The effect of training on spontaneous blink rate in dogs

Effekten av träning på spontan blinkfrekvens hos hundar

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ABSTRACT

The spontaneous blink rate is found to be a non-invasive but functional, reliable and useful way to monitor central dopaminergic activity. Dopamine is associated with a range of functions such as reward, motivation and learning. It plays an important role in goal-directed behavior and in maintaining a behavior once a task is learned. As far as the author knows, there are today no publications that discuss the topic regarding the possible link between training and spontaneous blink rate in dogs. Therefore, eight privately owned dogs were included in this study to determine if there was a significant difference in the spontaneous blink rate before and after the process of learning a task. If there was a significant difference in the spontaneous blink rate, a secondary aim was to determine if the change showed a consistent pattern during the training process. It was predicted that the spontaneous blink rate would increase in accordance with changes in dopaminergic activity associated with the activation of neuro-affective circuits associated with reward acquisition during the establishment of the response and furthermore that the spontaneous blink rate would decrease as the behaviour shifted from a goal directed behaviour to a habitual response. For the two dogs that reached criterion the data showed a similar trend where the blink rate per minute was decreased during training and returned towards baseline levels post threshold criterion. A significant difference was found in blink rate between baseline and training for the two dogs that reached criterion. From this study it was not possible to conclude that the spontaneous blink rate reflects dopaminergic activity. However, one conclusion that can be drawn from this study was that there is a change in blink rate during training compared to baseline for the two dogs that reached criterion. Further studies on larger sample sizes are necessary to assess the effect of training on spontaneous blink rate in dogs. For any further studies, it is strongly recommended accurate and careful recruitment of individuals is essential to ensure that they are suitable for such training.

SAMMANFATTNING

Den spontana blinkfrekvensen har visat sig vara ett icke invasivt men ett funktionellt, pålitligt och användbart sätt att övervaka den centrala dopminaktiviteten. Dopamin är associerat med en rad funktioner såsom belöning, motivation och inlärning. Det spelar en viktig roll inom målinriktade beteenden och upprätthållandet av inlärda beteenden. Såvitt författaren vet finns inga publikationer som behandlar den möjliga länken mellan träning och spontan blinkfrekvens hos hund. Åtta privatägda hundar deltog därför i denna studie för att fastställa om det fanns någon signifikant skillnad i den spontana blinkfrekvensen före och efter en inlärningsprocess. Om det fanns en signifikant skillnad i den spontana blinkfrekvensen var ett sekundärt syfte med studien att fastställa om skillnaden visade ett konsekvent mönster under inlärningsprocessen. Det var väntat att den spontana blinkfrekvensen skulle öka i enlighet med förändringar i dopaminaktivitet som är associerat med aktiveringen av neuroaffektiva kretsar associerade med mottagandet av en belöning under etableringen av responsen och dessutom att den spontana blinkfrekvensen skulle avta när beteendet gick från ett målinriktat beteende till en invand respons. Data för de två hundar som nådde kriteriet visade samma trend, där blinkfrekvensen per minut avtog under träning och återvände mot baslinjen post kriterium. Det fanns en signifikant skillnad i blinkfrekvens mellan baslinjen och under träning för de två hundar som nådde kriteriet. Det var inte möjligt utifrån den här studien att dra slutsatsen att den spontana blinkfrekvensen speglar dopaminaktiviteten. Däremot, den slutsats som kan dras är att det fanns en skillnad i blinkfrekvens under träning i jämförelse med baslinjen för de två hundar som nådde kriteriet. Ytterligare studier med större stickprov är nödvändiga för att bedöma effekten av träning på den spontana blinkfrekvensen hos hund. Det är starkt rekommenderat att rekryteringen av individer för vidare studier är noggrann för att säkerställa att hundarna är lämpliga för den här typen av träning.

1. INTRODUCTION

Dopamine is used at all times by the brain to control nerve signals which in turn controls the movements of the body. A lack of dopamine thus leads to an impairment of the control of body movements. A blink is a body movement that is affected by dopamine levels. There are different types of blinks which are voluntary, triggered by reflex or spontaneous. A spontaneous eye-blink differs from voluntary and reflexive blinks in terms of a shorter duration. The spontaneous blink rate is usually recorded during several minutes and expressed as blinks per minute.

The spontaneous blink rate differs between individuals (Blin et al., 1990; Chen et al., 2003; Colzato et al., 2008) species, age (Blount, 1927) and time of the day (Barbato et al., 2000) etc. In addition, it has been found that the spontaneous blink rate is affected not only by corneal drying but by behavioral states and physiological factors such as arousal (Stern et al., 1984; Tanaka and Yamaoka, 1993), nervousness (Hall, 1945), anger (Ponder and Kennedy, 1927), anxiety (Stern et al., 1984; Tanaka and Yamaoka, 1993) sleepiness (Stern et al., 1984; Barbato et al., 2007), clinical status (Tecce et al., 1978) and cognitive workload such as speaking (Hall, 1945; Karson, 1983) reading, memorizing (Karson, 1983) and task performance (Stern et al., 1984; Tanaka and Yamaoka, 1993; Chermahini and Hommel, 2010). It has been found that human individuals performing stereotypic behaviours may have an altered blink rate. Elderly adults performing sterotyped behavior have a reduction in blink rate compared to individuals not engaged in any stereotyped behavior (Roebel and MacLean, 2007). Results from a study made by (Lethbridge, 2011) indicate that horses performing stereotypic behavior such as crib-biting show an elevated spontaneous blink rate.

The spontaneous blink rate is a non-invasive but functional, reliable and useful way to monitor central dopaminergic activity (Karson, 1983; Blin et al., 1990; Taylor et al., 1999; Colzato et al., 2008; Colzato et al., 2009; Barbato et al., 2012). In addition, spontaneous blink rate can be used to reliably predict behavioural performance in cognitive tasks associated with dopaminergic function (Colzato et al., 2007). Individuals with a high spontaneous blink rate also have high basal dopaminergic activity (Colzato et al., 2008). As it is believed that spontaneous blink rate reflects the tonic dopamine level one can also assume that a high blink rate reflects a high tonic dopamine level (Colzato et al., 2008). A high tonic dopamine level enables high phasic levels which in turn results in more efficient gating (Colzato et al., 2008).

Stern et al. (1984) concluded that attention will lead to a reduced blink rate and where the reduction is proportionate to the level of attention needed. A blink tends to occur when the attention is decreased and thus marks a short interruption of information intake (Stern et al., 1984). A more recent report (Tanaka and Yamaoka, 1993) found that an eye blink occur after information processing in the brain and thus when a task is more difficult (higher level of attention) more information needs to be processed and more blinks occur. The conclusions are contradictory and whether the blink rate will increase or decrease might be due to the type of task and for example what senses the task focuses on. The tasks included in the study made by Tanaka and Yamaoka (1993) was mental arithmetic tasks. Stern et al. (1984) discussed both mental arithmetic tasks and tasks such as reading.

