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"Exposure to controlled challenges increases stress resilience in dog puppies"

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1 Abstract

The socialization period in dog puppies is one of the most important periods in determining later behavior. Here I aimed to test the effect of providing early stimulation beyond mere stimulus presentation on stress resilience in dog puppies. I tested 83 dog puppies from 12 litters. Half of each litter formed treatment group, the other half the control group. Treatment group puppies received age-appropriate 'challenges '12 days between the ages of 3-5 weeks. Treatment included exposure to noises, novel objects, and problem solving tasks. The control group did not receive any training. Between 40 and 52 days of age, all puppies were tested in a behavior test. A nonlinear Principal Component Analysis over coded behaviors yielded four principal components, two of which differed significantly between treatment groups, revealed by a linear mixed effects model. Firstly, "Response to Novelty" ($F_{1,70}$=8.75, p=0.0042), meaning that treatment puppies solved the problem solving task faster, showed more exploration and less seeking of humans and whimpering. Secondly "Social-Startle" ($F_{1,70}$=8.93, p=0.0039), indicating that treatment puppies showed a reduced startle reaction after a loud noise; however, the control group showed a higher interest in a friendly stranger - perhaps increased handling experienced by the control group had beneficial effects on their sociability.

To conclude, the presentation of a diversity of exercises, noises and objects at an early age seemed to enable the treatment puppies to cope better when confronted with surprises, which leads me to the assumption that this treatment is increasing their stress resilience.

2 Introduction

2.1 Theoretical Background

Behavior is known to be influenced by biological and experiential factors. Genes set the base, but environmental and maternal influence affect how this genetic potential is utilized (Wilsson, 2016).

2.1.1 Stress Coping Ability

The development of stress coping abilities can be a crucial factor in adapting to environmental changes more easily. Early life experiences appear to be particularly influential for the development of the ability of coping with stressful situations throughout an individual's life, as has been shown in numerous studies on rodents and monkeys. Literature suggests that early life experiences, for example environmental enrichment, and the handling of neonates, can affect behaviour and stress coping beneficially in the long-term (Bray et al., 2017; Fernandez-Teruel et al., 1991, 1992; Levine et al., 1967; Núñez et al., 1996; Plotsky & Meaney, 1993; Reul & Kloet, 1985).

2.1.2 Environmental Enrichment and Stress Inoculation Training

Especially environmental enrichment in early life stages seems to help with the development of stress resilience, which is known as ability to bounce back after an aversive experience and is studied intensively in humans and several other species (Nuaimi et al., 2012; Harlow, 1958; Harlow & Suomi, 1971; Kozorovitskiy et al., 2005; Scheffer et al., 2018). There seems to be a certain period of time in an early life stage, when careful exposure to stressors leads to the development of arousal regulation and stress resilience, and therefore, better adaptation to future stressful experiences. This procedure is known as stress inoculation training (Lyons et al., 2009, 2010). In squirrel monkeys, stress inoculation training in form of early life separations correlates with reduced anxiety, increased exploration of novel situations and decreased levels of cortisol (Levine & Mody, 2003; Lyons et al., 2000; Lyons et al., 1999). This stress inoculation training seems not only to be responsible for changes

in behavior, but also has a broad influence on neurological factors, such as an expansion of the prefrontal cortex (Lyons et al., 2009), which might play a major role in inhibitory control (Tapp et al., 2003).

However, inducing stress early in life can have devastating consequences and therefore the level of stress has to be managed carefully (Parker & Maestripieri, 2011). Lyons & Parker (2007) found that presenting squirrel monkeys with solvable challenges induced adaptations such as increases in cognitive control, emotional processing, curiosity and neuroendocrine regulation.

2.2 Domestic Dogs and Stress

In domestic dogs, adaptation to stress and novel stimuli could have a major effect on welfare, performance, and living in stressful environments like cities. In order to set them up for a successful life without anxiety, aggression, or other stress related problem behaviors, it is important to evaluate how experiences during the first weeks of life shape future behaviors (Foyer et al., 2013).

2.2.1 Puppies' main developmental stages

Since general literature suggests that early life experiences are crucial for influencing the development of stress resilience and the ability for environmental adaptation, I take a closer look into puppies' development. In puppies' development, there are different early age stages: first the ‘primary‘, ‘neonatal' or ‘initial‘ phase; secondly, the ‘socialization‘ period, and thirdly, the ‘enrichment‘ or ‘juvenile‘ period.

The first period, also known as the ‘primary‘ phase, lasts from birth until an age of about three weeks. The sensory capabilities are not yet fully developed, which means puppies display limited interest in novelty as well as a limited capacity for conditioning (Battaglia, 2009; Lindsay, 2013; Serpell & Jagoe, 1995).

The second important stage is the ‘socialization' period, which starts when the puppies are about three weeks old, and start moving around the den once they become aware of their surroundings as their eyes and ears open up for full functionality (John Paul Scott & Fuller, 1974). During this period, their sensory system develops rapidly

(Battaglia, 2009; Scott, 1958), puppies become interested in novel objects, and start interacting socially with other living creatures (Scott, 1958). When this period ends between the age of 12-14 weeks, fear or avoidance behaviors occur more often when puppies are confronted with novel stimuli and new situations (Serpell et al., 2016). Third, there is the ‘enrichment' or ‘juvenile' period, which lasts until the dog is about 12 months of age (Battaglia, 2009; Serpell & Jagoe, 1995).

All three stages are essential to the process of socialization, but the ‘socialization’ period is the most important for the development and maintenance of long-term relationships with humans and the development of the ability to adapt to the environment (Howell et al., 2015). This stage especially, is also known as a sensitive period, in that experiences the puppies have appear to have a greater effect on development and coping style than experiences made outside of this stage (Overall & others, 1997; Serpell & Jagoe, 1995). In order to avoid fearful behaviors later in life, exposing puppies to a range of different stimuli, for example noises, visual stimuli and other sensory experiences including social interactions with conspecifics and other species (e.g. humans), is important during this period of rapid neurological and emotional development (Scott & Fuller, 1965).

2.2.2 Influence of Maternal Care

Different maternal styles seem to have an effect on the development of stress resilience in puppies. Recent studies of maternal care in dogs have found that higher levels of early care are positively correlated with better explorative tendencies and lower stress responses in puppies at the age of eight weeks (Guardini et al., 2016), and increased social and physical engagement and aggression later in life at an age of 15 to 18 months (Foyer et al., 2016). Not only the level of maternal care, but also the amount of vertical nursing appears to affect the development of stress resilience in puppies. Bray et al. (2017) show that puppies who nursed vertically more frequently, were more likely to fail a guide dog training program later in life. The authors suggest that puppies who had to work for being nursed, showed improvement in the development of stress resilience. The mothers of those puppies who were successful in the guide dog training program spent more time apart from their offspring and nursed while sitting or standing instead of lying down. Hence, puppies had to overcome

small challenges during an early life stage. While this – amongst other factors - may have prepared them for greater challenges later in life, a possible correlation still needs further investigation.

2.2.3 Influence of Challenges

Previous studies of dog and other species and their development of stress resilience mainly focused on presenting novel stimuli and sounds but did not include confrontation with small challenges. Regarding stress resilience in dogs, there is some anecdotal evidence indicating that exposure to small challenges has positive effects on dogs' behavioral development even beyond that achieved solely through exposure to novel stimuli (Killion, 2014). In children, it has been suggested that frustration tolerance can be improved, and aggression reduced by presenting challenges and giving the opportunity to learn how to successfully solve problems (Webster-Stratton et al., 2001). One pilot study with a small sample size indicated that problem solving games as mental / physical challenges can reduce fear in adult dogs (Zilocchi et al., 2012), but no further research has been done to this date.

2.2.4 Influence of Sounds, Novel Objects and tactile Stimuli

Considering the extremely high prevalence of noise sensitivities in dogs, i.e. questionnaire studies indicating that around half of all dogs fear the sound of fireworks (Riemer, 2019a, 2019b), preventing the development of noise sensitivities is an area with great potential to enhance the welfare of a large number of dogs.

In general, the startle response in puppies first appears at an average of 19.5 days (J P Scott & Fuller, 1965). It seems that at the age from three to seven weeks they exhibit a reflexive startle reaction, which is followed by an immediate recovery. The reaction is not comparable with an adult-like, active fear-related response (Morrow et al., 2015).

