altered intensity and frequency may indicate the opposite emotional state (e.g. lip licking) (Csoltova, 2020). Therefore, the positive sounds used in this experiment seemingly increased the emotional arousal of dogs, but the valence of that emotional arousal remains unknown.

Further research is needed to understand if this ambiguity is related to the procedure which could be lacking of appropriateness: positive states as happiness are considered long term emotions (Boissy, 2007), so an auditory stimulus lasting 30 seconds may not be enough to produce the emotion targeted; or because of the choice of the sounds used, since they've been assumed capable to evoke positive emotional states, but no relevance was provided.

Since the procedure did not allow to identify a clear indication of pleasure when exposed to presumptively positive sounds, a novel procedure was devised that allowed to observe a clear choice to be exposed to such sounds, rather than silence.

3.2 Maze Test

This procedure was aimed at the identification of an objective preference of dogs towards a sound. The preference was based on dogs' choice to enter an arm of a maze where sound was produced or one where no sound was produced.

3.2.1 Subjects

This test was done also on the same subjects of the OF to see if any correlation could be done within the choices done in the maze and the free behaviours performed in the Open Field Test.

The dogs included in this study were varied in breed, sex and age; the recruitment criteria were the same of the OF.

The subjects included in this study consisted of 7 dogs. Six of them were habituated to stay in the lab while one of them has been invited occasionally.

3.2.2 Equipment and setting

The setting was composed by a maze with two arms and a starting area where both arms were visible and accessible. Both arms were bent so that their ends were not visible from the entrance. At the end of each arm there was a bowl full of food. In that location a hole was created for the operators to refill the food bowls (Figure 5).

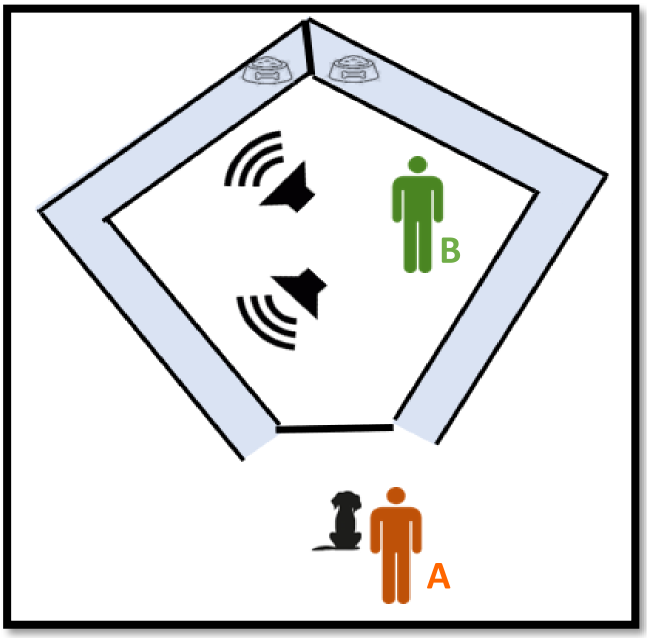

Figure 5: configuration of the Maze Area 1 with the position of the subject, speakers, bowls and experimenters (A = dog operator, B = sound operator).

One arm was approximately 4.80 m long built with 1.50 m high panels. Two speakers were in the middle, in an area which was out of the dogs' reach. They were directed towards one of the two arms of the maze, designated for each dog as "the Sound Arm" (the left one in the example reported in Figure 5). The dogs could have access to the two arms but not in the middle. The floor was fully washed before and in between the experiments, to reduce bias in dog behaviours due to foreign odors or remains.

3.2.3 Procedure

For this test two experimenters were needed: the sound operator was responsible for triggering the sound and was not seen by the dogs; the dog operator was entering the test room with the dog and was keeping him in the proper position at the entrance before the two arms (Figure 6).

Before starting the procedure one arm was chosen to be the Sound Arm (SA): every time the subjects entered such arm the sound was played. The other was the Silent Arm (SiA), and no sound was played when the subjects entered it. SA and SiA were fixed and not switchable for each subject but semi-randomly attributed to them, in order to approximately half of the dogs experiencing the SA on the left and the other half on the opposite side.

Figure 6: picture of the two entrances, Maze Test 1.

