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Playful activity post-learning improves training performance in Labrador Retriever dogs (*Canis lupus familiaris*)

Nadja Affenzeller [a,b,*], Rupert Palme [c], Helen Zulch [a]

[a] *Animal Behaviour, Cognition and Welfare Group, School of Life Sciences, University of Lincoln, Green Lane, Lincoln, LN6 7DL, United Kingdom*
[b] *Department of Companion Animals, Clinical Unit of Internal Medicine Small Animals, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria*
[c] *Department of Biomedical Sciences, Unit of Physiology, Pathophysiology and Experimental Endocrinology, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria*

HIGHLIGHTS

- Enhancement of memory in dogs through post-learning activity was investigated.
- Playful activity post-learning improved memory in an object discrimination paradigm.
- Cortisol significantly decreased after play when compared to a control group.
- More studies are needed to evaluate implications for the field of dog training.

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ABSTRACT

Situations that are emotional and arousing have an effect on cognitive performance. It is thought that beta adrenergic activation and the release of stress hormones enhance memory consolidation and lead to an increase in memorability of emotional events. This beneficial effect has been shown in humans, non-human primates and rodents. Techniques which could enhance memory for learning specific tasks would be highly valuable, especially in dogs, which are extensively trained to aid humans.

A pseudo-randomized, counterbalanced, between subject study designs was utilised and 16 Labrador Retrievers ranging from 1 to 9 years of age were trained in a 2-choice discrimination paradigm. After task acquisition, either a playful activity intervention ($N = 8$) or a resting period ($N = 8$) took place, lasting for 30 min.

A range of factors including age, sex, training experience and trials to criterion on each day was subjected to a multiple factor/covariate General Linear Model analysis. The results show that playful activity post-learning improved training performance evidenced by fewer trials needed to re-learn the task 24 h after initial acquisition (playful activity group: mean number of trials 26, SD 6; resting group: mean number of trials 43, SD 19, effect size 1.2). Average heart rate, as a measure of arousal, during the intervention was significantly higher in the playful activity group (143 beats/min, SD 16) versus the resting group (86 beats/min, SD 19, $P < 0.001$). Salivary cortisol did not significantly differ between groups during training, however a significant decrease (T: −4.1 $P < 0.01$) was seen after the playful activity.

To our knowledge this is the first evidence that posttraining activity may influence training performance in dogs.

1. Introduction

It is well known that emotional and arousing stressful situations often create long lasting memories in humans. From an evolutionary point of view this might serve an adaptive function highlighting salient stimuli so as to be prepared for similar future occasions [33]. Stress induced arousal can be defined as an emotional and physiological reaction to stimuli which leads to an activation of the sympathetic, autonomic, and/or the endocrine system [63].

Within the last decade, studies on emotional arousal in both human and animal literature explored the role and interplay of different neuroanatomical structures, neural pathways and their activating and deactivating neurotransmitters and neuromodulators (review [35,36,47]).

The critical role of adrenal hormones such as adrenaline, noradrenaline and glucocorticoids on memory for emotional events has been documented in various animal and human studies ([1,7,56]; review [55], review [35]). Additionally, enhanced recognition memory has also

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[*] Corresponding author at: Animal Behaviour, Cognition and Welfare Group, School of Life Sciences, University of Lincoln, Green Lane, Lincoln, LN6 7DL, United Kingdom.
E-mail address: naffenzeller@lincoln.ac.uk (N. Affenzeller).

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been found in people with a high heart rate response following a stressful event occurring shortly after learning [25]. More specifically, it has been shown that adrenaline, beta adrenergic receptor activation and noradrenergic activation of the amygdala are essential to improve memorability of stressful situations, with specific beta receptor antagonist medication being able to block these positive effects of arousal on memory consolidation [8,23,44,57]. Similar effects have been found when administering corticosterone, glucocorticoid agonists and corticoid receptor antagonist in rats and chickens ([58,70], review [69]). Most importantly, activation of the sympathetic nervous system through beta adrenergic activation can be induced by both aversive stimuli (review [35] for laboratory animals) but also pleasant stimuli ([38,51]; both in human studies).

In summary, it is thought that the concurrent beta adrenergic activation and the release of adrenal hormones enhance the consolidation process and hence lead to this increase in memorability of emotionally arousing events ([57]; review [35]).

This beneficial effect of arousal has mainly been found in research on declarative memory [66]. Declarative memory is responsible for being able to remember single events and supports the learning of relationships between items [3], for example learning to discriminate between objects. Neuroanatomically, the hippocampal formation has been found to be an important structure in declarative memory [62] and together with the amygdala plays a central role in processing emotion (review [30]).

Enhancement of memory through arousal is not only dependent on the form of memory, the activated neuroanatomical structures and involved neurotransmitters but also that arousal itself is occurring close in time to learning [66]. Existing human literature on post-learning intervention suggests that pleasant events causing arousal enhance long term memory if they take place within 30 minutes post-learning [46]. Similar crucial time dependant effects have been found in laboratory animals when administering different drugs and hormones to simulate arousal (e.g. amphetamines, adrenaline, corticosterone). The most pronounced effect on memory consolidation was seen when these substances were applied shortly after training but not after a more prolonged delay (review [55]).

It is important to point out that any kind of arousing intervention before and during learning can influence attention, coding and consolidation [4], however manipulation through an emotionally arousing event after learning allows for a clear attribution to consolidation mechanisms [25,46]. Thus, post-learning intervention is one effective way to selectively test effects of positive arousal on memory consolidation [6,61].

Interest in the field of learning and memory has grown rapidly [9] with studies mainly performed on humans, non-human primates and rodents. Little information is known about companion animals, especially dogs. Dogs are trained to fulfil specific tasks, for instance detection of explosives and drugs in the professional sector or for guide- and assistance dogs in the private sector. Given the time and money invested in such training [26], further information about factors influencing memory, and ultimately training performance, would be valuable.

This study investigated the effects of an acute, post-learning, positively arousing event on memory in dogs. Heart rate and salivary cortisol were measured as an indicator of physiological arousal. A 2-choice discrimination task was used, that is thought to engage declarative memory mechanism across mammals, with a playful activity intervention taking place within the 30 min following initial acquisition of the task. The control group experienced a resting period post-learning.

Only Labrador Retrievers were chosen to avoid possible learning differences between breeds [19,41]. It was hypothesised that positive arousal in the form of a playful event would improve memory consolidation and hence training performance of this newly learned task in dogs, evidenced by fewer trials needed to meet criteria for the task 24 h after initial acquisition.

2. Materials and methods

2.1. Study design

This study was a between subject design following a methodologically standardized approach with a pseudo-randomized object location and two groups, balanced for trained object, intervention type, age and cognitive testing experience. The number of dogs enrolled was based on previously published papers on object discrimination (OD) learning ranging from 15 to 25 dogs [17,20,26,61]. The study met the ethical guidelines of the University of Lincoln, UK.

2.2. Study group

Nineteen purebred Labrador Retrievers were recruited for this study (see Table 1 for individual information). Seven males (3 intact, 4 neutered) and 12 females (5 intact, 7 spayed) ranging from 1 to 9 years were tested at the Riseholme Campus, University of Lincoln, UK. All dogs were privately owned pet dogs, and informed consent was obtained after explaining the procedures and objectives of the study to each owner. All dogs had to be reported healthy by their owners and not be taking medication. Naïve dogs were defined as having no further dog training experience besides standard obedience training. Gundogs were defined as dogs undergoing standard obedience training and competing as field trial and working gundogs [65]. Experienced dogs were defined as having participated in cognitive testing before. Exclusion criteria were; dogs younger than 1 year or older than 10 years, visual lameness during habituation, a history of reluctance to engage in playful activity and/or a history of aggressive behaviour towards unfamiliar people. Additionally, dogs needing <3 sessions to meet criterion for OD-training on day 1 were excluded due to a suspected strong object preference.