Alves et al. (2018) exposed puppies at an early stage to auditory stimulation, and contrary to their expectations found no effect on noise sensitivity. Instead, the authors found an effect on the behavioral reaction to manipulations, different environmental stimuli and interaction with humans.

In another study, 37 German shepherd puppies were exposed to radio programs three times a day for 20 minutes during their first seven weeks of life and seemed to show better response to intense sudden noise (Chaloupková et al., 2018).

Recently, Vaterlaws-Whiteside & Hartmann (2017) showed positive short and long-term effects on puppy development with an early socialization program which included the presentation of stimuli to one to six-week old dog puppies five times a week. They studied six litters, in total 34 puppies, raised under standardized conditions and exposed them to a variety of novel stimuli and handling exercises, based on existing puppy nest stimulation theories. In total, they presented three challenges and problem-solving tasks to the puppies; but mainly focused on tactile, auditory and visual stimuli, and human interaction. This is the first study that found positive short and long-term effects on individual dog behavioral traits. I wanted to not only validate their findings, but also extend the training regime by presenting a variety of different exercises to the puppies; focusing mainly on age-appropriate 'challenge' exercises (12 in total), as well as exposure to single loud noises and mobile novel objects when puppies were three to five weeks old.

2.2.5 Hypothesis and Prediction

Vaterlaws' findings lead us to the us to the hypothesis that the development of stress resilience in dog puppies can be improved through targeted 'challenge' exercises during the socialization period, including exposure to novel (and mobile) objects, potentially startling stimuli, problem solving tasks, and short periods of separation.

Within my study, one half of twelve litters of eight different breeds were exposed to a diversity of novel sounds, objects and challenges early in life.

I predicted that puppies from the treatment group would show:

- bolder behaviour when exposed to new situations
- a reduced startle reaction and faster recovery following a loud noise
- a shorter success latency and greater persistence in a problem-solving task
- more exploring and less whimpering when left alone in a novel room with a strange human

compared to a control group whose members did not receive any challenge exercises. These predicted differentiations in puppy behaviors could be indicative of an improved development of stress resilience in the treatment group.

3 Material and Methods

3.1 Participants

Subjects of this study were 83 dog puppies (*Canis familiaris*) of eight different breeds. Private breeders in Eastern Austria were recruited through advertisements on the internet (dog breed clubs, web sites, Facebook) and were contacted via E-Mail or phone. In this study, 11 Breeders with 12 litters of 4 to 11 puppies were selected (for details see supplementary table 1). All puppies came from small-scale breeders and were bred according to FCI (Féderation Cynologique Internationale) standards. The puppies spent most of their time in the house (housing condition). To minimize stress as well as hygiene risks, all training and testing took place at the breeders' homes.

3.2 Treatment and Control Group

Each litter was divided into a treatment and a control group. Puppies were randomly selected, and sexes equally distributed. Training situations were standardized to reduce variability between treatment and control group. Therefore, puppies in both groups were handled and fed similarly and by the same person, for example for some exercises, puppies in the treatment group were picked up or fed and therefore, puppies in the control group were also handled and fed. The experimenter spent approximately the same amount of time with both groups of puppies and either interacted with them or stayed neutral and watched them.

3.3 Training Phases

For every litter, the training of both groups started four to ten days after eye-opening, and lasted for three weeks. I chose this short period for the treatment phase, to be able to conduct the behavior test at around six weeks, well before the start of the fear period at eight weeks (John Paul Scott & Fuller, 2012). During this time, puppies were trained four times a week, in total 12 times.

The training phases included:

1. two to four days of pre-habituation phase, where the litter and their mother became habituated to a novel room;
2. two days of habituation phase, during which the litter was split into training and control group and the puppies were separated for the first time;
3. three weeks of treatment phase with exercises and presentation of novel objects and sounds for the treatment group, and
4. one day of behavioral testing.

3.3.1 Pre-Habituation Phase

In preparation for separating the litter into treatment and control group during the challenge exercises (so that only the treatment group was exposed to a stimulus), the breeder habituated both mother and puppies to two different rooms, starting when the puppies opened their eyes for a few days (at around two weeks old). Over several days, the breeder increased the time that mother and litter spent in a different room to approximately 30 to 60 minutes each day. All breeders performed this task for two to four days before the experimenter became involved.

3.3.2 Habituation Phase

After the puppies opened their eyes and ears (they were about two to three weeks old), both the treatment group and the control group were habituated to a separation from the other half of the litter and the mother (but not individual separation) on two consecutive days for 30 minutes each day. While one group remained in their familiar home area, the other group was taken to the room they had been previously habituated to. Dependent on the need and comfort of the mother or the puppies, the mother was either with them or not present. During those 30 minutes, the experimenter stayed with the group and stroked and talked to the puppies. For counterbalancing, in half the litters the control-group was taken to the other room on the first day and remained in the home area on the second day and vice versa for the other half of the litters. During these first two days of separation, no exercises were presented, so that

both groups had the same repertoire of experience when the treatment phase started.

3.3.3 Treatment Phase

Once the habituation phase was completed and the puppies had successfully acclimatized to the new situation, the treatment phase started. On each day of training, the treatment group was presented with four to eight exercises: one presentation of a novel object, one problem solving exercise and two to ten startle response & recovery exercises. The order of these exercises was adjusted to the activity levels of the puppies. An example of one exercise is given below.

A noise stimulus (for example dropping a heavy book within a distance of 300cm to the puppies) was presented to all puppies of the treatment group at the same time; first quietly then a little louder (with approximately 5-30 seconds between presentations). These noise stimuli were expected to induce a light startle response that should be followed by immediate recovery. When one puppy showed a strong startle response, which meant neither immediate recovery nor recovering within the following 30 seconds, a break was taken for about one minute. The same sound was then presented once again from a greater distance and with lower volume. If a puppy showed an exaggerated startle response (startle recovery took longer than 30 seconds), training was terminated for the day for this puppy, and next time the experimenter worked separately with this puppy, with a lower intensity exposure, which happened once. When the puppies didn't show a short startle response, either the volume was increased or the distance decreased. Sometimes puppies were sleeping deeply during the sound testing, so that they did not show any startle response. After the noise presentations, the puppies in the treatment group were separated from their littermates individually by using a grid barrier. The puppies in the treatment group remained in the same room to minimize stress, and in the beginning of the treatment phase they could see each other through the grid. Over several training sessions, the level of separation was increased by gradually blocking the puppies view by putting a towel over the grid. Each individual separately received an age-appropriate problem-solving-game (exercises partly from Jane Killion, Puppy Culture DVD) (see figures 1 and 2). Following as a third exercise, all puppies from the treat-

ment group were placed together again and a second noise stimulus (different from the first) was presented twice. Finally, a novel-object was presented to the puppies (see figure 2). The sequence of presentation of each exercise varied from day to day, dependent on the puppyies level of activity. In total, there were 12 different novel objects, 11 different problem solving tasks and 16 different sounds presented to the puppies during the treatment phase (for details on presentation for each week look at supplementary table 2).

Figure 1: A three-week old puppy is placed on a cold smooth plate and has to has to find a way off by itself. The problem-solving task was completed when all four feet were off the plate.

Figure 2: The four-week-old puppies explore novel objects placed in their room.

Figure 3: The four week old puppies have to climb over an obstacle to reach the feeding bowl.

In total, one training session for an entire treatment group lasted about 10 to 40 minutes (depending on the exercises and how many puppies were in each litter), with each puppy being trained for an average of one to ten minutes. After the training

session, the puppy groups were swapped between rooms and the experimenter spent the same amount of time with the control group puppies. The experimenter started either with the treatment or with the control group - half of the trainings began with the treatment group, and the other half began with the control group. To spend the same time with the puppies from control group and with the puppies from treatment group, the experimenter took the average time of the amount of time, that was needed for the exercises the days before, when starting with control group.

3.4 Behavioural Testing

All puppies were tested at the age of 40 to 51 days with a specifically designed behavioral test (adapted from Riemer et al., 2014) consisting of six subtests: a novel room, a friendly unfamiliar person, a novel object, a brief isolation period in a novel room, a problem solving task and exposure to a loud noise. All five subtests were performed in one session and took about 20 minutes for each puppy. In five out of six subtests there were three people present during the tests, the breeder, the experimenter and the cameraperson, who was unfamiliar to the puppies and was blind to their group assignment (for descriptions of each subtest see table 1). During the first exercise, which was the exploration subtest, there was only the videographer present, a female unfamiliar to the puppies.