Dopamine effects and regulates some cognitive processes (Chermahini and Hommel, 2010) and has a key role in cognitive function, including the working memory processes (Williams and Goldman-Rakic, 1995; Goldman-Rakic et al., 2000; Braver and Barch, 2002; Hazy et al., 2006). Dopamine is associated with a range of functions such as reward (Goldman-Rakic et al., 2000), motivation and learning (Wise, 2004). Learning is defined as a change in response to a specific stimulus and memory is the modification in the cells which mediates that change (Arias-Carrión et al., 2010). Williams and Goldman-Rakic (1995) found that the parts of prefrontal cortex that are referred to as the "memory fields" are modulated by dopamine. These findings reveal how normal cognitive processes are modulated by endogenous factors (Goldman-Rakic et al., 2000). Dopamine is one of the endogenous substances that take part in the forming of memories (Arias-Carrión et al., 2010) by projecting the prefrontal cortex and through modulations of neurons and the dynamics (Braver et al., 1999). In simple terms, this is done by modulations and synchronization in activity of specific nucleus in the brain, such as nuclei accumbens, where dopamine favor memory of events or happenings that are of motivational significance (Arias-Carrión et al., 2010). Mostly, an individual is motivated to return to a site, situation or to cues that lead to rewards and dopamine plays an important part in the selection between a neutral stimulus and reward giving ones (Wise, 2004; Arias-Carrión et al., 2010). This system serves a learning function since it may determine what information shall be preserved or not and also the regulation of when information is updated (Colzato et al., 2008). By increased cortical dopamine activity information is updated in the working memory, where it is maintained until the next phasic dopamine increase. Thus, equilibrium between stability (ability to ignore any distractions) and flexibility (ability to change attention) is believed to be maintained by the level of dopamine. Dopamine is therefore an important factor in attentional selection (Colzato et al., 2008).

When a response is established it stays somewhat autonomous unless the motivational stimulus has dropped in value for the individual or disappeared (Arias-Carrión et al., 2010). Wise (2004) drew the conclusion that dopamine plays an important role in goal-directed behavior and in maintaining a behavior once a task is learned. This seems to be done through associations to response-reward and stimulus-reward that are important for controlling the motivated behavior of experiences from the past (Wise, 2004). Stimuli which are associated with a reward tend to induce bigger dopamine increases, and therefore it is more likely that they will be attended. Therefore dopaminergic systems are involved when positive reinforcement is used in training (Overall, 2011).

When using positive reinforcement there is an increase in central dopaminergic level which enhances learning and makes the individual learn even more about the positive effect of their behaviour (Hazy et al., 2006). Associations of stimulus-reward during Pavlovian conditioning fall flat when blocking dopamine systems (Wise, 2004). This means that when dopamine is impaired or blocked the association between a normally reinforcing stimuli, such as food, and an event fail to occur.

For this study, continuous reinforcement was used which refers to reinforcing the dog for each correct response, since this results in the most rapid assimilation of an initial response (Overall, 2011). However, dogs were not reinforced after a certain fixed time period but variably. In other words, dogs were reinforced for every correct response of chin targeting and were the mean time spent in contact with target was gradually increased (variable), adapted to every individual.

1.1 Aim

As far as the author knows, there are today no publications that discuss the topic regarding the possible link between training and spontaneous blink rate in dogs. Therefore, the aim of this study was to determine if there was a significant difference in the spontaneous blink rate before and after the process of learning a task in dogs. If there was a significant difference in the spontaneous blink rate, a secondary aim was to determine if the change showed a consistent pattern during the training process. It was predicted that the spontaneous blink rate would increase in accordance with changes in dopaminergic activity associated with the activation of neuro-affective circuits associated with reward acquisition during the establishment of the response and furthermore that the spontaneous blink rate would decrease as the behaviour shifted from a goal directed behaviour to a habitual response, i.e. when the dog had learnet the task.

The project was approved by the School of Life Sciences research ethics committee, with application reference no: SLS1/12.

2. PILOT STUDY

2.1 Preliminary skills training

To familiarize with the equipment, to practise using the Solomon Coder program and to find out important information for the recording of blink rate in animals, such as angle and light intensity, some video recordings of two privately owned cats and their faces were taken.

2.2 Material and Method

2.2.1 Subjects

Two privately owned dogs (Table 1) recruited from the staff at the University of Lincoln, Lincoln, United Kingdom, were used for the pilot study, one Labrador retriever and one Saluki Lurcher. The Labrador had previous experience of both clicker training and chin targeting and was therefore not a candidate in question for the final experiment but used for the initial part of the pilot study. The Saluki Lurcher had also previous experience of clicker training but not chin targeting and was therefore a good candidate for the latter part of the pilot study.

Table 1. The two dogs included in the pilot study with information about their gender, breed and age.

Individual	Gender	Breed	Age
1	Female	Labrador	12 years
2	Female	Saluki Lurcher	10 months

2.2.2 Material

Two video cameras were used to record the training of the dogs and their blink rate. One Panasonic (SDR-H100, SD/HDD) and one JVC (GZ-MG155EK, hard disk camcorder).

The light intensity in the room was measured using a digital lux meter (LX1330B). The target was made of Pro Driver car sponges (22cm*12cm*6cm) split in two lengthwise which were attached to a brick (16cm*12cm*6cm) using rubber bands. Before the car sponges were split they were soaked in water for 18 hours and the water was changed once after six hours, this was done to remove possible chemicals or unpleasant smell from the new sponges. Underside each sponge half the dogs' name was written to be enable to distinguish which sponge belonged to which dog. This means that the dogs had their own sponge throughout the training. Brown pieces of tape were attached to the floor to facilitate the positioning of the tripods with video cameras and the target so that the dogs and cameras would be at the approximate same position of the room every session. However, the positions of the cameras were adjusted according to the position of the dog for each trial. A green plastic barrier (Z4025/HDLG: Standard flex display rectangle set, size: 1.2m wide, 0.6m high and 1.5mm thick) was used for some dogs during forced baseline blink rate recordings to minimize the risk that the dogs' would lie down outside the range of the video camera and by that not obtain a recording of blink rate. Forced baseline blink rate refers to recording of the dogs spontaneous blink rate while in a lying position and held in its collar/harness by the trainer.

During the pilot work with the dogs it was difficult to clearly see the eyes and blink rate on a dark dog with dark eyes, like the case with the Labrador. Therefore a table lamp with a 20W light bulb was used to see if an extra light source could solve this issue. However, the extra light source only helped slightly and that was if it was placed within a meter of the dog and was directed straight at the dogs face. A light bulb with higher Watt could possibly make a difference but it was decided that an extra light source was yet another factor to take into account during the experiment. Therefore, no extra light source was used during the experiment. Instead the cameras were focused to record close-ups of the dogs faces. A mobile phone (Sony Ericsson WT19i) was used to know the time when a session started, ended and also for the start and end of an outdoor break. A stopwatch (Fisher Scientific) was used to keep track of the time between trials and during the forced baseline blink rate recording. The treats used depended on the individual. During the pilot study Schmackos (Pedigree) were used. To ensure that the dogs were not thirsty during training, it was decided that a bowl with water should be placed in the training room during the final experiment.