2.3. Materials

All dogs were trained under daylight conditions in a room (5.0 m × 3.5 m) with solid anti-slip flooring, a temperature maintained at 22 ± 2 °C and fresh water freely available at all times. An in-between trial waiting area was separated from the training area using a barrier to prevent the dogs' visual contact during trial set up (see Fig. 1).

Dogs were trained in a 2-choice discrimination paradigm to differentiate between two objects differing in; odour (cat litter vs. woodchip),

Table 1
Key demographics of individual dogs.

	Dog	Dog handler	Age (months)	Intervention	Trained object	Sex	Training status
1	Guy	Assistant 2	101	Play	Green	m	Experienced
2	Dennis	Owner	26	Play	Green	m	Gundog
3	Meg	Assistant 2	74	Play	Green	fs	Experienced
4	Kess	Assistant 1	31	Play	Green	fs	Experienced
5	Bramble	Owner	37	Play	Blue	fs	Experienced
6	Penny	Owner	24	Play	Blue	f	Experienced
7	Eyla	Owner	108	Play	Blue	fs	Gundog
8	Poppy	Assistant 1	15	Play	Blue	f	Naive
9	Bruno	Assistant 2	108	Rest	Green	mn	Experienced
10	Moya	Assistant 2	23	Rest	Green	f	Experienced
11	Wren	Owner	18	Rest	Green	f	Gundog
12	Hope	Assistant 1	91	Rest	Green	fs	Experienced
13	Max	Owner	83	Rest	Blue	m	Naive
14	Edith	Owner	14	Rest	Blue	f	Gundog
15	Poppet	Owner	60	Rest	Blue	fs	Gundog
16	Monty	Owner	66	Rest	Blue	mn	Experienced
17	Mollie	Owner	84	NA	Blue	fs	Naive
18	Jupiter	Owner	26	NA	Blue	mn	Experienced
19	George	Owner	31	NA	Blue	mn	Experienced

Blue: dogs being trained to go to the blue basket, f: female, fs: female spayed, green: dogs being trained to go to the green box, NA: not available, m: male, mn: male neutered.

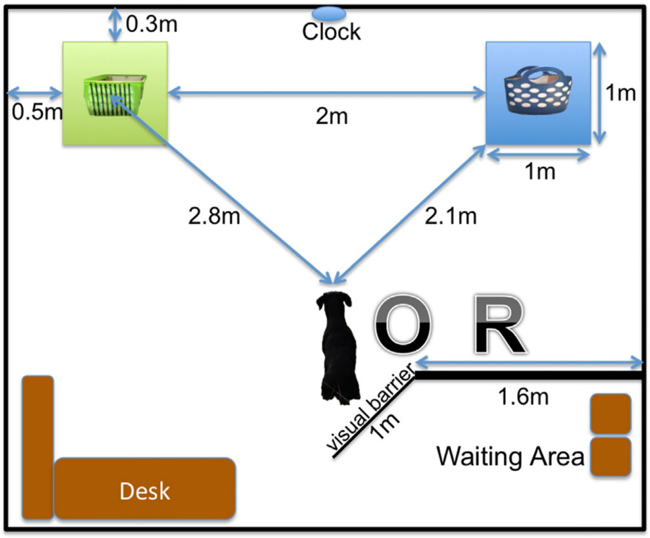

Fig. 1. Experimental setup and dimensions of the testing area.

pattern (black stripes vs. white dots), size and shape (box vs. basket) and colour (light green vs. dark blue) as shown in Fig. 1. The blue basket with white dots (30 × 14 × 21 cm, white dots diameter: 3.5 × 1.5 cm) was filled with woodchips (Durstons© Large Chip Bark, Durston Garden Products Limited, Somerset, UK) and the green box with black stripes (35 × 14 × 26 cm, black stripes: 0.5 × 14 cm) was filled with cat litter (Msavers© Cat Litter, Morrison Supermarkets PLC, Bradford, UK) each to a depth of approximately 5 cm. Each object was placed in the middle of a 1 × 1 m cardboard square covered in a cotton towel, the colour of which corresponded to the object colour. Each dog was trained to correctly indicate one of the two objects, the object assigned to each dog was pseudo-randomized.

During the training all dogs were wearing a Polar© RS800CX heart rate monitor (Polar Electro Oy, Kempele, Finland) consisting of a watch, receiving and storing the data, and an electrode belt and transmitter (Polar, Wear Link Smart Fabric sensor W.I.N.D©), which has been shown to reliably measure heart rate in dogs [14]. This device measures heart beats at a frequency of 1000 Hz with a transfer rate of 2.4 GHz between belt and heart rate monitor. Ultrasound gel (Konix© Ultrasound Gel, Turkuaz Ltd., Istanbul, Turkey) was used to promote conductivity. Heart rate data were transmitted at the end of each day to a laptop computer using the Polar software Protrainer 5©. Each dog's heart rate data were exported as a text file into Kubios© HRV software Version 2.2. (University of Eastern Finland, Kuopio, Finland, [64]).

Either the owners or one of two research assistants (both female, blonde, similar height, aged from 22 to 26 years) handled the dogs. The researcher was responsible for training the dogs.

O: designated area of the owner, R: designated area of the researcher.

2.4. Procedure

Owners were asked to follow their daily morning routine with their dogs before coming to the training facility between 8 am and 10 am. All dogs were allowed to freely explore the room for at least 5 min during which time the training objects were not present. Then the electrode belt was strapped around the chest of the dog and fixed with another strap (3 M© Vetrap Bandaging Tape, 3 M Animal Care Products, St. Paul, USA) around the shoulders. The transmitter was placed ventrally with the electrodes positioned on each side of the sternum. Ultrasound gel was applied liberally between electrodes and fur until a signal was transmitted to the watch. All training was continuously recorded with a video camera (HC-V130, Panasonic©, Panasonic Corporation, Osaka, Japan).

2.4.1. Saliva sampling and cortisol analysis

Salivary cortisol samples were taken on three occasions: 20–30 min after entering the room (after the habituation process and procedure summary for the owners), immediately after successfully acquiring the task and finally, 10 min after the intervention finished to allow for a reported 20-30 min time delay in salivary cortisol concentrations [5,68]. Salivation was stimulated with inaccessible sausage held in one hand in front of the dog's nose while gently holding a cotton swab (Eurotubo® Collection swab, Deltalab, Rubì, Spain) between the lip and the gum at the caudal commissure of the lips for 3 min. Dogs were allowed to freely move to avoid restraint induced stress.

Saliva was recovered by squeezing the cotton swab into a microtube (microtube 1.5 ml, Sarstedt, Germany) with the researcher wearing gloves. If the minimum sample size of 0.5 ml was not achieved, the dog underwent a second 3 minutes sampling procedure as described above. All saliva samples were frozen at −20 °C within 4 h after collection and analysed for cortisol content using an enzyme immunoassay (for details see: [49]) that has previously been used in dogs [16,18].

2.4.2. Set up

The owner was asked to walk the dog from the waiting area to the designated starting point (marked with an X on the floor), and then stand next to the dog, looking ahead at a clock placed centrally on the wall (see Fig. 1). Owner and researcher were wearing dark sunglasses at all times. The researcher was standing in a marked, designated area on the right hand side of the owner, looking towards the X on the floor. This allowed the researcher to view the dog, and ensured that dogs continuously looked towards the setting for at least 2 s before the researcher verbally cued the owner to release the dog on a "Go" command. All dogs were kept on a 3 m long lead, which allowed the dogs to freely access the objects. After the dog had made a choice, the researcher used either a click sound followed by a reward (one piece, approximately 0.5 cm^3, of pork or chicken sausage per correct choice based on individual dietary sensitivities) or a spoken "Wrong" in a neutral voice. After each trial the owner and dog returned to the waiting area until being called back in by the researcher for the next trial.