Table 1: Personality Test at 40-51 days of age (c.f. Riemer et al., 2014)			
Nr.	**Subtest**	**Description**	**Duration**
1.	Room exploration	The puppy was allowed to explore the unfamiliar room for one minute. In most cases, only the videographer, a female unfamiliar to the puppies, was in the room, while the experimenter and the breeder stayed outside. Except of two litters, where puppies behaved more anxiously than the other puppies, and therefore, the breeder stayed to provide social support to increase the puppies' confidence.	60 sec
2.	Greeting Test	A female unfamiliar to the puppies sat on the floor and stayed neutral in a predefined position for 30 seconds without making noises or movements. The puppy was placed in the room as well. After 30 seconds the unfamiliar person initiated contact for five seconds by calling the puppy's name, chatting in a friendly voice or clicking her tongue. After that, the person behaved neutrally for five seconds, then initiated contact again. When the puppy approached, she petted the puppy and talked to them in a friendly way for. The interaction and ignore sequences alternated - in total there were five times of interactions per minute.	90 sec
3.	Problem Solving	The materials used for the problem-solving task were several pieces of food (dependent on the puppies' diet), a piece of cardboard (20x30cm) with two holes with a diameter of 7 and 9 cm and a paper cup with small holes of 1 cm diameter, so that the puppy could smell food put inside. First, the experimenter placed some pieces of food under the cup, which the pup had to knock over to obtain the food (=solvable). During preparation, the puppy could watch the experimenter. Second, the experimenter did that again, but fixated the cup with the piece of carton, so that the puppy could not get it off (=unsolvable). Each puppy had two minutes to solve the solvable problem and two minutes to solve the unsolvable.	120 sec

Nr.	Subtest	Description	Duration
4.	Startle Test	A balloon was burst approximately three meters away from the puppy. The breeder remained in close approximity to the puppy (about one meter) for the reason of social support, and tried to engage the puppy in play after the noise. Because of differing levels of noise sensitivity amongst the different litters, different noises was chosen for some litters. The average decibel of the balloon burst noise was 90dB. This was louder than any noise the experimenter had presented to the puppies before. Some litters had more noise sensitive puppies, or the test was done in a very small room so that the balloon burst would have been extremely loud. Those litters were instead presented with a different novel noise (either an eye-case lid that was closed quickly, or a plastic bowl that hit the ground) with lower dB (average was 50 dB). As I tested the puppies within their sensitive period, I did not want to risk any future anxieties because of this experience. Nine litters were exposed to a burst balloon, one to the eye-case lid closing and two to the plastic bowl that fell onto the ground.	60 sec
5.	Novel Object	A battery-powered toy comparable with a paper bag, approx. 20 x 10 x 5 cm, was placed in a predefined position to assess the puppy's reactions to the novel object's erratic movements.	120 sec

3.5 Video analysis

The puppies' behaviors in the test were videotaped and subsequently coded via Solomon Coder (© András Péter) by a blinded coder, who was unaware of the details of the treatments and the group allocation. Statistical analysis was carried out using SPSS (Statistical Programme, IBM®) and R Version 3.6.1. (2019). Coded behavior variables mostly consisted of durations, such as time spent exploring, whimpering, being near a person, having direct body contact to a stranger, touching the novel object, solving the problem-solving task, and touching the object of the problem solving task. Some variables consisted of scores, such as tail position, approach towards stranger, startle reaction, activity and play after startle. It is important to elaborate, that ‚startle reaction' addresses how strong the puppies' first reaction was after the noise. 'Startle activity' contains the summary scores of further reactions after the startle: freeze, flee, seek comfort, lower tail. The 'startle play' score shows how fast and whether the puppy starts to play after the startle (for more details on which behavior variables were coded, see table 2).

Table 2: Definitions, scores and durations of the behaviors that was coded during the behavioral test when puppies were six weeks old.

Variable	Type of original variable	Possible Values	Description
Exploration			
Tail	Score (every 30 seconds)	3	Tail mostly high: tail is above base of tail
		2	Tail mostly medium: tail is in line with base of tail
		1	Tail mostly low: tail is lower than base of tail
Active	Duration		Move or explore: Move includes forwards or backwards movements, coding starts when dog starts to move leg, followed by body movement. Does not include if dog moves legs but does not change spatial position. Exploring includes when the puppy's nose is <5cm from ground or from objects, apparently sniffing, mouthing, manipulating, or scratching objects with the paw.
Whimper	Duration		Puppy is whimpering.
Near Stranger	Duration		Puppy's head is <50cm in proximity to the stranger.
Out of sight	Duration		Puppy is out of sight.
Start	Marker		5 seconds after breeders hand removed from puppy
Stop	Marker		60 seconds after start marker
Greeting			
Latency to Approach	Score	0	Does not approach the stranger (10cm from stranger's hands) within 45 seconds
		1	Approaches the stranger within 21-45 seconds after she started calling
		2	Approaches the stranger within 11-20 seconds after she started calling
		3	Approaches the stranger within 10 seconds after she started calling
		4	Puppy is already in contact with stranger
Whimper	Duration		Puppy is whimpering.
Near Stranger	Duration		Puppy's head is <50cm in proximity to the stranger.
Direct Body Contact	Duration		Direct body contact with stranger (only when puppy initiates contact)
Out of sight	Duration		Puppy is out of sight.

Variable	Type of original variable	Possible Values	Description
Stranger starts to ignore puppy	Marker		When puppy's paws first meet the ground, and the stranger sits on the floor calmly and ignores the puppy or when stranger stops the interaction and starts ignoring the puppy
Stranger starts to interact with puppy	Marker		When stranger starts soliciting the puppy's attention
Stop	Marker		90 seconds after puppy's paws first meet the ground, 60 seconds after first, stranger starts to ignore puppy
Problem solvable			
Whimper	Duration		Puppy is whimpering
Invisible	Duration		Puppy is out of sight
Problem Solved	Marker		Problem sSolved when puppy is eating food
Start	Marker		Starts when the puppy is in full view and the person carrying them has completely removed her hands - puppy able to move independently
Stop	Marker		2 minutes after „start" when food was not available
Problem unsolvable			
Whimper	Duration		Puppy is whimpering
Touch Object	Duration		Puppy touches cardboard or paper cup with a body part; If puppy touching object AND near a person, code touch object
Invisible	Duration		Puppy is out of sight
Near Breeder	Duration		Puppy's head is within 10 cm from Breeder
Near Lisa	Duration		Puppy's head is within 10 cm from Experimenter
Near Stranger	Duration		Puppy's head is within 10 cm from Stranger
Start	Marker		Starts when the puppy is in full view and the person carrying them has completely removed her hands - puppy able to move independently
Stop	Marker		2 minutes after „start" when food was not available
Novel object			
Approach	Score	2	Approaches within 20 cm of the novel object within 5 seconds
		1	Approaches within 20 cm of the novel object after 5 seconds

Variable	Type of original variable	Possible Values	Description
		0	Does not approach the novel object within 30 seconds
Touch Object	Duration		Puppy touches novel object with a body part
Out of sight	Duration		Puppy is out of sight
Whimper	Duration		Puppy is whimpering
Near Breeder	Duration		Puppy's head is within 10 cm from Breeder
Near Lisa	Duration		Puppy's head is within 10 cm from Experimenter
Near Stranger	Duration		Puppy's head is within 10 cm from Stranger
Start	Marker		Starts when the puppy is in full view and the person carrying them has completely removed her hands - puppy able to move independently
Stop	Marker		2 minutes after Start
		Startle	
Reaction	Score	0	No visible reaction
		1	Weak reaction: only one body part moves (e.g. ears or head)
		2	Strong reaction: > 1 Bodyparts are moving and changing position (e.g. head plus limbs)
		3	Very strong reaction: Puppy lowers complete body, their belly touches the floor OR puppy makes a sudden move with all body parts
Activity of the puppy	Summary score with 1 point each for the below variables	0	Puppy does not change activity and keeps doing what they did before or runs towards noise
		1	Puppy changes activity and does not run towards noise
		1	Freeze (stops movement for more than 2 seconds) only codable when dog moved before
		1	Flees (runs away from the direction from the noise)
		1	Seeks comfort from breeder (Hides behind the breeder or tries to elicit attention)
		1	Tail lowered for at least two seconds after noise
Play	Score	0	Puppy plays and moves again within 15 seconds after noise
		1	Puppy does not play or moves again for > 15 seconds after noise

3.6 Reliability

The inter-rated reliability of behavioral codings was checked with one randomly picked puppy from each breeder (in total: 12 puppies) and results were adequate for all variables (see supplementary table 3 and 4).