2.2.3 Experimental area

The pilot study took place at the University of Lincoln, Riseholme Park, Lincoln, United Kingdom during four consecutive weeks between May 28th and June 24th in 2012. All training sessions took place in the same room which was about 7.5m long, 5.4m wide and reached 2.60m to the ceiling. The room had two windows with blinds which were closed during all sessions. In addition, the door had a window with a height and width of 0.90m and 0.17m respectively. This window had no blinds but was covered with white paper.

The room was equipped with a large cupboard (1.80m high, 1.10m wide and 0.60m deep), a smaller cabinet on wheels (1.22m high, 0.63m wide and 0.53m deep) and a large table (2.20m*0.90m) each in separate corners of the room (Figure 1). Beside the large cupboard there was a small refrigerator and next to that a small table. The cupboard and cabinet were always closed and under and on top of the large table several chairs were placed. In the fourth corner of the room there was a low round table and two soft chairs next to each other on one side and four piles of chairs on the other side of the table. Near the cabinet on wheels another similar piece of furniture (0.95m high, 0.70m wide and 0.65m deep) was positioned with a computer screen and other computer accessories on top. This cabinet on wheels was used to place equipment such as mobile phone and treats used during a session.

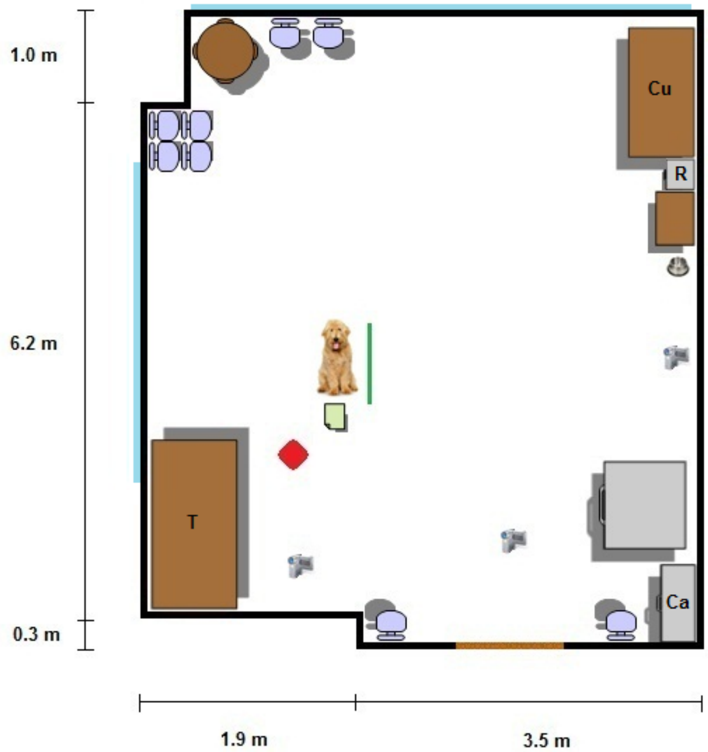

Figure 1.Overview of the test area. The blue lines represent the windows and the brown line the door. The brown rectangles represent the large table (T), the cupboard (Cu) and the small table (no letter). The brown circle represent the round table. The grey rectangles represent the refrigerator (R), the small cabinet (Ca) and the cabinet (no letter) used for placing equipment on top during the experiment. The chairs in the room show were the chairs were positioned, the green line represents the barrier and the water bowl shows were it was placed in the room. The video cameras show were they were positioned during different parts of the experiment, the red romb show were the trainer sat during training, the dog is facing the trainer and the beige rectangle is the tape attached to the floor to give the trainer a point of reference of were to ask the dog to lie down and present the target.

2.2.4 Experimental part of pilot study

The initial approach was to make the dogs put their heads through an opening in a wall where a camera was fixed to record their blink rate. This was planned to be done by lure and by reinforcing with treats, as often as needed, to make the dog hold its position for 60 seconds. Thereafter the plan was to gradually decrease the number of reinforcements until the dogs put their head through the opening and stayed there until reinforced once after 60 seconds. For several reasons such as the need to individualize the height of the opening, the dog being in a separate room from the trainer during sessions and the risk of dogs feeling tired of standing up during all sessions this approach was not chosen. The approach chosen instead was to record blink rate while dogs were lying down.

Dogs were let into the test room, their lead was removed and the dog was let to explore freely. The dog was ignored, apart from occasional eye contact and some physical contact in the case that the dog sought attention/contact. Once the dog lied down (settled) by itself for the first time and stayed down for at least 5 seconds, the baseline blink rate recording started. The JVC video camera was used during this phase since the Panasonic video camera made sounds whenever the record/pause button was pressed and sound may attract the dogs' attention etc. However, both cameras were used during the rest of the pilot study. When more than a minute had elapsed the dog was asked to stand up or lured up and given a treat. The blink rate was recorded again once the dog lied down (settled) the second time for at least five seconds and so on.

During the forced baseline blink rate phase it turned out to be easier to put the lead back on and lead the dog to the position of the recording of blink rate instead of holding the dogs' collar/harness and lead it. In addition it proved to be difficult to get recordings of the dogs' face for the forced baseline blink rate since the dogs did mostly not lie down in the exact area of recording or looked straight ahead. To minimize that problem a barrier was put up to the dogs' left side while the trainer always was positioned at the dogs' right side. In addition, the position of the two video cameras was changed. Instead of being placed directly in front of the dog and at 90 degrees to the left of the dog one was placed slightly to the right of the dog and the other at about a 45 degree angle to the dogs' left (Figure 1).

The initial training technique was to present the target, lure the dog to put its head on the target and give it treats, as often as needed, to make it hold its' position on the target for 60 seconds. Once that was accomplished the idea was to successively increase the time to 90 seconds and thereafter successively reduce the number of treats until the dog put its' head on the target, once presented, and stayed in contact with target until reinforced, once, after 90 seconds. The reason why 90 seconds was chosen was that the dog would not expect any treat before 60 seconds and a one minute block of data would be collected and still a variable reinforcement schedule (between 60 and 90 seconds) would be possible.

To facilitate recording of the dog's blink rate newspaper was taped to the floor, shaped as a rectangle with the shorter side facing the door, in the middle of the room. The idea was that the dogs would be asked to lie down in this area only, during training, facing the camera. It was found that by reinforcing to keep the dog in position on the target was not a preferable method since it made the dog too food focused and did not pay attention to the target and did not seem to attend to the position of its' chin in relation to target and time of reinforcement. Therefore another approach was used - based on clicker training (Shaw, 2009). However, instead of a clicker, a word was used. To make sure it was a new word to the dogs and a word that was not similar to any word they knew the Swedish word for "good" ("bra") was chosen. In addition, it was important that the dogs would be able to lie down and stay still with its chin on the target until hearing the word "bra" even if the trainer held treats in her hands and therefore another two phases were added to the training schedule. During these two pree-training phases the dogs learned to wait for the treat to come to them, and not try to take a treat until the word "bra" was said. The dogs could initially chose in which position they wanted to wait but during the second phase they had to wait from a lying position. To make sure all dogs had the same minimum basic experience regarding liying and staying down for a few seconds, when asked or when lured, an additional (third) pre-training phase was added.