In between trials the researcher always walked down the centre of the room towards the objects and then walked a figure of eight either relocating or leaving the objects in their designated areas as required to give consistent human scent and auditory cues. At the end of each training day, objects were wiped with a disinfectant towel, and the cat litter and wood chips discarded.

2.4.3. Pre-training

All dogs were first trained to go to an object (a Jar: 15 × 11 × 17 cm) after a release cue "Go" had been given by the owner. The jar was placed in an alternating order on either of the two spots where the objects were later placed during OD-training. On the first two occasions a piece of sausage was visibly placed on top of the jar, by the researcher, to motivate the dogs to approach it. Criterion was met when the dogs had at least two paws within 0.5 m of the jar in 4 out of 5 trials. A dog needing >10 sessions with 5 trials each to meet criterion was excluded from participating further.

2.4.4. OD-Training

The same setup as previously described was applied. On the first two occasions a piece of sausage was visibly placed inside the correct object, which was placed on the right or left designated area (both sides baited for every dog), to motivate the dogs to approach it after the release cue. After that the location of the objects was pseudo-randomized using the free online software Research Randomizer [67] such that each object was presented on the left and right side an equal number of times; but not on the same side for more than two consecutive trials, to prevent the development of a side bias by the dogs.

The dog was considered to have made a choice when 2 paws were placed on the cardboard square of the object. No choice was defined as the dog not having two paws on either square within 30 s after the release cue. Trials where no choice was made were not counted as correct or incorrect, instead the same trial was repeated. Three consecutive no choices were followed by a break. Another 3 consecutive no choices after a break led to the exclusion of the dog from the study. After a correct choice the researcher continued with the next trial, whereas after a wrong choice the same trial was presented again until the dog made a correct choice. Three consecutive wrong choices in the same trial resulted in the owner walking the dog over to the correct object and were then followed by the next trial. Criterion was met when the dog had an 80% or higher correct choice in two consecutive sessions, each session consisting of 10 trials.

2.4.5. Breaks

Breaks were mandatory after every finished session. The dogs were walked outside or kept in the waiting area on an alternate basis. Owners/assistants were allowed to interact (petting, talking) with their dogs during breaks. Additional breaks were given when no choices were made three times in a row (see above).

2.4.6. Intervention

Meeting criterion in the OD-training was followed by either a 30 minute resting period or a playful activity consisting of a 10 minute walk, 10 minutes off lead play, and another 10 minute walk. The dogs in the resting group were asked to lie down on a dog bed, while the researcher engaged the owner/assistant in a conversation to prevent further attention or interference with the dog. However, when laying their head on the floor dogs were called their name and/or touched to prevent them from falling asleep. The dogs in the playful activity group were allowed to explore the surroundings while being walked to a fenced in area (20.5 × 33.5 m). Play consisted of fetching a ball, running after Frisbees, and playing tug-of-war depending on each dog's preferred play style, reported by its owner or chosen by the individual dog. Dogs in the playful activity group not going above a heart rate of 120 beats/min [59] were excluded from the study. After the intervention period, dogs were walked back to the training facility, heart rate equipment was removed, the last saliva sample taken and owners and dogs left and were asked to follow their normal daily routine. Owners were requested not to take part in any other formal training that day.

2.4.7. OD-Training day 2

Dogs were trained in the same way as the previous day until criterion was met, starting approximately 24 h after day 1.

2.5. Data analysis

Total number of trials needed to meet criteria for pre-training as well as training on day 1 and day 2 were counted. Average heart rate and length of time during training sessions, breaks and intervention was calculated on day 1. On day 2, average heart rate during re-training was calculated. Salivary cortisol concentrations were expressed as nanogramm per millilitre (ng/ml). If not stated otherwise levels are reported as mean ± SD.

Statistical analysis was performed using Minitab© Statistical Software (version 17.2.1). A multiple factor/covariate General Linear Model analysis was performed followed by backwards stepwise simplification, where non- significant highest to lowest order main effects were excluded first. Residuals had to be normally distributed.

A 2- sample *t*-test assuming equal variances was used for parametric variables. For non-parametric variables a Mann Whitney *U* test was performed. A Repeated Measure Analysis was performed for within subject average heart rate and cortisol differences.

The size of effect for significant results between both groups was calculated using Cohen's d [10].

A two-tailed binomial test [31] was conducted to identify criterion for an individual's performance that was significantly above chance level (16 out of 20, 80% or higher, $P = 0.01$).

A *p*-value < 0.05 was considered significant.

Dogs that failed to meet criterion on day 1 were excluded from further statistical analysis.

3. Results

3.1. Study group

Three dogs (dogs 17–19, see Table 1) had to be excluded from the study. Two dogs ("Mollie", "Jupiter") were removed due to excessive no choice trials on day 1, despite breaks. One dog ("George") met criterion in the OD-training within 2 sessions on day 1, a performance indicating strong object preference. All further results do not include data from the three excluded dogs.

Sixteen dogs with a median age of 49 months met criterion on both days. Median age for dogs in the resting group was 63 months, in the playful activity group 32 months, in the blue basket trained object 49 months and in the green box trained object 50 months, respectively (see Fig. 2).

Blue: blue basket; Green: green box, Play: playful activity group, Rest: resting group.

There was no significant difference in age between the playful activity group and resting group (Mann-Whitney *U* test, $W = 67.5$, $P = 1$) or between trained objects (Mann-Whitney *U* test, $W = 73.5$, $P = 0.6$).

3.2. Pre-training

Median number of trials to meet criterion in pre-training was 5 (1st quartile 5, 3rd quartile 8). There was no overall significant effect of age, sex, training experience, dog handler or cortisol levels after habituation on the absolute trial number to meet pre-training criterion. However, following model simplification, number of trials needed to finish pre-training was significantly correlated to training experience ($F_{2,13} = 6.88$, $P = 0.01$). Dogs that already had cognitive testing experience needed fewer trials than naïve or gundog trained dogs (see Fig. 3).

Mean cortisol levels after habituation were 9.2 ± 5.5 ng/ml, with dogs in the playful activity group having 7.8 ± 3.7 ng/ml and in the resting group 10.5 ± 6.7 ng/ml, respectively. There was no significant difference between the groups (see Fig. 4).

3.3. OD-Training day 1

Mean absolute trials to reach criterion was 83 ± 39. There were no overall effects of age, sex, training experience, dog handler, trained object, number of trials to criterion in pre-training, average heart rate, cortisol levels after habituation and cortisol levels after training to reach criterion on day 1 on absolute trial numbers on day 1 (General Linear Model: $p > 0.1$). However, following model simplification, training experience was significantly correlated to absolute trial number on day 1 ($F_{1,14} = 3.97$, $P = 0.045$). Naïve dogs and experienced dogs needed a mean number of 58 ± 25 and 70 ± 21 trials, respectively. Gundog trained dogs needed significantly longer than experienced dogs (117 ± 49 trials, unpaired *t*-test, $t\ (12) = 2.6$, $P = 0.02$). Individual learning curves separated into group performance can be seen in Fig. 4a and b.