3.7 Statistical analysis

The different behavioral variables from the behavioral test (adapted from Riemer et al., 2014) were reduced to four factors with non-linear Principal Component Analysis (PCA) (Linting & van der Kooij, 2012). The effects of the treatment, age at testing, sex and litter size on the four factors were investigated using linear mixed effects models, with litter as random effect. Except for models with no significant predictors, models were reduced step by step until only significant variables remained in the model. To assess the influence of litter, the final reduced model for each dependent variable with litter included as a random factor, was compared with a model without litter. If they differed significantly from each other, litter was retained in the final model.

Model assumptions were checked by visual inspection of residual plots, and if necessary, the analysis was repeated with transformed variables and assumptions checked again. No transformation was needed for the components ‘Explore‘ and ‘Novel Object‘, except that the Novel Object component was multiplied by -1 to facilitate interpretation of this component, such that higher values on this component reflected boldness, rather than fearfulness. The ‘Social-Startle‘ component was transformed by adding a constant of 6 (to obtain all positive values enabling further transformation) and subsequently squaring the values. The Whimper component was transformed as $(\text{original value} + 6)^{-1}$. As for Social-Startle, a constant of 6 was added prior to transformation to achieve positive values.

3.8 Ethics statement/ animal experimentation license

According to the Austrian Animal Experiments Act (§ 2, Federal Law Gazette No. 501/1989), such non-invasive behavioral studies are not considered as animal experiments and no special permission for use of animals in such studies is required. The project was presented to the ethics commission at the University of Vienna and confirmed that no ethical approval is required to carry out the study. All procedures comply with the ‘Guidelines for the Treatment of Animals in Behavioral Research and Teaching‘ of the Association for the Study of Animal Behavior (ASAB). All breeders signed an informed consent form prior to participation in the study.

4 Results

A nonlinear PCA over the 19 behaviour variables was performed (table 3). Based on the maximum number of components with acceptable internal consistency (Cronbach's alpha >0.6), four factors were extracted explaining 55.8% of variance in the data (table 3). Considering a cut-off point of 0.4, there were only two cross-loadings (table 4).

Table 3: Results of the Nonlinear PCA with Varimax rotation

Components	Cronbach's alpha	Eigenvalue	Variance explained
PC 1 - Social-Startle	0.691	3.086	0.162
PC 2 - Whimpering	0.674	2.746	0.145
PC 3 - Novel Object	0.660	2.527	0.133
PC 4 - Exploration	0.602	2.251	0.118
Total	0.956[b]	10.610	0.558

Table 4: Components and component loadings of the CATPCA (Varimax rotation); variable loadings >0.3 are bolded.

Nr.	Variables	PC 1 - Social-Startle	PC 2 - Whimpering	PC 3 - Novel Object	PC 4 - Explo-ration
1	Exploration - whimper	0.216	**0.383**	0.177	**-0.567**
2	Exploration - near stranger	0.141	-0.165	0.026	**0.575**
3	Exploration - tail position	-0.059	0.176	0.077	**0.694**
4	Exploration - activity	0.117	0.127	-0.120	**0.850**
5	Greeting test – whimper / ignored	0.053	**0.832**	-0.072	-0.131
6	Greeting test – body contact/ ignored	**0.875**	0.137	-0.091	0.090
8	Greeting test – body contact/ interaction	**0.845**	-0.155	-0.071	0.103
9	Greeting test - person approach latency	**0.840**	0.132	-0.060	0.099
10	Problem solving latency	-0.037	-0.100	**0.313**	**-0.469**
11	Problem solving - whimper	-0.023	**0.675**	0.103	0.178
12	Problem solving - Touch object (unsolvable)	**-0.366**	-0.151	-0.068	0.134
13	Startle reaction	**0.496**	-0.145	0.012	0.059
14	Startle – Play	0.117	-0.248	**0.615**	-0.086
15	Startle - activity of the puppy	**0.464**	-0.176	**0.377**	-0.242
16	Touch novel object	0.178	-0.263	**-0.744**	0.005
17	Novel Object - whimper	0.172	**0.556**	**0.581**	-0.029
18	Novel Object - near person	0.111	0.045	**0.699**	-0.075
19	Novel Object approach latency	**0.350**	-0.135	**-0.661**	0.037

4.1 PC 1 - Social-Startle

The first principal component labelled ‘PC 1 - Social-Startle’, explained 16.2% of variance and had high positive loadings for the time spent in body contact with the stranger during the greeting test, both when ignored and when the stranger initiated the interaction, a short latency to approach the stranger in the greeting test and negative loadings for the time spent touching the object of the problem solving task, the startle reaction and response. The second component labelled ‘PC 2 – Whimpering’, explained 14,5% of variance and had positive loadings for whimpering in all subtests (explore, greeting, problem solving and novel object). The third component labelled ‘PC 3 - Novel Object‘, explained 13,3% of variance and had positive loadings for the latency to solve the problem, the activity and recovery after the startle, the whimpering and the time spent near a person during the novel object test and negative loadings for time spent touching and the latency to approach the novel object. The fourth component labelled ‘PC 4 - Exploration‘, explained 11,8% of variance and had positive loadings for the time spent near the stranger during the exploration test, as well as the tail position and the activity during exploration and negative loadings for time spent on whimpering during the unsolvable problem solving task and the latency to solve the solvable problem (table 4).

A linear model testing for the effects of treatment, age, litter and sex demonstrated a highly significant difference between the two experimental groups (treatment vs control) in the ‘PC 1 - Social-Startle‘ and in ‘PC 3 - Novel Object‘ and additionally, a significant influence of the puppies’ age in the ‘PC 2 - Whimpering‘.

To start with, the treatment group had significantly lower values for the ‘PC 1 - Social-Startle‘ ($F_{1,70}$=8.93, p=0.0039) than the control group (figure 4, table 5). This means that the treatment group spent less time in body contact with the stranger during the greeting test, both when ignored and when the stranger interacted. Puppies from treatment group also had a longer latency till they approached the stranger during that test situation than the control group puppies. The treatment group puppies had a weaker startle reaction and better scores in activity during or right after the startle. This ‘activity after startle’ variable combines several behaviors I measured after the noise during the startle test: flight reactions were measured as well as comfort seeking, tail lowering, running towards noise, and activity change after the startle (for ex-

planations of each variable view table 4).

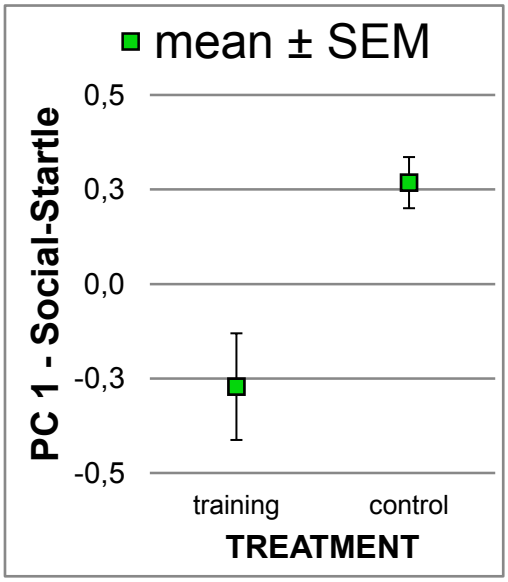

Figure 4.: Mean values ± SEM for the factor 'PC 1 - Social-Startle' of training (n=42) and control group (n=41)

Table 5. Statistical results of the full and final reduced model of the effect of treatment on 'PC 1 - Social-Startle' factor.