At first, a clock was used to keep visual track of time during trials but it turned out to be more difficult than expected to look at the clock and stay focused on the dog and the training, even though the clock was placed in front of the trainer. Therefore the clock was placed on top of the table, close to the trainer so that the ticking sound of the second hand helped the trainer to keep track of time and still be able observe the dogs. Notes about each session, such as day, time, type of treat, number of trials etc. were written in a notepad. The pilot study helped find out the necessary questions to ask the dog owners for the main experiment.

3. MATERIAL AND METHOD

3.1 Subjects

Eight dogs were included in this study, four males and four females (Table 2). They were privately owned and recruited from the staff at the University of Lincoln, Lincoln, United Kingdom. Before the onset of the experiment every owner had to ensure that they gave their full consent for their dog to participate in this study by signing an informed consent form (Appendix 1) and also answer questions about their dog according to a form (Appendix 2) written by the experimenter/trainer.

Table 2. The eight privately owned dogs included in the study with information about their gender, breed and age.

Individual	Gender	Breed	Age
1	Male	Border Collie	8 months
2	Male	Labrador	7 years
3	Female	Beagle - Cross	5 years
4	Female	Staffie - Cross	6 years
5	Female	Terrier - Cross	2 years
6	Male	Border Collie	6 years
7	Female	Border Collie	3,5 years
8	Male	Collie - Cross	10 years

3.2 Material

The same material which was used during the pilot study was used during the experiment. However, some adjustments were done. Another target was made for the Terrier-Cross, due to the individuals’ small size, using a plastic container filled with soil (about 11.5cm*10.0cm*4.5cm) and a sponge (11cm*10cm*3cm) attached to it with rubber bands. Dogs had access to water from a bowl inside the training area. The bowl was rinsed and filled with new water every morning and refilled during the day if needed.

3.3 Experimental area

The study took place at the University of Lincoln, Riseholme Park, Lincoln, England during five consecutive weeks between June and August in 2012. The same room which was used during the pilot study was used during the experiment.

3.4 Experiment

Dogs were trained twice a week, on Mondays and Wednesdays or on Tuesdays and Thursdays (Table 3). Two individuals were trained in the morning and two individuals in the afternoon. Each dog was trained approximately the same time both days, every week, wherever possible. All recordings took place between 9.30 in the morning and 5.00 in the afternoon since the spontaneous blink rate is stable throughout the day, from morning until afternoon, with a significant increase around 8.30 pm (Barbato et al., 2000). Training started on the 25^{th} of June and continued for about five weeks, until the 30^{th} of July.

Table 3.The schedule for the eight dogs where each dog was trained approximately the same time both days, every week, wherever possible.

Time	Monday	Tuesday	Wednesday	Thursday	Friday
9.30 – 10.30	Dog 1	Dog 4	Dog 1	Dog 4	
11.00 – 12.00	Dog 2	Dog 3	Dog 2	Dog 3	
13.15 – 14.15	Dog 7	Dog 5	Dog 7	Dog 5	
14.45 – 15.45	Dog 8	Dog 6	Dog 8	Dog 6	

The dogs were picked up and returned to the owners' office or the university kennel by the trainer and walked to and from the experimental facility, also at campus. The exceptions were dog 7 which was brought to and picked up at the experimental facility by her owner and dog 8 which was left at the experimental facility by his owner.

The treats used were dependent on the individual dog, using information from the owner data form (appendix 2) the dog owners filled in before the experiment and also depending on how the dogs responded each training trial and session. Light intensity was measured twice per session, at the beginning and at the end. This was done on the same spot every time, which was on the tape on the floor placed there to give the trainer a point of reference of where to ask the dog to lie down and present the target.

Each training session did not exceed 60 minutes, including at least one 5-minute break outdoors with shorter (60 seconds) breaks indoors between the trials. Dog number seven did not have any outdoor breaks during any session but had at least one 5-minute break indoor instead. The nervous and fearful traits of dog 7 were the reason why the owner asked the trainer to exclude outdoor breaks during training sessions. All individuals were given time to habituate to the test arena together with the trainer prior to the experiment to get a chance to explore the novel place and trainer. The time to habituate was until the dog settled by itself for the first time and lied down for at least 5 seconds.

3.4.1 Baseline blink rate

The same procedure was used as for the pilot study, aiming for four separate intervals of one minute recordings or eight separate intervals of 30 seconds. There had to be a minimum of 30 seconds between the recordings.

3.4.2 Forced baseline blink rate

Dogs were, during this phase of recording, positioned at approximately the same location as for coming training, facing the door (Figure 1). Video cameras were attached to tripods. One video camera was placed in front of but slightly to the right of the dog and the other at about a 45 degree angle to the dogs' left. For some of the dogs, if needed, a barrier was placed at the dogs left side to prevent the dog from moving outside the recording area and the trainer was always positioned to the dogs' right side.

The trainer put the lead on and walked the dog to the position of recording for the forced baseline blink rate. The dog was held by its collar/harness, on its right side, until it settled (lied down). The trainer was sitting on the floor while holding the collar/harness. If the dog did not settle (lie down) within five minutes it was released and given another trial after a two minute break. If the dog wiggled and twisted and would not stop within a two minute period it was released and given another trial after a two minute break. Video recording the dogs blink rate aiming for four intervals of one minute or eight recordings of 30 seconds. After each recorded period the dog was asked to stand up or lured to stand up and given a break before next recording. There was always a minimum of 60 seconds between recordings.

3.4.3 Pre-training

Stage 1

Dogs were during all training facing the trainer and the trainer was always positioned facing away from the door. Training of the dogs was video recorded from the dog's left side without any focus on recording the blink rate. Dogs were trained to wait for the treat to come to them without attempts to take the treat. The dogs could chose in which position they wanted to wait. Trainer stood in front of the dog and raised the right hand while holding a treat and then slowly moving the treat vertically down towards the dogs face. When the dog was still and waited for the treat and its head was still the trainer said the Swedish word for "good" ("Bra") and gave the treat. "Bra" was then used throughout the training, instead of a clicker, as an indicator for the dog that the trial was completed and treat would come. When the dog moved towards the treat or tried to take the treat the arm went up in a raised position again. When the dog waited for the treat to come to it without any attempts to take the treat before the word "bra", eight out of ten consecutive times, the training proceeded to the next step.

Stage 2

Training of the dogs was video recorded in the same way as in stage 1. While in a sitting position on the floor, the trainer asked the dog to lie down or lured the dog into a lying position in front of the trainer. When the dog scored 3 from the scoring system (Table 4) and stayed down for a minimum of four seconds for a treat, eight out of ten consecutive times, the training proceeded to the next step. The same scoring system was also used during stage 4, 5, 6 and 7.

Table 4. Response scores and their definitions, modified from Fukuzawa et al., 2005.

Score	Definition
5	A complete and instant response to the cue.
4	A complete but delayed response to the cue with the delay to completion not exceeding 5 seconds.
3	A complete but delayed response to the cue with the delay to completion not exceeding 15 seconds.
2	An incomplete response to the cue; e.g. the dog sits or stands up from a lying position or make contact with the target with its' nose.
1	A nonspecific response to the cue; e.g. the dog orients towards the trainer, raises a paw or bark.
0	No response within 15 seconds of the cue.