Mean average time spent in OD training was 73 ± 32 min across all dogs, for dogs assigned to the resting group 79 ± 29 and for dogs assigned to the playful activity group 67 ± 35 min, respectively. Mean average time spent in breaks was 44 ± 21 min with the resting group having 45 ± 19 and playful activity group having 42 ± 24 min, respectively. There was no significant difference between both groups when comparing length of time spent in training (2 sample *t*-test, $t\ (13) = -0.77$, $P = 0.5$) and time spent in breaks (2 sample *t*-test, *t*

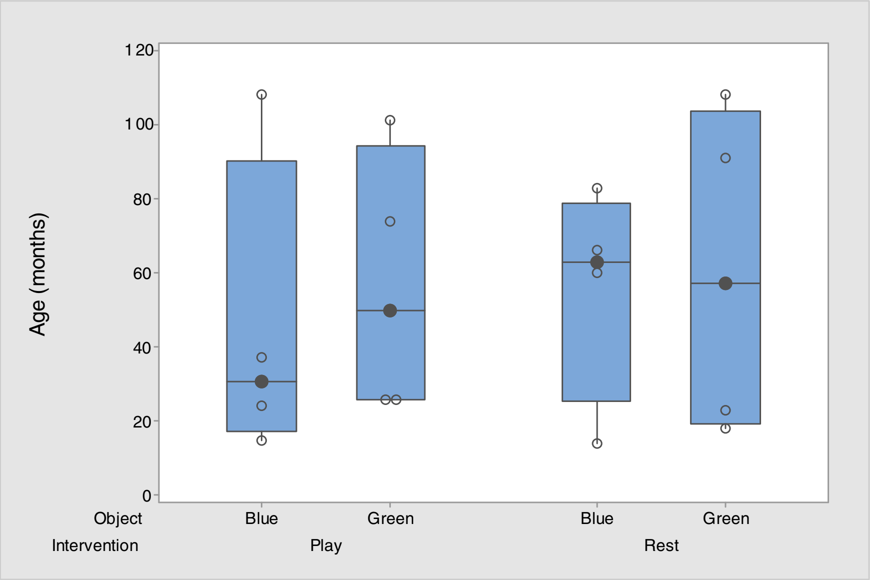

Fig. 2. Box plot of age distribution in dogs based on intervention group and object the dogs were trained to. Box plots show the median and interquartile range from the 25th to the 75th interquartile. Open circles represent individual dogs.

(*13*) = −0.26, $P = 0.8$). Please see Table 2 for individual data per dog on training and breaks.

Mean average heart rate was 111 ± 17 beats/min across all dogs, for dogs assigned to the resting group 110 ± 10 beats/min and for dogs assigned to the playful activity group 112 ± 23 beats/min, respectively. Mean average heart rate during OD training was 102 ± 16 beats/min (resting group: 98 ± 11, playful activity group 106 ± 20), mean average heart rate during breaks was 106 ± 12 beats/min (resting group: 103 ± 8, playful activity group 109 ± 14). No significant difference with respect to mean average heart rate was found between the groups (2 sample *t*-test, $P > 0.1$). In addition, no significant difference was found within individuals when comparing average heart rate during training and during breaks (paired *t*-test, $P > 0.01$). Please see Table 2 for individual mean average heart rate data per dog during training and breaks.

Mean cortisol levels after training on day 1 was 9.3 ± 5.0 ng/ml with dogs assigned to the playful activity group 9.1 ± 4.2 ng/ml and the resting group 9.4 ± 4.2 ng/ml, respectively. There was no significant difference between both groups before the intervention took place (2 sample *t*-test, $P > 0.1$, see Fig. 5).

Play: playful activity group; Rest: resting group.

3.4. Intervention

All dogs in the resting group were able to settle on the dog bed or lie down next to the owner/assistant and all dogs in the playful activity group engaged in play with the researcher. In the resting group all owners/assistants engaged in talking to the researcher and all dogs remained responsive with no dog observed to have fallen asleep.

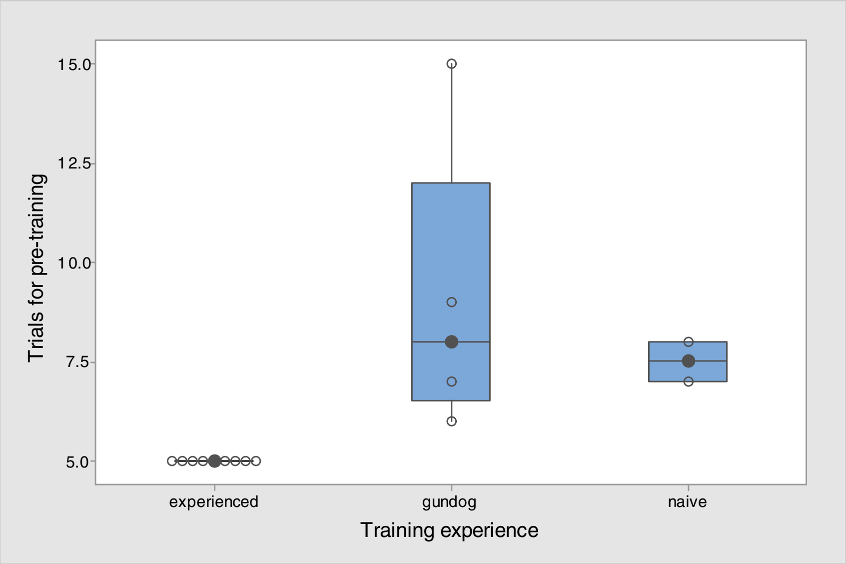

Fig. 3. Box plot of number of trials needed to meet criterion in the pre-training based on training experience. Box plots show the median and interquartile range from the 25th to the 75th interquartile. Open circles represent individual dogs.