Full model										
Factor	**Predictor**	**Value**	**Std. Error**	**CI -95%**	**CI +95%**	**Cohens'D**	**numDF**	**denDF**	**F**	**p**
PC 1 - Social-Startle	Treat-ment	-0.49	1.64	-8.15	-1.59	-0.71	1	69	-2.96	0.0042*
PC 1 - Social-Startle	Age	-0.63	0.48	-1.72	0.46	-0.87	1	9	-1.30	0.2245
PC 1 - Social-Startle	litter_size	1.29	0.81	-0.53	3.11	1.06	1	9	1.6	0.1443
PC 1 - Social-Startle	Sex	0.59	1.75	-2.89	4.08	0.08	1	69	0.34	0.7342
Reduced model										
Factor	**Predictor**	**Estimate**	**Std. Error**	**CI -95%**	**CI +95%**	**Cohens'D**	**numDF**	**denDF**	**Effect of litter**	**F**	**p**
PC 1 - Social-Startle	Treat-ment	-4.9	1.64	-8.17	-1.63	-0.71	1	70	Significant	8.93	0.0039*

4.2 PC 3 - Novel Object

Second, the treatment group had significantly higher values for the ‘PC 3 - Novel Object‘ ($F_{1,70}$=8.75, p=0.0042) than the control group (Fig. 5, Tab. 6). This means that the treatment group solved the solvable problem task faster than the control group, had better scores in ‘activity after startle‘, and was quicker to begin playing after the startle. Within the novel object test they touched the novel object for a longer period of time, approached it faster, whimpered less and spent less time near a person.

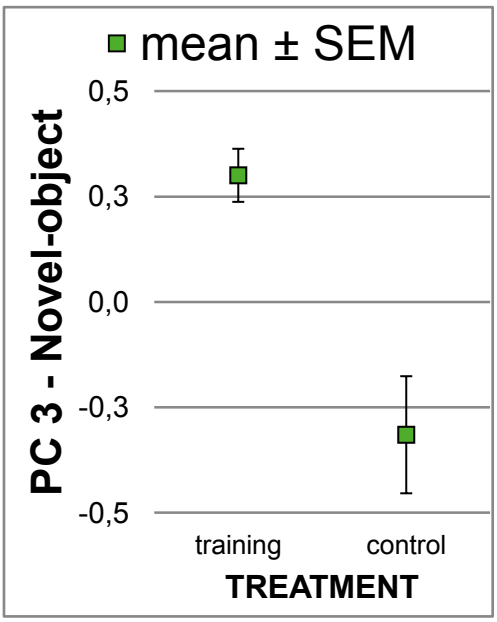

Figure 5.: Mean values ± SEM for the factor ‘PC 3 - Novel Object‘ of training (n=42) and control group (n=41)

Table 6. Statistical results of the full and final reduced model of the effect of treatment on ‘PC 3 - Novel Object‘ factor.

Full model										
Factor	**Predictor**	**Value**	**Std. Error**	**CI - 95%**	**CI +95%**	**Cohens’D**	**numDF**	**den DF**	**F**	**p**
PC 3 - Novel Object	Treatment	0.58	0.19	0.19	0.97	0.72	1	69	2.98	0.0039*
PC 3 - Novel Object	Age	-0.02	0.05	-0.14	0.1	-0.24	1	9	-0.36	0.7250
PC 3 - Novel Object	Littersize	-0.09	0.09	-0.29	0.1	-0.71	1	9	-1.06	0.3140
PC 3 - Novel Object	Sex	-0.27	0.21	-0.68	0.15	-0.31	1	69	-1.29	0.2017
Reduced model										
Factor	**Predictor**	**Estimate**	**Std. Error**	**CI - 95%**	**CI +95%**	**Cohens’D**	**num DF**	**den DF**	**Effect of litter**	**F**	**p**
PC 3 - Novel Object	Treatment	0.58	0.20	0.19	0.97	0.71	1	70	Not significant	8.76	0.0042*

4.3 PC 2 - Whimpering and PC 4 - Exploration

I could not find any treatment-effects for the ‘PC 2 - Whimpering‘ ($F_{1,69}$=1.7, p=0.1962) and ‘PC 4 - Exploration‘ ($F_{1,69}$=0.04, p=0.8511) (Tab. 7, 8)

Table 7.: Statistical results of the full model of the effect of treatment, litter-size, age and sex ‘PC 4 - Exploration‘ factor.

Factor	Predictor	Value	Std. Error	CI -95%	CI +95%	Cohens’D	numDF	denDF	F	p
PC 4 - Explo-ration	Treatment	0.04	0.21	-0.38	0.46	0.05	1	69	0.2	0.8414
PC 4 - Explo-ration	Littersize	-0.1	0.08	-0.28	0.09	-0.8	1	9	-1.2	0.2593
PC 4 - Explo-ration	Age	-0.04	0.05	-0.15	0.07	-0.56	1	9	-0.85	0.4189
PC 4 - Explo-ration	Sex	-0.15	0.22	-0.6	0.3	-0.16	1	69	-0.68	0.4956

Table 8.: Statistical results of the full and final reduced model of the effect of age on ‘PC 2 - Whimpering‘ factor.

Full model										
Factor	Predictor	Va-lue	Std. Er-ror	CI -95%	CI +95%	Cohens’D	numDF	denDF	F	p
PC 2 - Whimp ering	Age	0.002	0.001	-0.2	-0.001	-1.53	1	9	2.19	0.0566
PC 2 - Whimp ering	Treat-ment	0.006	0.005	-0.68	0.15	-0.3	1	69	1.28	0.2040
PC 2 - Whimp ering	Littersize	0.0009	0.002	-0.23	0.11	-0.56	1	9	0.5	0.6305
PC 2 - Whimp ering	Sex	0.003	0.004	-0.5	0.37	-0.07	1	69	0.55	0.5820

Reduced model											
Factor	**Predictor**	**Esti ma- te**	**Std. Er- ror**	**CI - 95%**	**CI +95%**	**Co he ns' D**	**Effect of litter**	**numDF**	**denDF**	**F**	**p**
PC 2 - Whimp ering	Age	2	0.00 1	-0.74	0.14	- 0.3	Not signific ant	1	10	5.36	0.0432*

4.4 Age effect for PC 2 - Whimpering

Additionally, I found an age-effect for 'PC 2 - Whimpering' ($F_{1,10}$=5.36, p=0.043), which means that younger puppies whimpered significantly more than older puppies within all subtests (figure 6, table 8).

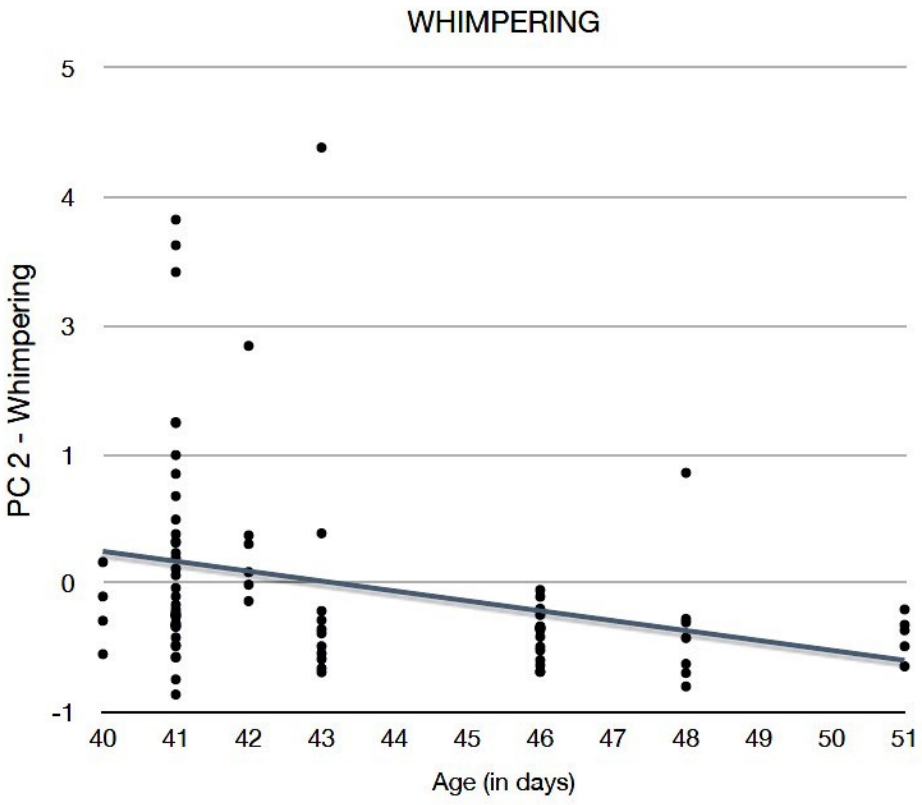

Figure 6: The loadings for each puppy for factor 'PC 2 - Whimpering' dependent on their age (40-51 days).