This test required a learning phase that started when the dog and the dog operator entered in the room. The operator positioned the dog toward one entrance and unleashed it to let the subject free to explore that arm and to reach the bowl full of food at its end. At that phase, the dog needed to enter in sequence twice each arm directed by the operator, so without being free to choose an arm rather than the other (ex. R,L,R,L). The sound was always produced towards the side decided (left in figure 5), and nothing happened in the other arm. This phase was intended to make sure the dog experienced that sound was played whenever he entered one of the two arms and not the other. Then, the test procedure could begin: just after the learning phase, the dog operator put the dog in front of the middle panel, equal distance from the two entrances, released the dog and waited that he entered one arm. They were free to reach the bowl at the end of the arm, eat, explore if wanted and come back. When the subjects chose the sound side, the sound was produced until the dog got out of the arm. Between one trial and the next the dog operator recalled the dog if it didn't exit the arm spontaneously and held it, meanwhile the sound operator put food in the bowl chosen previously. These steps were repeated until 10 choices have been made by each dog.

The subjects repeated the test procedure two times on different days, because two different types of positive sounds were used:

- Human voice: owner voice or stranger voice greeting the dog. These audio files were recorded previously by the lab experimenters.

- Classical music: Bach or Beethoven. These stimuli were the same used during the OF.

3.2.4 Results and discussion

Table 2: data collected from Maze Test. It shows the Sound arm selected for each dog (L=left or R=right) and the number of choices of the sound performed by dogs when presented with either human sound or music, out of 10 total entrances.

Dog	Sound used	Sound Choice	Sound arm
Ares	Owner voice	NA	R
	Bach	10/10	R
Axel	Owner voice	4/10	L
	Beethoven	7/10	L
Millo	Owner voice	2/10	R
	Beethoven	0/10	R
Mustu	Human voice	3/10	R
	Beethoven	1/10	R
Reïko	Human voice	6/10	L
	Bach	10/10	L
Kai	Human voice	4/10	L
	Beethoven	5/10	L
Mafalda	Human voice	6/10	L
	Beethoven	0/10	L

The number of choices for the sound arm on the 10 trials was on average 4.5. Single sample Wilcoxon test showed no significant difference in number of choices for the sound arm compared to a hypothetical random choice of 50% ($p=0.553$).

It is possible that this is due to individual preferences that are variable among subjects, or by inherent problems of the procedure, which might have hindered the possibility to observe a true preference. For instance, several of the dogs chose always or almost always either the silent or the sound arm, without even considering the other, and it was the first arm visited by them. While this might reflect a clear preference, it is highly possible that this reflects instead a strong spatial bias. It is a common observation whenever dogs must acquire a strategy for choosing between two spatially separated options, and it's called the primacy effect. This primacy effect indicates that initial experience can result in hypotheses about regularities in displays which then become resistant to updating (Jungé, 2017). Moreover, in the dogs that did not choose clearly one or the other arm, often alternation was observed. Spontaneous alternation is a measure of exploratory behaviour that has been evaluated mainly in rodents. Rats and mice, as do cats and chickens, normally alternate at levels significantly above chance, indicating their willingness to explore novel environmental stimuli (Lalonde, 2002). It may be that this exploratory strategy has been used also by dogs to complete the Maze Test.

The learning phase seemed not appropriate since its goal was to make the dogs confident with the apparatus and to create the association between the side of the arm and the sound/silence, still ensuring reward (bowl full of food) for either arm/choice. About the confidence with the apparatus, most of the dogs tested were used to the lab and didn't show any fear signs, while the dogs invited occasionally didn't succeed straight away to go to the maze and showed signs of discomfort. Furthermore, it is not clear if the dogs based their choices on the presence/absence of the auditory stimulus or on the learning and exploratory spatial strategies.

The setting of this test also resulted not appropriate, since it was really hard for the dog operator to create a standard position for himself and the dog, as the space before the two entrances was really small. In addition, during the procedure phase the dog operator had the neutral role to release the dog without giving any indication/suggestion about an arm instead of the other, but it was really difficult to keep the dog in a neutral position and to let him free when it was not looking/focused on one entrance.

In conclusion, while the idea of obtaining a clear preference from dogs seemed promising, the constrains of the maze and of the procedure impacted on the possibility to observe a clear response linked to the sound playback.

3.3 Two - side Room Test

The Two-side Room test (2SR) was devised in order to observe a clear preference, as it was hoped to observe with the maze, but at the same time to allow dogs to freely express their behaviour, as in the OF test.

3.3.1 Subjects

For this experiment ten dogs were recruited. Recruitment criteria included that the dogs were of different breeds and sex, of age between 1 and 12 and not hearing impaired.