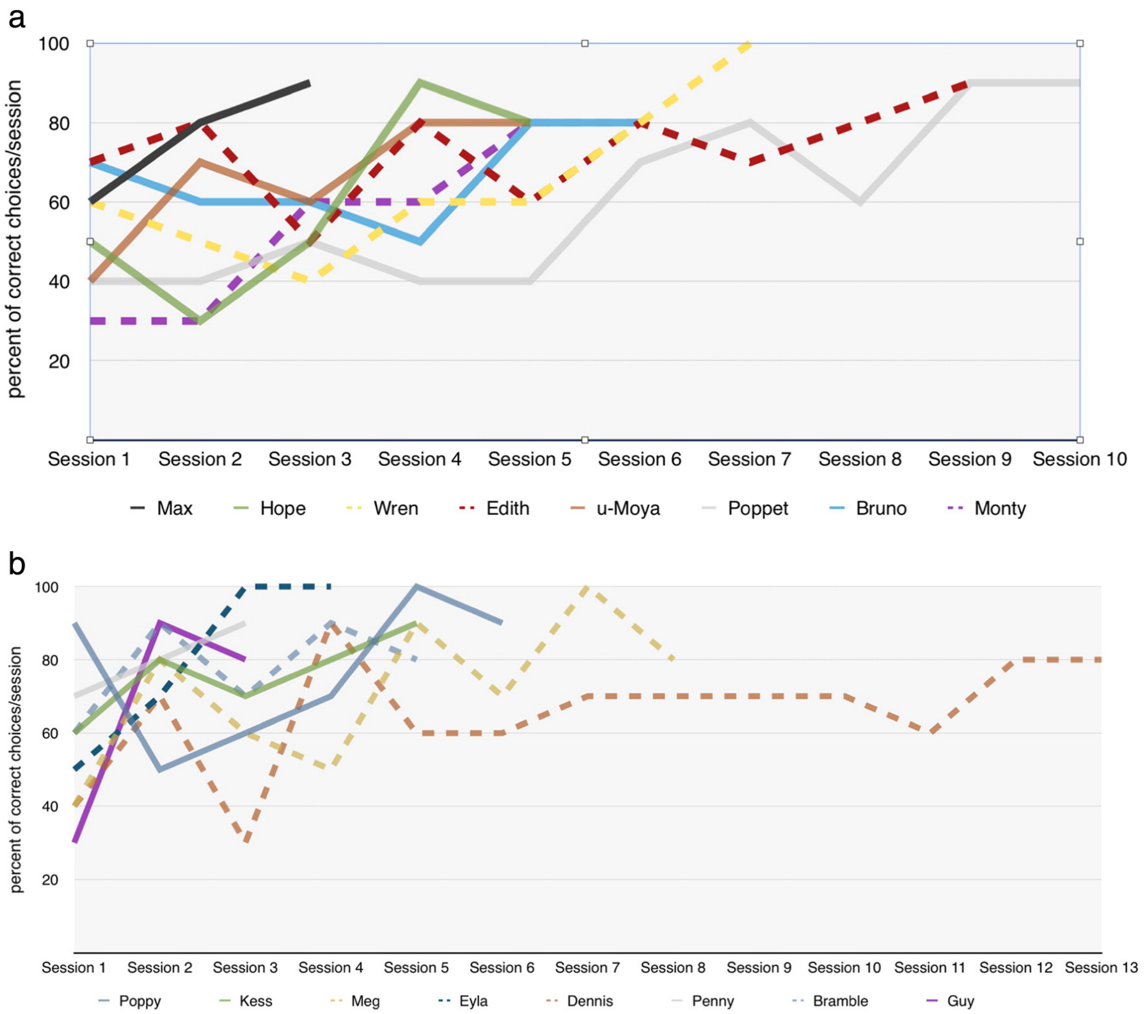

Fig. 4. a. Individual learning curves of dogs assigned to the resting group. Number of correct choices per training session is shown in per cent. Solid and dashed lines for visual clarity. b. Individual learning curves of dogs assigned to the playful activity group. Number of correct choices per training session is shown in per cent. Solid and dashed lines for visual clarity.

Average heart rate during the intervention was significantly affected by the type of intervention ($F_{1,14} = 42.93$, $P < 0.001$, effect size $d = 3.2$). Mean average heart rate in the resting group was 86 ± 19 beats/min and in the playful activity group 143 ± 16 beats/min.

In addition, a significant difference within group with respect to the mean average heart during training (including breaks) and the average heart rate during the intervention was found: heart rate of the resting group decreased (paired t-test, $t = -4.02$, $P < 0.01$) while heart rate of the playful activity group increased (paired t-test, $t = 5.2$, $P < 0.01$ detailed in Fig. 6).

a. Heart rate intervention: average heart rate during either of two intervention types; m.a. heart rate Day 1: mean average heart rate during

Table 2
Key data of object discrimination training, breaks and heart rate of individuals.

	Dog	Intervention	Time OD training day1	Time breaks day 1 abs. (%)	m.a. HR OD training day 1	m.a. HR OD training day 2	m.a. HR breaks day 1	m.a. HR breaks day 2	Average heart rate session 1 day 2
1	Guy	Play	30	15 (48)	130 ± 13	125 ± 6	132 ± 11	135 ± 2	119 ± 20
2	Dennis	Play	136	85 (63)	109 ± 12	117 ± 1	113 ± 17	130 ± 30	116 ± 19
3	Meg	Play	71	44 (63)	130 ± 6	120 ± 4	123 ± 19	130 ± 4	116 ± 17
4	Kess	Play	53	26 (48)	79 ± 2	76 ± 4	103 ± 8	* 89 ± 20	79 ± 20
5	Bramble	Play	66	48 (73)	79 ± 13	70 ± 16	86 ± 11	84 ± 23	70 ± 16
6	Penny	Play	38	30 (79)	96 ± 6	99 ± 0	100 ± 38	* 83 ± 16	99 ± 16
7	Eyla	Play	41	25 (61)	108 ± 11	110 ± 4	112 ± 13	* 120 ± 11	113 ± 15
8	Poppy	Play	98	65 (66)	114 ± 14	99 ± 0	107 ± 12	* 108 ± 19	99 ± 11
9	Bruno	Rest	58	37 (64)	93 ± 5	101 ± 2	94 ± 7	* 115 ± 16	102 ± 13
10	Moya	Rest	53	29 (55)	96 ± 9	97 ± 8	97 ± 14	96 ± 19	107 ± 20
11	Wren	Rest	77	35 (46)	107 ± 8	102 ± 3	114 ± 20	*141 ± 26	104 ± 14
12	Hope	Rest	60	27 (45)	83 ± 10	80 ± 3	98 ± 13	94 ± 5	75 ± 19
13	Max	Rest	57	33 (58)	113 ± 12	111 ± 11	113 ± 3	106 ± 5	118 ± 15
14	Edith	Rest	117	55 (47)	100 ± 10	108 ± 5	107 ± 21	*126 ± 10	111 ± 15
15	Poppet	Rest	130	62 (48)	107 ± 7	106 ± 6	105 ± 12	105 ± 9	116 ± 18
16	Monty	Rest	82	82 (100)	86 ± 10	90 ± 8	97 ± 22	113 ± 34	81 ± 17

a.: average; HR: heart rate, m.a.: mean average, time is presented in minutes and breaks in minutes and percent (%) of training, * represent average HR data from single breaks.

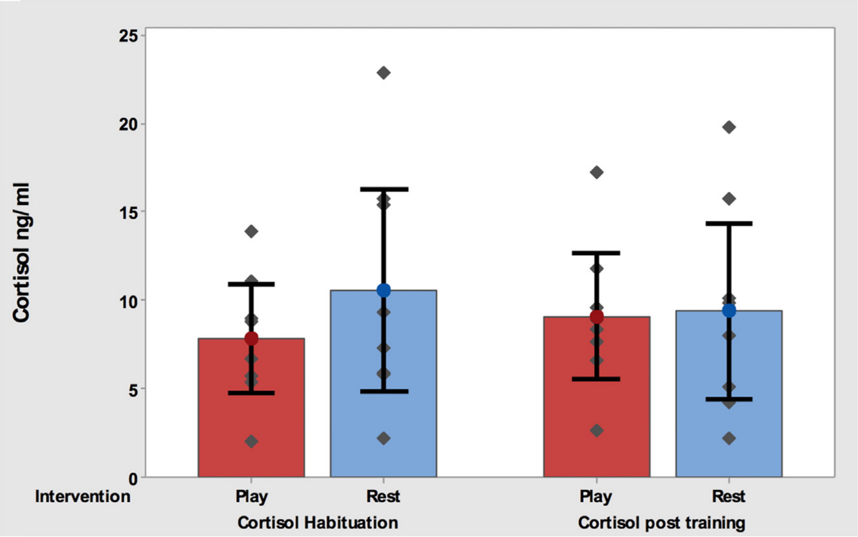

Fig. 5. Individual value plot and bar chart of cortisol concentrations (ng/ml) in samples taken after the habituation process (Cortisol Habituation) and after acquiring the task (Cortisol post training). Playful activity group represented in red, resting group represented in blue. Error bars express the 95% confidence interval of the mean. Solid grey diamonds represent individual dogs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

training (including breaks) during either of two intervention types; play: playful activity group; rest: resting group.

Cortisol levels after the intervention were significantly affected by the type of intervention ($F_{1,14} = 8.26$, $P = 0.01$, effect size d = 1.43). Cortisol levels after the playful activity intervention significantly decreased to a mean of 3.7 ± 2.0 ng/ml (paired *t*-test, $t = -4.1$, $P < 0.01$) whereas mean cortisol levels after resting showed a marginally non-significant increase to a mean of 14.6 ± 10.6 ng/ml (paired *t*-test, $t = -2.4$ $P = 0.05$, see Fig. 7). In addition, a significant difference in cortisol levels between the two groups was found post intervention (2 sample *t*-test, $t\ (7) = -2.8$, $P = 0.01$, effect size d = 1.4, detailed in Fig. 7).