Stage 3

Training of the dogs was video recorded in the same way as in stage 1 and 2. While in a sitting position on the floor, the trainer asked the dog to lie down or lured the dog into a lying position in front of the trainer. Dogs were trained to wait for the treat to come to them without attempts to take the treat from a lying position. The trainer sat in front of the dog and withdrew the right hand while holding a treat and then slowly moving the treat horizontally towards the dogs face. If the dog moved towards the treat or tried to take the treat the arm was withdrawn again. When the dog waited for the treat to come to it without any attempts to take the treat before the word "bra" was said, eight out of ten consecutive times, the training proceeded to the next step.

3.4.4 Training

Stage 4

During all following training the video camera was focused on the dog's face while in contact with the target. The dogs were asked to lie down or lured into a lying position in front of the trainer, near the tape mark on the floor. Target was presented and the dog was lured to put its chin on the target and once in the correct position, reinforced. This procedure was repeated five times and the sixth time a test trial was made. During a test trial the trainer presented the target and waited 15 seconds for the dogs' own initiative to chin target. If the dog scored at least a 3 from the scoring system during the test trial another test trial was made. In the case that the dog made three mistakes (less than 3 from the scoring system) in a row the trainer went back to do another five consecutive trials with a lure. After five trials with a lure, another test trial was made, and so on. Between each trial the target was removed from its position and placed where dogs did not have access to it, and that was done after every trial for all further training. When the dog scored at least a 3 from the scoring system, eight out of ten consecutive times, with or without lure, the training proceeded to the next step.

Stage 5

The dogs were asked to lie down or lured into a lying position in front of the trainer, near the tape mark on the floor. The target was presented and when the dog put its chin on the target, without any lure, it was reinforced immediately. The mean time the dog had its chin on the target was successively increased. If the dog removed itself from the target by sitting/standing up (score 2), responded in a nonspecific way to the target (score 1) or did not show interest in target for more than 15 seconds from presentation of the target (score 0), that trial was ended and target removed. When the dog scored at least a 3 from the scoring system and stayed in contact with the target for 90 seconds eight out of ten consecutive times the training proceeded to the next step.

Stage 6
A variable hold was then used on the response, according to a predetermined numerical sequence, where each number represented a 5 second window for reinforcement.

1=60-65 sec,
2=66-70 sec,
3=71-75 sec,
4=76-80 sec,
5=81-85 sec and
6=86-90 sec.

The time for reinforcement varied between 60 and 90 seconds, in the following random generated sequence: 3, 4, 4, 2, 2, 5, 1, 6, 3, 2, 6, 1, 4, 5, 5, 4, 5, 2, 5, 3, 6, 1, 5, 6, 2, 2, 3, 5, 4, 4, 2, 1.

3.4.5 Post criterion

When the dog scored a 5 from the scoring system and stayed with its chin in contact with the target until reinforced (60 to 90 seconds), nine out of ten times, the dog had reached the threshold for when the response was established.

Stage 7
The target continued to be presented and the dog was reinforced between 60 and 90 seconds, according to the random generated sequence presented at stage 6, for the remaining trials of the experiment.

3.5 Data coding methods

Dogs were videotaped during all training sessions. Video files were run in the Solomon Coder program and scoring began when target was presented and coding began when the dog put its chin on the target and until the word “bra” was said or the dog removed itself from target. To be able to make the coding needed for this experiment it was important to have clear definitions of a blink. Modified definitions (Table 5) from Blount (1927) were used. Blink and half-blink were registered as a blink while wink and full closure were not.

Table 5. Eye lid movements and their definitions, modified from Blount, 1927.

Eye lid movements	Definition
A blink	A temporary closure of both eyes, involving movements of the upper and lower eyelids. The pupil is momentarily hidden from view, but the eyeball does not necessarily move its position to an observable extent.
A wink	A similar movement to “a blink” but of one eye only.
A half-blink	May or may not involve the lower eyelids. The upper eyelids are always lowered and approach the lower lids. Half or less than half of the eyeball may be visible.
A full closure	Is much the same as a prolonged blink, but the edges of the eyelids remain together for a considerable time.

3.6 Statistical analysis

Out of the eight dogs which were included in this study, two dogs (number 1 and 8) did not learn (did not reach 15 seconds in contact with target), two dogs (number 2 and 5) learned slowly (reached about 30 seconds in contact with target) and time ran out and they had to be excluded from analysis. Two dogs (number 6 and 7) had to restart training because they started to offer new or complementing behaviours to the point that the training had to go back to scratch, and did not reach criterion and were therefore excluded as well. The two remaining individuals (number 3 and 4) learned efficiently and reached ninety seconds in contact with the target. Statistical analysis was focused on these two dogs.

The data collected was transferred into Microsoft Excel and blinks were changed into blinks per minute by dividing the number of blinks with the recorded duration in seconds and multiplying by 60. No recorded durations less than 15 seconds were used during data analysis since the risk of the calculated blink rate differing too much from the accurate frequency. For the baseline blink rate and the forced baseline blink rate only recordings from the first 30 seconds were used for all dogs. This was done to eliminate any differences in blink rate due to dissimilar length of data recordings and the change into blinks per minute.

Only data from trials where the dog seemed focused on the task, by staying in contact with the target until the word “bra” was said, were used. Dog 3 had a total of 258 training trials (excluding pre-training phases and baseline phases) and 73 trials (including 12 trials from baseline phases) were used for analysis. For dog 4 the total number of training trials (excluding pre-training phases and baseline phases) was 276 and 139 trials (including 14 trials from baseline phases) were used for analysis. The original threshold for when the response was established was when the dog scored a 5 from the scoring system and stayed with its chin in contact with the target until reinforced (60 to 90 seconds), nine out of ten times. Since dog 4 had few trials post criterion (16 trials) and dog 3 had none the threshold for criterion was changed to be able to analyse the data. The threshold used during data analysis was when the dog scored at least a 5 from the scoring system and stayed in contact with the target for 90 seconds, eight out of ten consecutive trials. That is, the threshold for stage 5.

The data collected was analysed in Minitab. Data was checked for normality using Kolmogorov-Smirnov test and also checked for homogenity of variance using Levene's test and was found to be normally distributed and with no difference in heterogeniety. To test statistical significance within dog the parametric ANOVA test was used, with training stage as a factor affecting blink rate, with post hoc Tukey tests for pariwise comparisons to identify the source of any significance. No analysis were done between individuals and ANOVA was therefore only used to analyze the variance within dog.

4. RESULTS

Data was divided into four phases: Baseline blink rate, forced baseline blink rate, training and post criterion. For the two dogs that reached criterion a significant difference was found in blink rate between baseline and training. For dog 3 the mean blink rate for the different phases are presented in table 6.

Table 6. Mean blink rate (± SD) for the different phases for the two dogs that reached criterion.