3.3.2 Equipment and setting

The room in which the experiment took place had two panels placed in the middle of the room, to divide it exactly in two halves, leaving an opening in the middle for the dog to pass and the owner to sit there. Straight in between the 2 panels, in the opening, a chair for the owner was positioned. The speaker was placed on a chair behind a curtain in correspondence with one of the two panels.

The four fixed ceiling cameras and the movable one were used to record the entire area and the dog movements and behaviours (as in Figure 8).

Figure 7: the view from "all cameras" of the test room.

3.3.3 Procedure

Before the beginning of the test the owner entered the test room with the dog on the leash. The owner was asked to sit on the chair and to let the dog loose only when one operator knocked the door. During the test the owner read the magazine or pretended to read it, avoided initiating any contact with the dog, did not speak and followed it with the gaze. The owner was allowed to briefly pet his dog if it insisted on getting attention. Out of the test room two experimenters were required: the dog operator followed the dog with the moving camera during the whole test. He was asked to focus on the dog when it was still and to zoom the image out when it was moving to have the entire dog all times. The sound operator was in charge of producing the sound. The sound side of the room was randomly determined for each dog. The sound was turned on every time the dog was in the pre-determined sound side, starting when both ears passed the middle line of the room. When both ears passed to the silent side, the sound operator stopped it immediately.

Two types of assumed positive sounds were used for this procedure representing the sound of dogs while greeting the owner, thus presumptively a positive valanced sound. The test lasted 10 minutes, starting from the moment the owner unleashed the dog. The whole test was video-recorded with all the cameras.

3.3.4 Data collection

The main parameter collected in this test was the side of the room in which the dog was at any given moment, i.e. either the sound half and the silent half (as shown in Figure 8).

3.3.5 Results

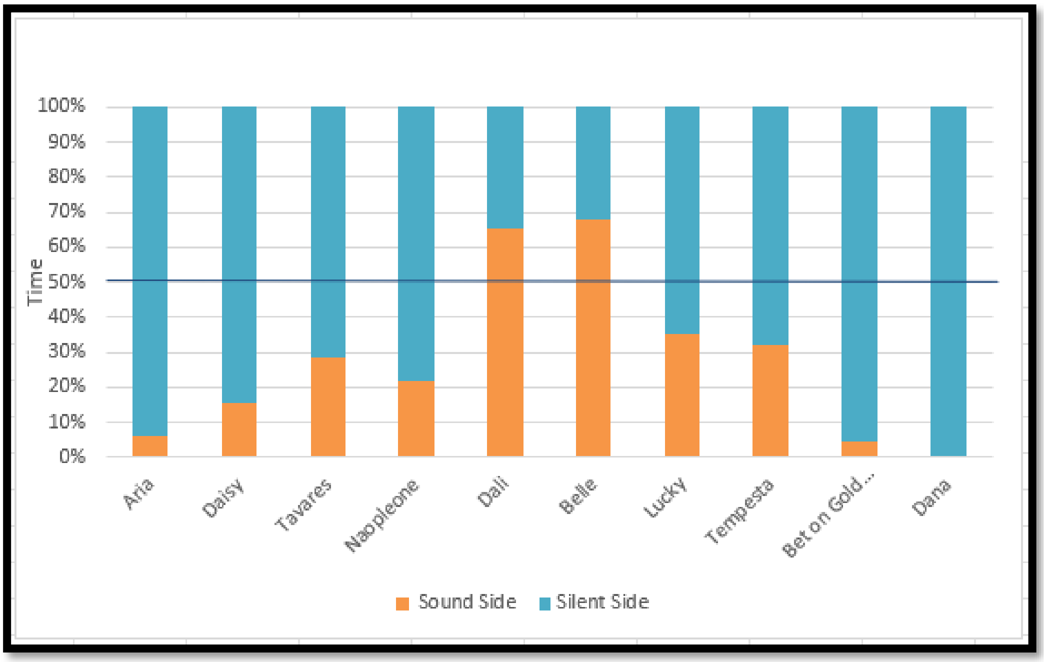

Figure 8: the percentages over the total duration of the test that dogs spent in the Sound Side vs. the Silent Side.

The mean percentage of time spent in the Sound Side was 28 ± 24%. The data was analysed using a Wilcoxon non-parametric one-sample test to detect if the dogs stayed significantly more on either sides compared to a hypothetic median of 50%, which can be interpreted as neutral mean preference for one side over the other. The results showed that dogs stayed significantly more in the side with no sound ($p=0.02$).