Play: playful activity; Rest: resting group.

3.5. OD-Training day 2

There were no overall effects of training experience, average heart rate during intervention, cortisol levels post intervention and intervention on absolute trial numbers to reach criterion on day 2 (General Linear Model: $P > 0.1$). However, following model simplification, number of trials on day 2 was significantly predicted by a single variable: the playful activity intervention ($F_{1,14} = 5.85$, $P = 0.03$, effect size 1.2). Dogs in the resting group needed 43 ± 19 trials and in the playful activity group 26 ± 6 trials to meet criterion on day 2, respectively (see Fig. 8).

Play = playful activity group; Rest = resting group.

Individual re-learning curves separated into groups are presented in Fig. 9a and b.

No significant interactions between intervention, average heart rate during intervention and cortisol post intervention were found (General Linear Model: $P > 0.1$).

Mean average heart rate during OD re-training was 101 ± 16 beats/min across all dogs, for the dogs assigned to the resting group 99 ± 11 beats/min and for the dogs assigned to the playful activity group 102 ± 20 beats/min, respectively. Mean average heart rate during time spent in breaks was 111 ± 19 beats/min (resting group: 112 ±

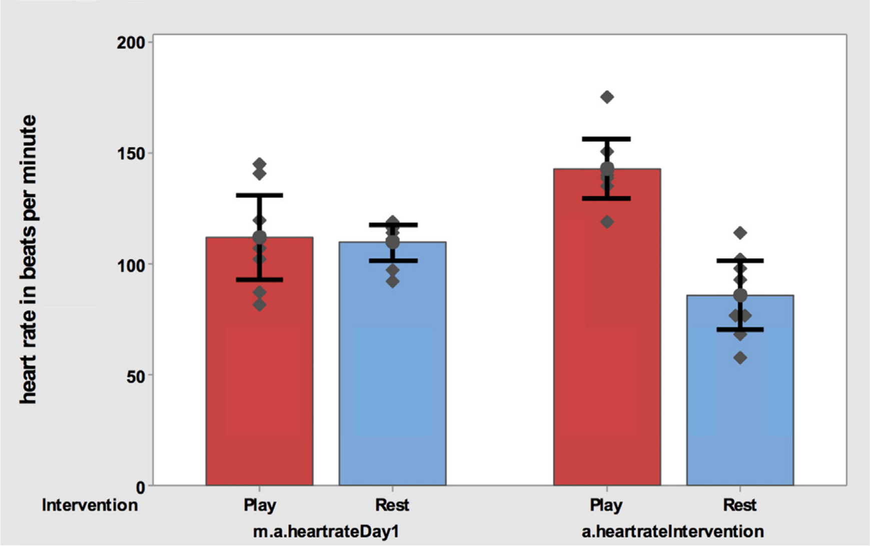

Fig. 6. Individual value plot and bar chart of heart rate data during training and intervention separated into groups. Playful activity group represented in red, resting group represented in blue. Error bars express the 95% confidence interval of the mean. Solid grey diamonds represent individual dogs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

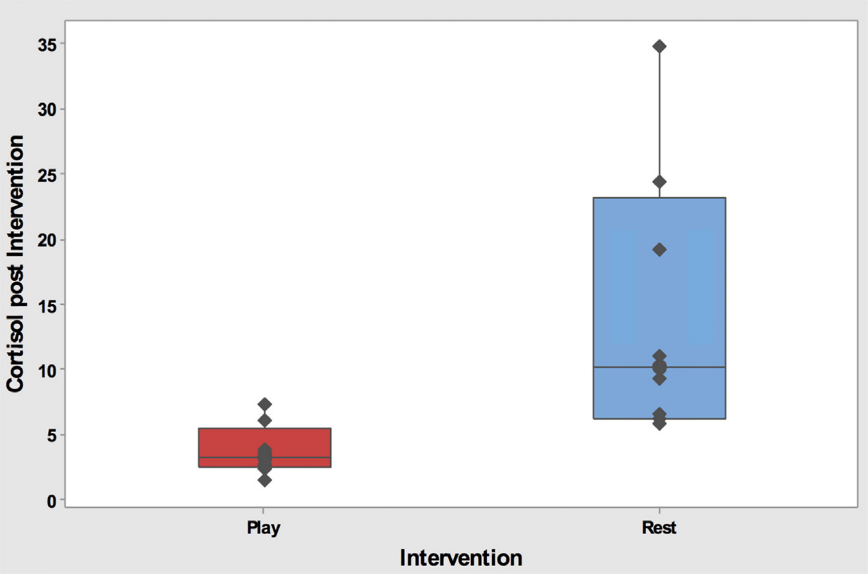

Fig. 7. Box plot of cortisol concentrations (ng/ml) after the intervention took place. Playful activity group represented in red, resting group represented in blue. Box plots show the median and interquartile range from the 25th to the 75th interquartile. Solid diamonds represent individual dogs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

16, playful activity group: 110 ± 22). There was no significant difference in the mean average heart rate between groups during re-training (2 sample *t*-test, $t\,(10) = -0.37$, $P = 0.7$) and breaks (2 sample *t*-test, $t\,(10) = -0.22$, $P = 0.8$). Additionally, there was no significant difference within groups with respect to mean average heart rate during training (including breaks) on day 1 and day 2 (paired *t*-test, resting group $t = 0.47$, $P = 0.7$, playful activity group $t = 2.12$, $P = 0.07$).

When comparing average heart rate during the first training session no significant difference was found between the two groups (resting group: 102 ± 16 beats/min, playful activity group: 101 ± 20 beats/min, 2 sample *t*-test, $t\,(13) = -0.14$, $P = 0.9$, see Table 2).

4. Discussion

The current study was designed to explore the role of an emotional and arousing event post-learning on training performance in dogs. The results show that engaging in playful activity for 30 min after successfully learning the task improved re-training performance, evidenced by fewer trials needed to meet task criteria 24 h after initial acquisition. This significant difference between the two groups not only suggests that the intervention is affecting long-term memory rather than an improved short-term memory [25,45,52,61], but also that pleasant arousal post-learning has similar effects on enhancing memory in dogs as it does in humans [13,28].

In the present study, the heart rate between the two groups only significantly differed during the intervention, with all dogs in the playful activity group experiencing a significant increase and all dogs in the resting group experiencing a significant decrease. Heart rate responses were used to indirectly measure catecholamine levels as it has been shown that administering both adrenaline and noradrenaline after learning can enhance memory (review [34]). Additionally, it is thought that the effect of adrenaline, concurrent beta adrenergic receptor

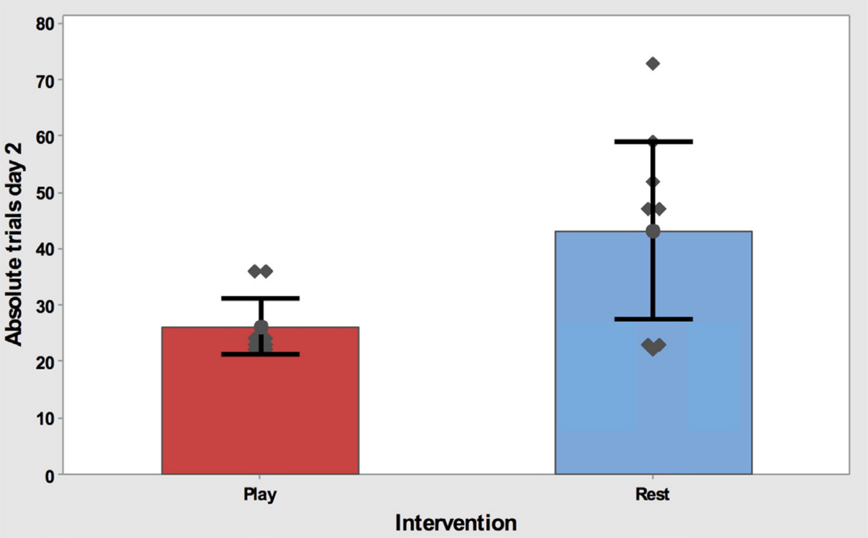

Fig. 8. Individual value plot and bar chart for absolute number of trials on day 2 needed to meet criterion based on type of intervention. Playful activity group represented in red, resting group represented in blue. Interval bars represent 95% confidence interval for the mean. Solid diamonds represent individual dogs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