Individual	Phase	Mean blink rate ± SD
Dog number 3	Baseline blink rate	16.86 ± 5.40
	Forced baseline blink rate	17.20 ± 8.67
	Training	7.46 ± 3.72
	Post criterion	11.00 ± 4.23
Dog number 4	Baseline blink rate	11.00 ± 4.00
	Forced baseline blink rate	10.00 ± 4.38
	Training	6.42 ± 4.61
	Post criterion	9.89 ± 4.05

A significant difference in blink rate between training stages was found ($F = 15.31$; $p = < 0.001$) for dog 3. Post hoc pairwise comparisons were made and significant difference in blink rate was found between baseline blink rate and training ($T = -5.244$; $p < 0.0001$) and also between baseline blink rate and post criterion ($T = -2.896$; $p = 0.0255$) for dog number three (Table 7). A siginifcant difference was also found between forced baseline blink rate and training ($T = -4.683$; $p = 0.0001$) and between forced baseline blink rate and post criterion ($T = -2.717$; $p = 0.0405$) for the same dog.

The mean blink rate for dog 4 and the four phases are presented in table 6. A significant difference in blink rate between training stages was found ($F = 6.83$; < 0.001) for dog 4. Post hoc pairwise comparisons were also made for dog 4 and a significant difference in blink rate was also found between baseline blink rate and training ($T = -2.789$; $p = 0.0305$) for this individual (Table 7). In addition, a significant difference was found between training and post criterion ($T = -3.625$; $p = 0.0023$). The data for dog 3 and 4 show a similar trend (Figure 2 and 3) where the blink rate per minute is decreased during training and return towards baseline levels post criterion.

Table 7. Summary of results from post hoc Tuckey test between phases within dog.

Individual	Comparison	Result
Dog number 3	Baseline blink rate vs. Forced baseline blink rate	ns
	Baseline blink rate vs. Training	T=− 5.244; p < 0.0001
	Baseline blink rate vs. Post criterion	T= − 2.896; p = 0.0255
	Forced baseline blink rate vs. Training	T= − 4.683; p = 0.0001
	Forced baseline blink rate vs. Post criterion	T= − 2.717; p = 0.0405
	Training vs. Post criterion	ns
Dog number 4	Baseline blink rate vs. Forced baseline blink rate	ns
	Baseline blink rate vs. Training	T= − 2.789; p = 0.0305
	Baseline blink rate vs. Post criterion	ns
	Forced baseline blink rate vs. Training	ns
	Forced baseline blink rate vs. Post criterion	ns
	Training vs. Post criterion	T= − 3.625; p = 0.0023

Key: ns = non significant (p > 0.05)

The two dogs (number 2 and 7) that learned slowly and reached a mean of 30-40 seconds of chin targeting had a total of 376 and 444 training trials respectively (Table 8). From the 376 training trials for dog number two 97 (including 13 trials from baseline phases) were used to calculate the mean blink rate per phase and the corresponding number for dog number seven was 144 (including 16 trials from baseline phases). Since they did not reach criterion they only had data for the first three phases: Baseline blink rate, forced baseline blink rate and training. The mean blink rate for dog 2 and the three phases was 10.86 ± 10.06, 22.67 ± 4.32 and 13.07 ± 6.91 respectively. For dog 7 the mean blink rate was 10.50 ± 5.63 (baseline blink rate), 7.50 ± 3.66 (forced baseline blink rate) and 5.77 ± 3.12 (training).

Pairwise comparisons were made and a significant difference in blink rate was found between baseline blink rate and forced baseline blink rate (T = 3.0129; p = 0.0092) and between forced baseline blink rate and training (T = − 3.223; p = 0.0049) for dog 2. For dog 7 no pairwise comparisons were made since the data was not homogenous (Levene's test, p = 0.002).

Light intensity was measured twice every session with a mean of 333 Lux (max = 355 Lux; min = 311 Lux) for the two dogs that reached criterion.

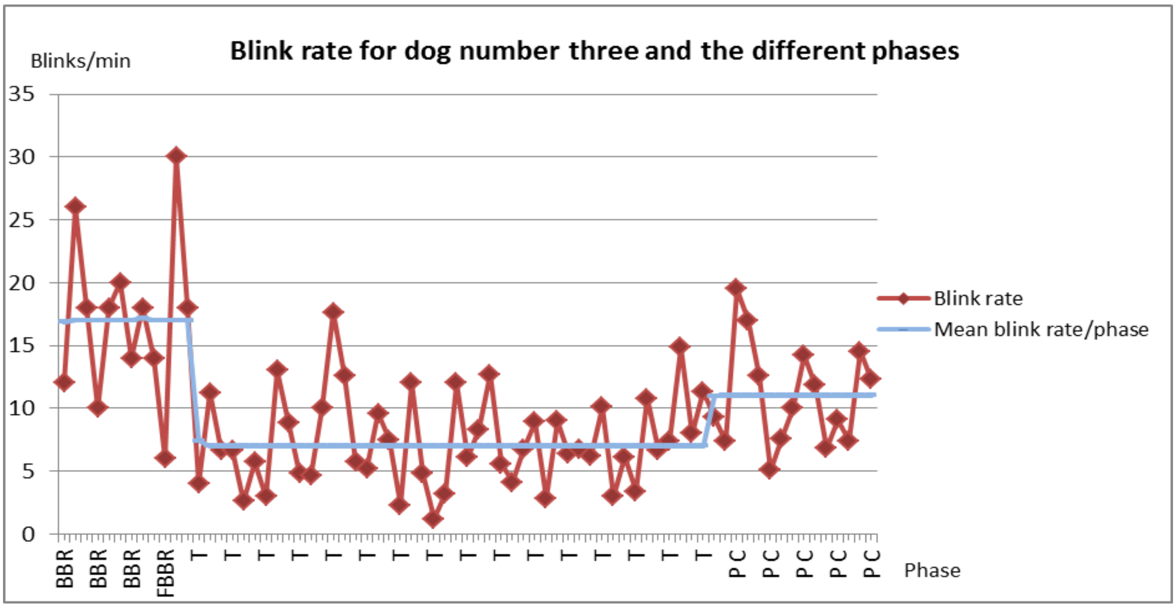

Figure 2. The blink rate per minute for each trial of the experiment and the mean blink rate per phase for dog number three. The phases are BBR = Baseline blink rate, FBBR = Forced baseline blink rate, T = training and P C = Post criterion. During the baseline blink rate phase recording took place when dogs settled (lied down) by themselves anywhere in the test area while during the forced baseline blink rate phase recordings took place while dogs were held by their collar/harness by the trainer. The training included chin targeting a sponge and no training trials shorter than 15 seconds of contact with target are included in this figure. Criterion was reached when dogs scored 5 (A complete and instant response to the cue) from the scoring system and stayed in contact with the target for 90 seconds, eight out of ten consecutive trials.