5 Discussion

I show that overcoming small challenges during the socialization period appears to improve the development of stress resilience when faced with new situations or startling stimuli. Although all puppies received basic handling and exposure to novel objects from the breeder already, I found that the short time of presenting challenges and surprises in form of problem solving games, novel objects, and loud noises had a positive effect. To discuss my findings in detail, the results are divided in three parts: 1) response to new situations and problem solving; 2) reaction to loud noises; 3) sociability to humans.

5.1 Response to new situations and problem solving

I hypothesized that puppies, who are exposed to new objects and problem solving exercises regularly, would learn to cope with new and frustrating events better. Results show that trained puppies were bolder when dealing with novel objects. In the novel object tasks, trained puppies also touched novel objects sooner and for a longer period of time. Additionally, they also whimpered less during the novel object task. Those findings go in line with the results of Vaterlaws and Westside's (2017) study. All of the participating puppies were presented with the same problems from Vaterlaws and Westside's study and therefore, show an effect beyond their findings. Since I did not only present novel objects, but also a variety of age-appropriate challenges, the results show an effect in dealing with frustrating situations when presenting an unsolvable problem task for the first time. When confronted with an unsolvable problem, puppies from the treatment group persisted in their problem-solving attempts for a longer period of time than their untrained littermates. This leads us to the assumption that they may have developed a better coping system when dealing with frustrating experiences and therefore, might have also developed a higher frustration tolerance than the puppies from the control group. Indeed, problem-solving and frustration tolerance have been shown to be consistent personality traits in dogs (Turcsán et al., 2018). In humans, there is evidence for a link between frustration and aggressive behaviors (Dollard et al., 1939; Fox & Spector, 1999). Also, frustrating

situations seem to lower the threshold for aggression in dogs (Borchelt, 1983). Therefore, I suppose that puppies who are trained in coping with controlled, mildly frustrating situations early in life, can also deal with these situations better later in life and even prevent aggressive behavior. Further investigation is needed to show whether there is a long term effect in puppies' behavior in frustrating situations later in life as adult dogs, and whether it could result in better abilities for working dogs.

5.2 Reaction to loud noises

I hypothesized that through repeated exposure of loud noises starting after puppies are opening eyes and ears till they are six weeks old, puppies could practice their startle response. I expected that puppies could recovery immediately after controlled exposure to noise and would be able to generalize speedy recoveries to novel sounds as well. My findings support this by showing that trained puppies had a better startle response and had a faster recovery time than the treatment puppies. I assume that the beneficial effects of controlled exposure to various novel stimuli during this brief phase in puppies' lives may extend to their later life experiences as well.

Although many studies have focused just on stress inoculation training with separation periods (Levine & Mody, 2003; Lyons et al., 2000; Lyons et al., 1999) or novel object presentations (Vaterlaws-Whiteside & Hartmann, 2017), more research is needed in regards to stress inoculation training including sound presentations in early life. In consideration of the extremely high prevalence of noise sensitivities in dogs, sound presentation to increase not only development of stress resilience in general but also prevent the development of noise fears is an area with a great potential for enhancing the welfare of a large number of dogs. Questionnaire studies indicate that around half of dogs suffer from firework fears (Blackwell et al., 2013; Riemer, 2019a, 2019b).

As far as I know, studies done with sound presentation in form of radio or video exposure have not been very effective in preparing puppies for the development of fast recovery responses after a sudden, loud noise. Alves et al., (2018) used a variety of sounds such as music and radio, including car noises, gunshots and more, which had no direct effect on puppies' reactions towards real noises.

The results of this study indicate that controlled exposure to sudden real-world sounds is preferable to using recordings, as it has measurable positive effects on puppies' startle reactions, recovery speed and activity after the startle. I do not know of any other studies about a punctual presentation of single loud noises during the important first three weeks after the ears have opened. As highly stressful or fearful experiences early in life can influence future experiences in a negative way (Luescher, 2011), I was especially considerate of the intensity of the noises I produced. There was no fear response involved, just a short startle and a fast recovery after. This seemed to prepare them for a very loud noise in a novel room without their littermates or mother. Further investigation is needed if this also makes a difference when puppies grow up and prevents puppies from developing noise sensitivities future in life. This would be very important for canine welfare, since fearfulness displayed at a young age (6 months) has been shown to further increase as dogs matures (Riemer et al., 2016).

5.3 Sociability towards humans

In the analysis of sociability of my study subjects, I expected no difference between treatment and control group because the experimenter spent the same amount of time with both groups, picked all puppies up and fed them the same way. Surprisingly, however, the puppies from the control group showed significantly more social behavior than the puppies from the treatment group. In the greeting test, the control group puppies approached the stranger sooner and interacted more. Although the coding of the video material did not take into account the possibility of avoidance motivation in the puppies as a reason for differences in sociability display, with the exception of one litter, I am sure that avoidance motivation was not a valid explanation for the different sociability results in the treatment and the control group. Thus, I can at this time provide no better explanation for the differences in sociability results, other than that the training with novel stimuli may have made them more intriguing than interactions with the experimenter or stranger, respectively. Further investigation is needed to explain these differences in sociability.

Foyer et al. (2013) showed that puppies from smaller litters scored better on sociability as adults, since they might receive more human attention in smaller litters. Alt-

hough I could not find such effect in my study, this could be a possible explanation, as my sample size was rather small. To sum up, the puppies from the control group showed significantly more social behavior than the puppies from the treatment group - an effect that was overriding that of litter size and needs to be further investigated upon.

5.4 Separation related and explorative behavior

What I could not find was a positive effect on separation related behavior during the exploration test or in the duration of whimpering in general. Puppies from the treatment group were not more active or whimpered for shorter periods, nor were there any differences in the tail position and time spent near a stranger when left alone in a novel room. Although I prepared puppies and separated them shortly from their littermates during most problem-solving tasks, they did not behave differently during the exploration test in which they were separated from familiar people as well as their mother and litter mates. With a similar procedure in separating puppies, Vaterlaws-Whiteside & Hartmann (2017) showed a decrease in separation related behavior on a long-term basis. Based on these findings, I need to wait for long-term behavioral data for finding effects here.

Separation related problems can be a huge problem for both humans and dogs, and make up 20% - 40% of all behavior consultations (Horwitz, 2000; Voith & Borchelt, 1985; Wright & Nesselrote, 1987). In rats, there is evidence of long-term benefits of briefly separating the mother from young pups. As adults, these rats where less reactive and more emotionally stable compared to controls (Levine et al., 1967). Within my study there was no difference found, but I expect future data to support the same positive effects of this early separation training, which I introduced very carefully.

5.5 Age seems to matter

Besides the effect of treatment, I tested the effects of age at test and the duration of whimpering. The results show the older they were, the less time puppies spent whimpering, which can be relevant in regard to exact timings of future puppy tests.

5.6 Litter Effect

Similar to the findings of Riemer et al. (2014), who found that litter effects affect several puppy test components such as exploration/inactivity, low boldness, playfulness, greeting, interaction, flight and struggle, I also found a litter effect for the principal component PC 1 ‚Social Startle'. The puppies from this study were the same age when tested. The presence of a litter effect in the data indicates that different effects such as genetic and maternal effects or the shared early environment might also influence behavior during the test of six-week-old puppies. To note, Strandberg et al. (2005) and Foyer et al. (2013) also found litter effects on the behavior of adult dogs tested, but they had a much larger sample size.

6 Conclusion

I conclude that controlled early exposure to a diversity of age-appropriate exercises and controlled exposure to noises and novel objects have a positive influence on the development of stress resilience in puppies. These findings are of interest to breeders, shelters, animal welfare facilities and organizations, as these results could help them prepare puppies for the stimulating city life, future assistance or working dog training. Further research is needed to investigate whether this early age training program can lead to the development of better stress coping abilities, greater resilience and as a result prevent future problem behavior in dogs.