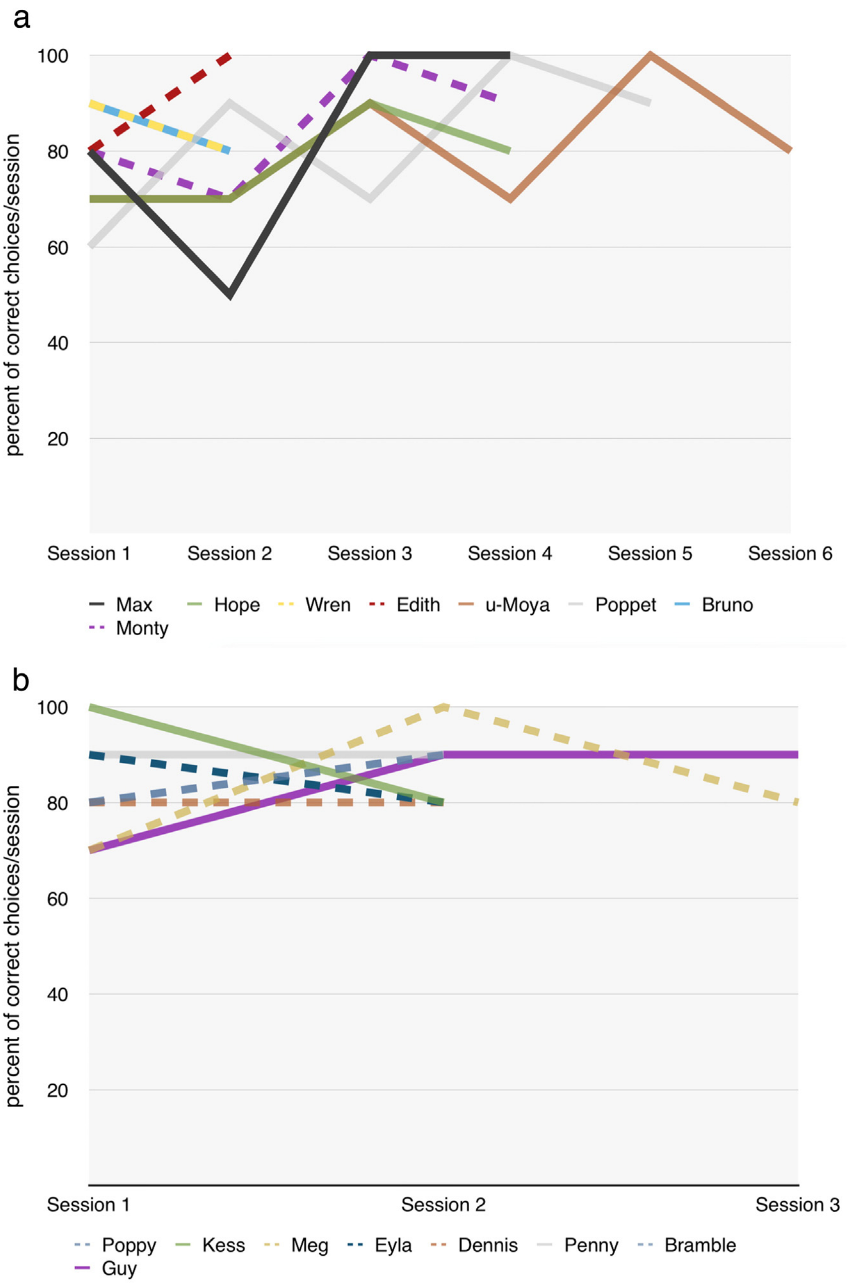

Fig. 9. a. Individual re-learning curves of dogs assigned to the resting group. Number of correct choices per training session is shown in per cent. Solid and dashed lines for visual clarity. b. Individual learning curves of dogs assigned to the playful activity group. Number of correct choices per training session is shown in per cent. Solid and dashed lines for visual clarity.

activation and noradrenaline activity in the amygdala affects memorability of emotional material ([6]; review [69]). Indeed, heart rate has been shown to respond readily during stressful situations, reliably indicating arousal in dogs [12]. The importance of a high, compared to a low, heart rate response when experiencing a stressful stimulus and its positive effect on memory has recently been evaluated in humans [25]. However, heart rate cannot be used to evaluate the different types and/or levels of stress [12].

There was no evidence found that average heart rate during the intervention was affecting absolute trial numbers on day 2. Furthermore, no interaction was found between the type of intervention and average heart rate during the intervention. Hence, it is concluded that the effect of beta-adrenergic activation alone was not strong enough to affect the training performance on day 2 in this small study population.

Alongside beta adrenergic activation, cortisol release has also been shown to facilitate memorability of emotional events (review [34]). A novel finding that emerges from this study is the significant decrease of salivary cortisol after playful activity. This is rather unexpected as it has been shown that emotional and arousing events of both positive and negative valence lead to a release of adrenal stress hormones, such as adrenaline and cortisol [33]. These findings might be explained by the locomotor activity and social context component of the playful activity intervention. Canine athletes participating in agility competitions had no significant increase in cortisol concentrations after running the course [5]. In addition, in humans, it has been documented that medium level exercise does not significantly alter cortisol levels [2] and even more interestingly that low level exercise leads to a reduction of circulating cortisol levels [21]. A similar phenomenon might have happened in the current dog study population, although the individually perceived strenuousness of the exercise component cannot be determined. Most importantly, all playful activity occurred in a human-dog social context. Beerda et al. [12] showed that stressful events in a social context (a human opening an umbrella and the dog being forcefully, physically restrained by a human) when compared to a non-social context (sound blasts and electric shocks) did not induce cortisol level increases despite a similar pronounced heart rate increase in all conditions. Interestingly, Horváth et al. (2008) found that differing social contexts during play with humans had contrasting effects on salivary cortisol levels in dogs. Disciplinary behaviour towards police dogs resulted in a significant increase in cortisol. In contrast, affiliative behaviour towards border control dogs significantly decreased circulating cortisol levels. This corroborates findings from the current study where it is speculated that social play with the researcher modulated cortisol levels, which led to the observed decrease in the playful activity group.

It is important to point out that glucocorticoids by themselves are not considered to be direct markers of emotion; memory studies using emotional words and pictures did not induce cortisol release in humans [69]. Indeed, it has been elegantly demonstrated that noradrenergic activity in the basolateral amygdala is key for memory enhancement during emotional arousal, with the amygdala playing a central role in processing emotions ([57]; review [35]). Therefore, it is hypothesised that the pleasant nature of the playful activity intervention led to a significant increase of heart rate, with a speculated noradrenergic activation of the amygdala ultimately causing the improved training performance on day 2.