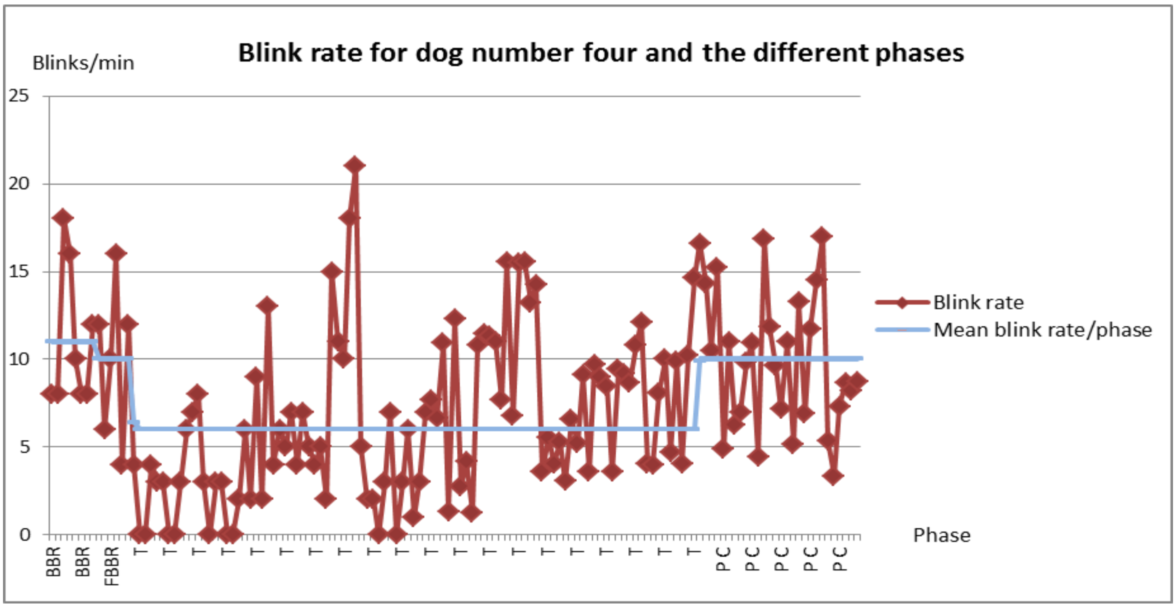

Figure 3. The blink rate per minute for each trial of the experiment and the mean blink rate per phase for dog number four. The phases are BBR = Baseline blink rate, FBBR = Forced baseline blink rate, T = training and P C = Post criterion. During the baseline blink rate phase recording took place when dogs settled (lied down) by themselves anywhere in the test area while during the forced baseline blink rate phase recordings took place while dogs were held by their collar/harness by the trainer. The training included chin targeting a sponge and no training trials shorter than 15 seconds of contact with target are included in this figure. Criterion was reached when dogs scored 5 (A complete and instant response to the cue) from the scoring system and stayed in contact with the target for 90 seconds, eight out of ten consecutive trials.

Table 8. Total number of training trials (excluding pre-training phases and baseline phases) and mean (last five completed trails) duration on target by the end of the study for all eight dogs.

Individual	Total number of training trials	Mean duration (sec) on target by end of study
1	210	3
2	376	37
3	258	Reached criterion
4	276	Reached criterion
5	320	19
6	478	9
7	444	33
8	240	4

5. DISCUSSION AND CONCLUSION

The central finding of this study was that training had an effect on blink rate in the two dogs that reached criterion. Results showed a significant difference in blink rate between baseline and training in the two dogs which reached criterion. The data for these two dogs showed a similar trend (Figure 2 and 3) where the blink rate per minute was decreased during training and returned towards baseline levels post criterion. This reduction in blink rate during training could be a focused attention effect (Stern et al., 1984).

During training operant conditioning was used which allows the dogs to influence the reward rate. However, the dogs need to find the treats or the training itself rewarding, otherwise they will not find the tradeoff between work and reward profitable. In addition, Rooney (2011) concluded that reward-based training is not only associated with benefits including improved ability when training a new task but also improved welfare of the individual. To be able to train all dogs twice a week it was not possible to recruit more than eight dogs, since owners had to bring the dogs to campus every day their dog had a training session and the trainer had to bring and return dogs to their office or kennel which is not time efficient. One day per week (Friday) was free from any scheduled training to enable to move a training session in case a dog was ill or the owner was unable to bring the dog to the campus etc.

Light intensity was found to be stable throughout the experimental period and thus would not have influenced the blink rate changes found between baseline levels and training in the two dogs that reached criterion.

The approach to use a word instead of a clicker was done solely to facilitate the practical training procedure since the trainer had both treats to hold and a heavy target to move. However, the experimenter was aware of the fact that the sound of a clicker is the same every time it is used while a spoken word is not.

The time given to the dogs to habituate in the training room was until the dogs settled by themselves for the first time and lied down for at least five seconds. This solution was chosen since dogs were not supposed to follow any directions or responding to a cue during baseline blink rate recordings. It was difficult to obtain blink rate recordings for some of the dogs during the baseline blink rate phases. The trainer, which also made the recordings, tried to make the dogs face the direction of the video camera by moving a bit from side to side, make sounds or scratch the carpet with the feet. During one session a person, that was unfamiliar to the dog, stood outside the training room and made sounds to make the dog face that direction to be able to obtain blink rate recordings. However, these recordings were not used for analysis since the individual in question did not reach over a 15 seconds hold on the target during training.

Dogs were held by their lead when taking them to the position for the forced baseline blink rate recordings because most dogs are more accustomed to that procedure than being held by the collar/harness when led.

The aim of this study was to determine if there was a significant difference in the spontaneous blink rate before and after the process of learning a task in dogs. Results indicate that there was a significant difference in blink rate between training and baseline in the two dogs that reached criterion. A secondary aim was to determine if the change showed a consistent pattern during the training process, predicting an increased blink rate during the establishment of the response and a decrease in blink rate once the behaviour shifted from a goal directed behaviour to a habitual response. Opposite to the prediction, the spontaneous blink rate decreased during training and returned towards baseline levels once the response was established. Reasons to this might be that the dogs stayed focused on the task and therefore the blink rate went down (Stern et al., 1984). Studies have shown that blink rate vary according to the type of task performed (Chermahini and Hommel, 2010; Tanaka and Yamaoka, 1993; Hall, 1945; Karson, 1983; Stern et al., 1984).

Results from this study can be seen as a valuable contribution to the subject of biology since it may help to understand the relationship between spontaneous blink rate and the process of training and learning in dogs. If we know more about blink rate and dopamine during the process of training and learning in dogs, blink rate might be a useful tool that may allow for the development of more effective training methods or ways to measure/indicate the level of knowledge obtained during training with the training goal as a reference. Results from this study may therefore be used to improve dog training techniques and by that maybe also improve their welfare during training. However, further research is needed to help understand what type of task that result in increases or decreases in spontaneous blink rate in dogs.

The two dogs that reached criterion, (dogs 3 and 4) had a total of 258 and 276 trials respectively. Dog 4 had previous experience of clicker training, was calm, patient, keen on training and had a will to please. Dog 3 was used to be handled and trained by different and unknown people. It was unclear whether this individual had any previous experience of clicker training so it was assumed that if this individual had such experience it was not much. However, training was seen as something fun and positive and treats as very rewarding to work for according to this individual. The data for dog 4 and dog 3 showed a similar trend, as presented earlier in Figures 2 and 3. Dog 3 had fewer number of trials and that may be a reason to why that graph did not show a clear trend as the graph for dog 4, where the blink rate returns to baseline levels at post criterion.