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9 Zusammenfassung

Die Sozialisierungsphase bei Hundewelpen ist eine der wichtigsten Phasen für späteres Verhalten. Kaum untersucht wurden bisher die Interventionen zur Verbesserung der Stressresilienz bei Hundewelpen. Mit dieser Studie werden die Effekte einer frühzeitigen Stimulation über die bloße Reizpräsentation hinaus auf die Stressresilienz von Welpen getestet. 83 Welpen aus 12 Würfen nahmen an der Studie teil. Die Hälfte jedes Wurfs bildete die Behandlungs- und die andere Hälfte die Kontrollgruppe. Die 3-5 Wochen alten Welpen in der Experimentalgruppe erhielten an insgesamt 12 Tagen altersgerechte Herausforderungen in Form von Exposition von plötzlichen Geräuschen, neuartigen Objekten und Aufgaben zur Problemlösung. Die Kontrollgruppe verbrachte Zeit mit dem Trainer ohne Aufgaben. Im Alter zwischen 40 und 52 Tagen wurden alle Welpen verhaltensgetestet.

Eine nichtlineare Hauptkomponentenanalyse über codiertes Verhalten ergab vier Hauptkomponenten, von denen sich zwei zwischen den beiden Gruppen signifikant unterschieden. Erstens: „Reaktion auf Neuheit“ ($F_{1,70}$=8,75,p=0,0042), bedeutet, dass die Welpen in der Experimentalgruppe die Problemlöseaufgaben schneller lösten, mehr Exploration an neuartigen Objekten zeigten, weniger Menschennähe aufsuchten und weniger winselten. Zweitens: „Social-Startle“ ($F_{1,70}$=8,93,p=0,0039), was darauf hinweist, dass die behandelten Welpen reduzierte Schreckreaktionen auf laute Geräusche zeigten; die Kontrollgruppe zeigte hingegen höheres Interesse an freundlichen Fremden - es ist möglich, dass die erhöhten Interaktionen der Kontrollgruppe mit dem Trainer positive Auswirkungen auf ihre Geselligkeit hatten.

Die frühe Konfrontation mit Herausforderungen, Überraschungen, neuartigen Objekten scheint es den Experimentalgruppe zu ermöglichen, besser mit neuartigen Reizen umzugehen, weniger ängstlich zu reagieren und sich nach lauten Geräuschen schneller zu erholen, sprich die Stressresilienz zu verbessern.

10 Appendix

Suppl. Table 1: Details about the subjects

total nr.	Litter	ID	sex	treat-ment	breed	age at test (days)
1	Sheltie1	Sheltie1 1	female	control	Shetland Sheepdog	43
2	Sheltie1	Sheltie1 2	female	control	Shetland Sheepdog	43
3	Sheltie1	Sheltie1 3	male	training	Shetland Sheepdog	43
4	Sheltie1	Sheltie1 4	female	training	Shetland Sheepdog	43
5	Sheltie2	Sheltie2 1	female	training	Shetland Sheepdog	51
6	Sheltie2	Sheltie2 2	male	control	Shetland Sheepdog	51
7	Sheltie2	Sheltie2 3	male	training	Shetland Sheepdog	51
8	Sheltie2	Sheltie2 4	male	training	Shetland Sheepdog	51
9	Sheltie2	Sheltie2 5	male	control	Shetland Sheepdog	51
10	MiniAussie1	MiniAussie1 1	male	control	Mini Australian Shepherd	41
11	MiniAussie1	MiniAussie1 2	female	training	Mini Australian Shepherd	41
12	MiniAussie1	MiniAussie1 3	female	control	Mini Australian Shepherd	41
13	MiniAussie1	MiniAussie1 4	male	control	Mini Australian Shepherd	41
14	MiniAussie1	MiniAussie1 5	female	training	Mini Australian Shepherd	41
15	MiniAussie1	MiniAussie1 6	female	control	Mini Australian Shepherd	41
16	MiniAussie1	MiniAussie1 7	male	training	Mini Australian Shepherd	41
17	Aussie1	Aussie1 1	female	control	Australian Shepherd	40
18	Aussie1	Aussie1 2	female	training	Australian Shepherd	40
19	Aussie1	Aussie1 3	female	training	Australian Shepherd	40
20	Aussie1	Aussie1 4	male	control	Australian Shepherd	40
21	Aussie2	Aussie2 1	female	control	Australian Shepherd	46
22	Aussie2	Aussie2 2	female	control	Australian Shepherd	46
23	Aussie2	Aussie2 3	female	control	Australian Shepherd	46
24	Aussie2	Aussie2 4	female	training	Australian Shepherd	46
25	Aussie2	Aussie2 5	female	training	Australian Shepherd	46
26	Aussie2	Aussie2 6	female	training	Australian Shepherd	46
27	Aussie2	Aussie2 7	female	training	Australian Shepherd	46
28	Aussie3	Aussie3 1	male	control	Australian Shepherd	46
29	Aussie3	Aussie3 2	female	training	Australian Shepherd	46

total nr.	Litter	ID	sex	treat-ment	breed	age at test (days)
30	Aussie3	Aussie3 3	male	training	Australian Shepherd	46
31	Aussie3	Aussie3 4	female	control	Australian Shepherd	46
32	Aussie3	Aussie3 5	female	control	Australian Shepherd	46
33	Aussie3	Aussie3 6	male	control	Australian Shepherd	46
34	Aussie3	Aussie3 7	female	training	Australian Shepherd	46
35	Aussie3	Aussie3 8	male	training	Australian Shepherd	46
36	Aussie4	Aussie4 1	female	training	Australian Shepherd	48
37	Aussie4	Aussie4 2	female	control	Australian Shepherd	48
38	Aussie4	Aussie4 3	male	training	Australian Shepherd	48
39	Aussie4	Aussie4 4	male	control	Australian Shepherd	48
40	Aussie4	Aussie4 5	female	control	Australian Shepherd	48
41	Aussie4	Aussie4 6	male	control	Australian Shepherd	48
42	Aussie4	Aussie4 7	male	training	Australian Shepherd	48
43	Labrador1	Labrador1 1	female	control	Labrador Retriever	41
44	Labrador1	Labrador1 2	male	training	Labrador Retriever	41
45	Labrador1	Labrador1 3	male	control	Labrador Retriever	41
46	Labrador1	Labrador1 4	male	control	Labrador Retriever	41
47	Labrador1	Labrador1 5	female	training	Labrador Retriever	41
48	Labrador1	Labrador1 6	female	training	Labrador Retriever	41
49	Labrador1	Labrador1 7	male	training	Labrador Retriever	41
50	Labrador1	Labrador1 8	female	control	Labrador Retriever	41
51	Labrador1	Labrador1 9	female	training	Labrador Retriever	41
52	Herder1	Herder1 1	female	control	Dutch Shepherd	41
53	Herder1	Herder1 2	female	control	Dutch Shepherd	41
54	Herder1	Herder1 3	female	training	Dutch Shepherd	41
55	Herder1	Herder1 4	male	training	Dutch Shepherd	41
56	Herder1	Herder1 5	male	training	Dutch Shepherd	41
57	Herder1	Herder1 6	female	control	Dutch Shepherd	41
58	Herder1	Herder1 7	female	training	Dutch Shepherd	41