The wider variation in training performance and cortisol concentrations in the resting group might be due to abruptly ending training after task acquisition, which has been shown to cause frustration in some individuals [24]. Indeed, monkeys and rats showed increased glucocorticoid activity when they did not find food rewards where expected [32]. Frustration might also be caused by a lack of further attention and social interaction (perceived as social withdrawal) and/or the removal of the possibility to earn food rewards. Dogs in the resting group may have had differing perceptions of the intervention itself, potentially causing varying frustration levels, thus affecting individual performance and the marginally non-significant increase in cortisol. However, average heart rates significantly decreased, remaining below physiological resting range of <120 beats per minute [59] in every dog, which does not seem to indicate frustration induced arousal.

A limiting factor of the study design is that cortisol levels have not been corrected for the effects of; haemoconcentration, fluid consumption, pH of the saliva and macromolecules ([2]; review [29]), factors all known to influence cortisol levels, as dogs had water available ad libitum and were fed treats during the training process. A time delay of 20–30 min for salivary cortisol is reported in dogs [5,68], therefore, the last samples in this study reflected cortisol concentrations during the mid-point of the interventions (off-lead social play and resting for the playful activity and resting groups respectively). Future studies should increase the frequency of saliva sampling to further evaluate the effects of long lasting interventions on glucocorticoid activity.

One major limitation of this study is the lack of a control for physical exercise, without an emotional component, as it has been shown that acute exercise impacts memory consolidation (see meta-analysis [54]). More specifically, exercise has been reported to have a positive impact on memory by increasing synaptic plasticity and long-term potentiation [27], both of which take place during the consolidation process. This is supported by recent studies suggesting that children engaging in physical activity during teaching had significantly greater learning gains (equating to 4 months over a 2-year study period) when compared to a control group [42,43]. Additionally, acute exercise post-learning improved memory recall of some training tasks in senior dogs [61]. For this reason, we cannot exclude that an exercise component of the arousing playful activity intervention contributed to the improved training performance. In order to elucidate this topic further, future studies should address this issue by incorporating a control group that experience only exercise.

No significant age effect on pre-training or OD-training was found, which is consistent with previous reports on dogs [19,41] and other species [53]. However, the median age between intervention groups differed and the observed non statistical significance might be attributed to the wide variations within the groups.

Unlike in human studies (review [11]) no sex dependent effects on memory were observed, which is in line with already reported data in dogs [20,26]. However, the high proportion of neutered individuals within this study population might have confounded the data. Therefore, future studies should be based on a larger sample size with more intact individuals to further address the effects of sex and neuter status on memory.

Another limiting factor of this study protocol is the presence of the handler and the researcher. This might lead to a Clever Hans Effect, where animals read human gestures and unconscious cues, thus improving their performance [50]; a phenomenon well known in dogs [40]. With the owner present the dog might also interpret the task in a social context, influencing them to be more confident and to maintain, or even improve, their performance [39]. However, in contrast, separating dogs from their owners might lead to emotional distress, which could also affect their performance [48]. Due to the lack of a double blinded study design, it cannot be totally excluded that the owner/assistant or researcher was inadvertently cueing the dog. Nevertheless, in this study no effect of dog handler on performance was seen, corroborating previous work by Heckler et al. [20]. Therefore, it is considered that the habituation procedure and the precise instructions given to the dog handlers (such as standing still with their arms at their side, where to look, and wearing sunglasses) were sufficient to minimise handler influence. As the post training activity used in this study engaged the dog in a game with a human, it needs to be pointed out that these findings can only be discussed in the context of interventions involving dog-human interactions. Thus, no conclusions can be drawn regarding alternative arousing interventions without a human being present (for example self-play or intra-species play).

Other factors which may influence learning and memory performance include those affecting; attention, sensory receptor sensitivity, motivation and general arousal level, all of which should be controlled for as fully as possible to avoid misinterpretation [9]. This study design controlled for some of these effects by balancing dogs for trained object, intervention, age and cognitive testing experience. Furthermore, all dogs started training between 8 and 10 am to minimise differences in attention and motivation based on general activity patterns of dogs, with activity levels being higher in the early morning and late afternoon hours [22]. One confounding factor might have been the same researcher conducting both the training and the interventions. If the playful activity intervention was positively associated with the researcher and served as reinforcement, the dogs' performance on day 2 might have been affected. In general, positive reinforcement with treats in the form of one piece of sausage per successful trial was used to keep dogs motivated to learn the task. Nevertheless, two dogs had to be excluded because of excessive no choice trials, which were interpreted as losing motivation or mental fatigue. Future studies should include different researchers for the training and intervention to further minimise perceived reinforcement, and the possible interference on re-training performance.

Interestingly, it has been shown that object features influence learning rate in dogs [17]. Therefore, to address individual sensory receptor sensitivities and to minimise individual physical object preferences, the chosen objects differed in shape, size, colour, pattern and odour. Mean overall learning rate across all dogs was 83 trials which is comparable to previous published papers on OD-training in dogs ranging from 65 trials [41] to 124 trials [26]. However, one dog had to be excluded from this study due to suspected object preference when solving the OD task within the first 2 sessions. Although no significant effect of trained object was found, future studies should perform an object preference test for each individual dog, and then conduct training with the non-preferred object.

Measuring average heart rate, which did not significantly differ between or within, groups on day 1 and day 2, respectively, has controlled for general arousal level both during training and memory recall. Hence, arousal levels during training and at the time of recalling information from day 1 on day 2 is unlikely to have affected memory formation and memory retrieval mechanisms [60].

Individual variability of learning and memory can also have a confounding effect on results [61]. In this study population, a significant effect was seen for the pre-training and the absolute trials on day 1 data. Dogs with cognitive testing experience needed fewer trials to meet criterion in the pre-training. This pre-training was meant to familiarise each dog with reward and object approach learning. Unfortunately, data regarding pre-training and cognitive testing experience is rarely statistically evaluated, which makes further comparison and conclusion highly speculative. Future studies should therefore balance their design for cognitive testing experience, and/or include it in statistical analysis.

Training experience also affected absolute number of trials on day 1, with gundogs needing significantly more trials than experienced dogs. Gundog training often relies heavily on subtle body cues from the trainer (such as pointing gestures), indeed it has been shown that working gundogs use visual cues from humans more successfully than pet dogs [37]. Therefore, gundogs may not have performed as well as pet dogs in the present study due to a lack of such cues from the owner/assistant/researcher, evident through a poorer performance on day 1. However, no significant effect of these parameters was seen on absolute trials on day 2, which is line with results from Nielson and Powless [46]. After grouping people in to good and poor learners based on their initial learning, they were able to show that learning rate did not affect response to the arousing intervention, with the pattern of enhancement by memory modulation being comparable. It is difficult to draw robust conclusions about the relationship between learning rate and memory in dogs based on the small sample size of this study.

More studies with adapted designs are needed to further investigate the questions raised above. This is particularly true when it comes to the number of dogs used in this study. The evaluation of a large number of covariates and factors on a rather small study population complicates statistical validation. Although widely used, General Linear Models, have the potential for false positive results arisen from effect size overestimation [15]. Hence, this study should be seen in the light of an exploratory data character.

Nevertheless, it is believed that the observed positive effects of playful activity post-learning on training performance in Labrador Retrievers confirm results of comparable human studies. Further research is needed to test for replication of the current results, with special emphasis on the role of amygdala activation during playful activity, exercise and the possibility of perceived frustration in a social context during training. In addition, better understanding of the most efficacious type of interventions leading to improved training performance in a wide range of training tasks (such as scent training for explosives detection and medical research) would be of tremendous practical use in the professional sector of dog training.

Conflict of interest

No actual or potential conflict of interest, including financial, personal or other relationships with people or organizations have inappropriately influenced, or were perceived to influence, this work.

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