3.3.6 Discussion

From the results it can be declared that the 2SR test is valuable for the identification of dog preference towards a sound or not. It allowed the dogs to move without limitations, explore, perform natural behaviours and stay where they preferred. It did not require any locomotor pattern and exploratory process, avoiding disturbances coming from spatial learning. The significance in the statistical test can be used as a base to analyse the dog behaviour through Observer and identify changes between when it was in its preferred side rather than the other. The statistical test showed a significant preference for the silent side rather than the sound one. It may be because what it has been assumed to be positive for the dogs (the sound of other dogs greeting their owner) actually it's not. It may be for different reasons, like the sound is coming from an unknown dog, or the impossibility to see/reach it is frustrating. It is also possible that, while the sound is actually evoking a positive emotional state, like one associated with curiosity and motivation to explore the source of the sound, the overall amount of time spent in the 'sound half' is not the best parameter to highlight such effect. Therefore, other behavioural reactions might be explored – such as latency of approach and attention towards the sound source – that might indicate an attraction towards the latter.

This test is a promising methodology for investigating dog emotional states and for the assessment of dog reactions to auditory stimuli with presumably positive valence. Nevertheless, detailed behaviours must be analysed and compared with other sound valences (negative and neutral) before concluding on the efficiency of the test.

4. CONCLUSIONS

This thesis was aimed at the development of a valuable methodology for the assessment of dog reactions to positively valenced auditory stimuli.

The first procedure designed was the OF test, to identify behavioural reactions characterizing the dog emotional state evoked by sounds with assumed positive valence. The data collected showed that the process of habituation took place when subjects repeated the test in multiple sessions, leading to altered results. In addition, no evident indicators of positive emotional state could be observed during the auditory stimulus; this could be related to two aspects: positive states are often more subtle and difficult to be recognised than negative ones; furthermore, positive emotional states, as happiness, are believed to be long-lasting emotions, so probably an auditory stimulus of 30 seconds length was not enough to evoke such status.

The second test designed was the Maze Test, with the aim to determine the appropriateness of the sound used in the OF, their valence and the objective preference of the dogs towards it or not. This test resulted strongly biased by the locomotor pattern needed to complete it, which required a learning process and an exploratory activity from the dogs that prevented them to focus on the arm-sound association and to choose according to their sound preference. The performance of this test has been challenged also by technical issues related to the apparatus shape and the influence of the owner presence on the willingness of the dogs to complete the trials.

To overcome previous limitations, the 2SR test has been designed. It foresaw only one test per dog to avoid habituation, a longer test to potentially see stronger reaction evoked by the positive auditory stimuli, the dogs being free to move wherever they wanted without any locomotor pattern required, and the passive presence of the owner, to reduce separation anxiety and discomfort.

Taken together, the overall results suggested that the implementation of a methodology to assess positive emotional status requires many steps and critical analysis, since it tends to be strongly altered by major biases. The 2SR represents a promising methodology to assess dog preference, to validate the valence of the auditory stimuli and to analyse behavioural reactions characterizing a positive emotional state evoked by sounds.

Future studies should refine the 2SR test and implement it with behavioural analysis through Observer, to try to identify behavioural indicators of positive emotional states. It could be useful to test as many sounds as possible, to eliminate assumptions and assess their actual valence for dogs. It may open a new frontier regarding dog behaviour and dog emotion.

5. REFERENCES

- Andics, A., Gácsi, M., Faragó, T., Kis, A., & Miklósi, Á. (2014). Voice-sensitive regions in the dog and human brain are revealed by comparative fMRI. *Current Biology*, *24*(5), 574-578.

- Beausoleil, N. J., Stratton, R. B., Guesgen, M. J., Sutherland, M. A., & Johnson, C. B. (2016). Scientific evaluation of animal emotions: Brief history and recent New Zealand contributions. *Journal of New Zealand Studies*, (22), 63-71.

- Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., ... & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & behavior*, *92*(3), 375-397.

- Bowman, A., Scottish, S. P. C. A., Dowell, F. J., & Evans, N. P. (2015*). 'Four Seasons' in an animal rescue centre; classical music reduces environmental stress in kennelled dogs*. *Physiology & behavior*, *143*, 70-82.

- Bradley, M. M., & Lang, P. J. (2000). Measuring emotion: Behavior, feeling, and physiology.