total nr.	Litter	ID	sex	treat-ment	breed	age at test (days)
59	Herder1	Herder1 8	male	control	Dutch Shepherd	41
60	Pitbull1	Pitbull1 1	female	training	American Pitbull Terrier	41
61	Pitbull1	Pitbull1 2	male	training	American Pitbull Terrier	41
62	Pitbull1	Pitbull1 3	male	training	American Pitbull Terrier	41
63	Pitbull1	Pitbull1 4	female	control	American Pitbull Terrier	41
64	Pitbull1	Pitbull1 5	male	control	American Pitbull Terrier	41
65	Pitbull1	Pitbull1 6	male	control	American Pitbull Terrier	41
66	Pitbull1	Pitbull1 7	female	control	American Pitbull Terrier	41
67	Pitbull1	Pitbull1 8	female	control	American Pitbull Terrier	41
68	Pitbull1	Pitbull1 9	male	control	American Pitbull Terrier	41
69	Pitbull1	Pitbull1 10	male	training	American Pitbull Terrier	41
70	Pitbull1	Pitbull1 11	female	training	American Pitbull Terrier	41
71	Icelandic1	Icelandic1 1	female	training	Icelandic Sheepdog	42
72	Icelandic1	Icelandic1 2	male	control	Icelandic Sheepdog	42
73	Icelandic1	Icelandic1 3	female	control	Icelandic Sheepdog	42
74	Icelandic1	Icelandic1 4	male	training	Icelandic Sheepdog	42
75	Icelandic1	Icelandic1 5	male	training	Icelandic Sheepdog	42
76	Icelandic1	Icelandic1 6	female	control	Icelandic Sheepdog	42
77	Setter1	Setter1 1	female	training	English Setter	43
78	Setter1	Setter1 2	male	control	English Setter	43
79	Setter1	Setter1 3	female	training	English Setter	43
80	Setter1	Setter1 4	male	training	English Setter	43
81	Setter1	Setter1 5	female	control	English Setter	43
82	Setter1	Setter1 6	female	control	English Setter	43
83	Setter1	Setter1 7	female	training	English Setter	43

Suppl. Table 2: Exemplary presentation of exercises for each week during the three week training period puppies (total 12 sessions per dog). Each session included presentation of a novel object, a problem solving task and two different sounds.

Exercises	Week 1 (3-4 weeks)	Week 2 (4-5 weeks)	Week 3 (5-6 weeks)
New Object Novel objects were presented to puppies in different ways. Puppies were able to explore them by themselves.	An empty trash bag (40x25cm) was filled with air and closed, till it formed an object similar to a ball. This was put into the puppy pen and moved and touched by the experimenter.	Several pieces of commercial white printer paper (A4) were crumbled up by the experimenter and thrown into the puppy pen.	A mirror (40x80cm) was either placed into the pen so that puppies could see themselves or was placed flat onto the floor.
	An umbrella was opened and closed several times next to the puppies and then opened up and placed into the puppy pen.	A red plastic cone (17.5x12cm) was put into the puppy pen.	An big empty plastic carrier bag (40x20x45cm) was put up inside the puppy pen and moved around by the experimenter.
	A cat toy was put into the puppy pen and moved. The toy (25x7cm) had a heavy, round body made out of plastic (looks like an egg) and it functioned like a tumbler. On top there was a feather. When puppies tried to catch the feather, it moved, but stayed in position. Inside of the plastic bowl, there was a little bell.	An empty paper carrier bag (32x17x44cm) was put up inside the puppy pen and moved around by the experimenter.	A roll up curtain (55x55cm) was put into the puppy pen and extended and retracted repeatedly.
	A 1.5 liter plastic water bottle filled with water was shaken, knocked over and moved next to the puppies and then put into the puppy pen either vertical or horizontal.	An inflatable plastic flamingo (18x11cm) was put into the puppy pen and moved around by the experimenter. Before puppies could bite it, it was removed from the puppy pen quickly.	A scooter (HUDORA 14708 BigWheel 205) was put in front of or into the puppy pen and puppies could either directly explore or take a look at it from a distance. The experimenter was either riding or moving it.

Exercises	Week 1 (3-4 weeks)	Week 2 (4-5 weeks)	Week 3 (5-6 weeks)
Problem Solving Puppies were confronted with age-appropriate problems which were solvable in the end. During problem solving puppies were usually put out of the puppy pen and separated from the other littermates for 1-5 minutes.	Puppy was placed on top of a book (17.5x25cm) and it had to find its way 3cm off the floor.	Puppy had to traverse an empty plastic carrier (40x20x45cm) to reach a human or their littermates.	Puppy had to traverse an empty carry bag out of paper (32x17x44cm) to reach food.
	Puppy was put on a cool plate with a diameter of 25cm and it had to find its way off (approx. 2cm off the floor). The plate was put into the fridge before for about ten minutes.	Puppy had to traverse a mirror (40x80cm) that was put flat on the floor to reach a human or food.	Shaping exercise part 1 - Food was put onto a chair or table so that puppy couldn't reach it. Then blanket was put onto the ground and each time puppy touched the blanked instead of trying to reach the food, it got clicked by a clicker and a food reward afterwards - this was repeated several times and all movements towards the ground - sitting, sniffing, lying down, … was clicked and reinforced. Exercise was done about 2-4 minutes.
	Puppy was put onto an elastic air cushion with a diameter of 34cm and it had to find its way approximately 4 cm off the floor.	Puppies had to climb over a barrier to get to littermates, human or food. The barrier was about 10-15 cm high and the material was a metal grid. To make it more comfortable for the puppies to climb over, a towel was put on top.	Shaping exercise repeated (see shaping exercise part 1)

	Week 1 (3-4 weeks)	Week 2 (4-5 weeks)	Week 3 (5-6 weeks)
	A kitchen towel (about 40x30cm) was placed over the body of the puppy and it had to find its way out.	Detour task: food was put behind a barrier in form of a metal grid. Puppies had to run around the grid in order to get to food. Different difficulties were done dependent on each litter and individual. Food was placed 2-20 cm from the edge of the grid and grid shape varied from V-shape to straight line.	
Sounds Different sounds with different volumes were presented from different distances - conditions changed for each litter adjusted to the reactions of the puppies. The sounds were presented from a distance between 4-323 cm and repeated 2-10 times and varied from 15-79 decibel, measured by the mobile phone app „Schallpegelmesser in Dezibel“. With each litter presentation started with high distance and low volume. Within time distance was decreased and volume increased.	A book weighing 1.3 kg was dropped on a tiled floor.	A cake tin weighing 314g with a diameter of 27cm was dropped on a tiled floor.	A firework recording from YouTube was presented to the puppies. The sound came out of a loudspeaker. https://www.youtube.com/watch?v=H7FANXaanG4
	A kitchen pot lid weighing 314g with a diameter of 22cm was banged against a metal kitchen pot weighing 1080g with a diameter of 22cm and a height of 17cm.	Eyeglasses case was dropped on the floor or opened and closed quickly.	A metal kitchen pot with a diameter of 22 and a hight of 17cm weighing 1080g was dropped on a tiled floor.
	A kitchen pot lid weighing 314g with a diameter of 22cm was dropped on a tiled floor.	A bicycle horn in form of a retro metal horn and an air filled rubber ball was squeezed (18.5x5cm).	A metal food bowl weighing 260g with a diameter of 20cm was dropped on a tiled floor.
	Several pieces of cutlery were dropped into a metal box (14x10x18cm).	A metal box (14x10x18cm) with several pieces of cutlery inside was shaken.	A metal clicker made for dog training (10.4x6x2.4cm) was clicked several times.
	A 1.5 liter plastic water bottle, filled with water, was dropped on a tiled floor from a distance of 33 cm.	It was drummed with a wooden spoon (30cm) onto a metal kitchen pot weighing 1080g with a diameter of 22 and a height of 17cm.	A gunshot recording from YouTube was presented to the puppies. The sound came out of a loudspeaker. https://www.youtube.com/watch?v=A5zwcsVsVz0

	Week 1 (3-4 weeks)	Week 2 (4-5 weeks)	Week 3 (5-6 weeks)
	A human was screaming for about 2 seconds with a high voice.		

Suppl. Table 3: Reliability for scores. All variables were reliable.

Cohen's kappa	
Variables	**weighted kappa**
Explore Tail mean	0.7
Greeting Approach	0.7
Novel Object Approach	0.88
Startle Reaction	0.67
Startle Activity of the puppy	0.64
Startle Play	1

Suppl. Table 4: Reliability for coded durations. All variables were reliable.

CRONBACH'S ALPHA	
Variables	std.Alpha
Explore - activity	0.92
Explore - whimper	1
Explore - near stranger	0.67
Greeting test – body contact/ ignored	0.99
Greeting test – body contact/ interaction	0.99
Greeting test – whimper / ignored	No variance
Novel object - whimper	0.99
Touch novel object	1
Novel object - near person	0.84
Problem solving - whimper	No variance
Problem solving latency	0.99
Problem solving - whimper (unsolvable)	0.83
Problem solving - Touch object (unsolvable)	0.99