As shown in Table 8 dogs' number 2, 5, 6 and 7 had between 320 and 478 numbers of trials but still did not reach criterion. One of the two individuals that reached over 30 seconds was dog number 2 which had 376 trials. This dog was a gun dog and had little previous experience of everyday training and no experience of clicker training. Dog 7 was the other dog that reached over 30 seconds with 444 trials. After 320 trials dog 5 had reached a mean of 19 seconds in contact with the target. This individual was lacking in sufficient training experience to understand the principles and also did not fully understand the release word, stated by the owner. A release word is used to inform the dog that a trial/task is over. In addition, this dog was high in energy and had difficulties concentrating. These things together caused some frustration at the beginning of the experiment.

Dog 6 had plenty of training experience, with familiar and unfamiliar persons, and was very much familiar with clicker training and shaping. Nine seconds was reached after 478 trials. This was due to the fact that this individual was used to offer numerous new behaviours and quite subtle behaviours as well. Since this dog was used to this training technique new behaviours or movements were offered very often and the gradual increase in time spent on target was done very slowly. Despite this, after passing 15 seconds on the target individual number 6 started to offer new or complementing behaviours to the point that the training had to go back to scratch. This meant that the dog was once again rewarded just for making contact with the target and slowly increasing the time in contact with the target, ending at nine seconds after a total of 478 trials.

Dog 8 reached a 4 second hold after 240 trials. Both dogs 7 and 8 were fearful and nervous and that was probably why they progressed slowly. Dog 1 reached three seconds in contact with the target after 210 trials and progressed at about the same speed as dog 8, however this was not due to nervousness but inability to focus on the task. The dog was only about 8 months old during the experiment and found it difficult to stay focused during training. It is not uncommon that dogs during the time of physical and/or mental maturity are less responsive to training and experience difficulties to focus, therefore dogs that have not reached physical and mental maturity should not participate in this study if replicated.

The target was attached to a piece of brick and it is recommended to use something lighter since it is moved and lifted numerous times per day and in the long run it becomes exhausting for the arm and hand performing the move. Pre-training sessions were included to be sure dogs had at least the same minimum knowledge/experience before the actual training started. As an example, all dogs did not have a cue or did not respond to the cue lie down, which was not believed to be a big issue at first since it was expected that dogs that did not have a cue for lie down would follow a lure. However, this was not the case and therefore, for any further studies, it should be a requirement that dogs respond to a cue for lie down to simplify the experimental process. In addition, to ensure that the dogs would wait for the reinforcement, even though the trainer had treats in front of them, sessions including training to wait for a treat was included. In retrospect, if all dogs had previous experience of clicker training and were acquainted with training situations these steps could have been excluded. However, these training sessions were perfect opportunities for the dogs to associate the new word “bra” with treats, which simplify the coming training.

Only two dogs reached criterion and one can discuss whether the criterion was set to high or not. For the same reason one can argue that two individuals actually reached criterion and therefore it was not an impossible goal to reach. What might be the issue here is the sample of individuals included in the experiment rather than the experiment itself. The selection of individuals should have been more precise in numerous ways, such as previous training experience, age, personality traits, fears etc.

For further studies, dogs shall have some previous basic training and even better, be familiar with and know the principles of clicker training. It will facilitate if dogs included in further studies have a cue for lie down (or the chosen position) and respond well to it. It simplifies the training if the dogs find treats/food as rewarding and worth working for.

Since behavioural states such as nervousness (Hall, 1945), and anxiety (Stern et al., 1984; Tanaka and Yamaoka, 1993) affect blink rate and stress may affect learning (Mendl, 1999; Schwabe and Wolf, 2010; Overall, 2011) it was perhaps not suitable to include dogs with such traits in a study like this, with limited time for training. Dogs that are too excited in a training situation and lack patience might not be suitable since the task involves a static behaviour performed during a long time (90 seconds) which might cause the dog frustration. In addition, Stern et al. (1984) concluded that increased arousal generally was associated with an increased spontaneous blink rate. Since the task includes an increase in time spent on the target it is of great importance not to include dogs that easily get bored or find the increased and prolonged wait for a reward further into the training as negative or a cue for incorrect behaviour. A dog that has much experience of clicker training and shaping, probably find this type of task easy. However, a dog that lacks patience or has little experience of static behaviours might find this type of task very difficult and frustrating.

Since it has been found that human individuals performing stereotypic behaviours may have an altered blink rate (Roebel and MacLean, 2007) and horses performing stereotypic behavior such as crib-biting show an elevated spontaneous blink rate (Lethbridge, 2011) dogs with stereotypic behavior should not be included in a study like this.

5.1 CONCLUSION

From this study it was not possible to conclude that the spontaneous blink rate reflect dopaminergic activity during training. However, one conclusion that can be drawn from this study was that there is a change in blink rate during training compared to baseline for the two dogs that reached criterion. Further studies on larger sample sizes are necessary to assess the effect of training on spontaneous blink rate in dogs. For any further studies, it is strongly recommended to be accurate and careful in the recruitment of individuals to ensure that they are suitable for such training. Over all, individuals included should be physically and mentally mature, stable and not fearful, anxious of aggressive. They should have previous experience of everyday training and clicker training. In addition, they should have a cue for lie down (or other chosen behaviour) and respond well to it. Another important trait in individuals included in any further studies is patience.

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Appendix 1

A study of the effect of training on blink rate in dogs

Informed consent form

This research is looking to see whether or not blink rate changes during training. The rationale for the work is that blink rate might reflect the level of an important neurotransmitter involved in reward seeking behaviour in the brain (dopamine). If blink rate changes this might give us a tool to understand what is going on inside the dog's head during training and perhaps adjust our training accordingly.

Please feel free to ask any questions if the information is unclear or you would like some further details.

The study involves training dogs to lie down and put their head on a sponge where their face will be videoed for about 75 seconds. We wish to look at how their blink rate changes with training. Food based rewards will be used throughout according to your dog's preference. We would need to train your dog on several days to establish the behaviour.

If your dog has any history of aggressive behaviour, is very nervous, prone to frustration or unable to cope without you, please do not volunteer him/ her for the research.

Each session should take no more than 60 minutes, including two outdoor breaks.

All of your contact details will remain confidential. Information supplied by you about your dog and data obtained from the study will remain anonymous.

You have the right to withdraw at any time and your data will be destroyed if you so wish.

I, (your name)

Being the owner of (dog's name)

Of (client's address)

Give consent for my dog to participate in the above study.

Signature & date: *(client must be over 18 years old)*

Appendix 2

Form to dog owner

Owner's name:
Telephone no:
Dog's name:
Breed:
Gender:
Age:
Is your dog currently suffering from, or has your dog a history of health problems/disease/ injury? Yes No Eyes Yes No Ears Yes No Joints Yes No If so, please give brief details; *such as approximate dates, condition, treatment, comment on the success of treatment and state today.*
Is your dog experiencing problems walking the stairs? Yes No Has your dog any experience of clicker training? Yes No

Do you prefer to provide your own treat for the training of your dog? Yes No Does your dog have a favourite treat? Yes No If so, please state the name of the product, brand and if it is an uncommon product please also state where it can be purchased below.
Has your dog a cue/word for sit or lie down? Yes No If so, please state the cues/words your dog know for sit and lie down below.
Has your dog a release cue/finished cue? Yes No If so, please state the release cue/finished cue below.
Anything else you would like to mention?

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