- Bulagang, A. F., Weng, N. G., Mountstephens, J., & Teo, J. (2020). A review of recent approaches for emotion classification using electrocardiography and electrodermography signals. *Informatics in Medicine Unlocked*, *20*, 100363.

- Cook, P. F. (2017). Studying dog emotion beyond expression and without concern for feeling. *Animal Sentience*, *2*(14), 15.

- Craig, M., Rand, J., Mesch, R., Shyan-Norwalt, M., Morton, J., & Flickinger, E. (2012). Domestic dogs (Canis familiaris) and the radial arm maze: Spatial memory and serial position effects. *Journal of Comparative Psychology*, *126*(3), 233.

- Csoltova, E., & Mehinagic, E. (2020). Where do we stand in the domestic dog (Canis familiaris) positive-emotion assessment: A state-of-the-art review and future directions. *Frontiers in Psychology*, 2131.

- Franzini de Souza, C. C., Dias, D. P. M., Souza, R. N. D., & Medeiros, M. A. D. (2018). Use of behavioural and physiological responses for scoring sound sensitivity in dogs. *PloS one*, *13*(8), e0200618.

- Franzoni, V., Milani, A., Biondi, G., & Micheli, F. (2019, October). A Preliminary work on dog emotion recognition. In *IEEE/WIC/ACM International Conference on Web Intelligence-Companion Volume* (pp. 91-96).

- Gerdes, A. B., Wieser, M. J., & Alpers, G. W. (2014). Emotional pictures and sounds: a review of multimodal interactions of emotion cues in multiple domains. *Frontiers in psychology*, *5*, 1351.

- Grigg, E. K., Chou, J., Parker, E., Gatesy-Davis, A., Clarkson, S. T., & Hart, L. A. (2021). Stress-Related Behaviors in Companion Dogs Exposed to Common Household Noises, and Owners' Interpretations of Their Dogs' Behaviors. *Frontiers in veterinary science*, 1345.

- Huber, A., Barber, A. L., Faragó, T., Müller, C. A., & Huber, L. (2017). Investigating emotional contagion in dogs (Canis familiaris) to emotional sounds of humans and conspecifics. *Animal Cognition*, *20*(4), 703-715.

- Izard, C. E. (2009). Emotion theory and research: Highlights, unanswered questions, and emerging issues. *Annual review of psychology*, *60*, 1-25.

- Jungé, J. A., Scholl, B. J., & Chun, M. M. (2007). How is spatial context learning integrated over signal versus noise? A primacy effect in contextual cueing. *Visual cognition*, *15*(1), 1-11.

- Kogan, L. R., Schoenfeld-Tacher, R., & Simon, A. A. (2012). Behavioral effects of auditory stimulation on kenneled dogs. *Journal of Veterinary Behavior*, *7*(5), 268-275.

- Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. *Neuroscience & Biobehavioral Reviews*, *26*(1), 91-104.

- Lindig, A. M., McGreevy, P. D., & Crean, A. J. (2020). Musical dogs: A review of the influence of auditory enrichment on canine health and behavior. *Animals*, *10*(1), 127.

- Mendl, M., Burman, O. H., & Paul, E. S. (2010). An integrative and functional framework for the study of animal emotion and mood. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1696), 2895-2904.

- Nardelli, M., Valenza, G., Greco, A., Lanata, A., & Scilingo, E. P. (2015). Recognizing emotions induced by affective sounds through heart rate variability. *IEEE Transactions on Affective Computing*, *6*(4), 385-394.

- Noldus, L. P. J. J. (1991). The Observer: a software system for collection and analysis of observational data. *Behavior Research Methods, Instruments, & Computers*, *23*(3), 415-429.
- Pitchforth, A. (2010). *Emotional response to auditory and visual stimuli*. Loma Linda University.
- Rolls, E. T. (1990). A theory of emotion, and its application to understanding the neural basis of emotion. *Cognition & Emotion*, *4*(3), 161-190.
- Smit, I., Szabo, D., & Kubinyi, E. (2019). Age-related positivity effect on behavioural responses of dogs to human vocalisations. *Scientific Reports*, *9*(1), 1-10.
- Tune, D. L. A. (2020). Dogs & Music: Do Dogs Like Music?.
- Wynne, C. D., & Udell, M. A. (2020). *Animal cognition: Evolution, behavior and cognition*. Bloomsbury Publishing.
- Yeates, J. W., & Main, D. C. (2008). Assessment of positive welfare: A review. *The Veterinary Journal*, *175*(3), 293-300.