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Social behavior in dog puppies: Breed differences and the effect of rearing conditions

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Introduction: Most of the studies investigating the effect of early rearing environment in dogs used laboratory dogs and reported that early experiences markedly affect the puppies' behavior. However, the subjects of these experiments cannot be considered as representatives of family dogs. *Methods:* In this study, we investigated whether different raising conditions shape social behavior toward humans in 8-week-old family dog puppies of two breeds, Labrador and Czechoslovakian wolf dog. The puppies were tested in a series of tests that represented typical situations of family dogs. *Results:* We found that Czechoslovakian wolf dog puppies were more active than Labrador puppies in general, as they were more likely to explore the environment and the objects and spent more time doing so. Tendency to gaze at humans also varied between breeds, but in a context-specific way. Additionally, puppies housed separately from their mother interacted more with toys, puppies housed in a kennel tended to stay closer to the experimenter than puppies raised in the house, and puppies housed in a kennel tended to stay in the proximity of the experimenter more than puppies raised in the house. *Conclusions:* Our results provide evidence for early keeping conditions influencing social behavior and also highlight breed differences in puppies' behavior. Whether these differences are due to different developmental patterns and/or behavioral predispositions remains to be explored.

INTRODUCTION

In altricial species, such as dogs, early experiences can have a large influence on future behavior (Foyer et al., 2014). In case of dogs, the most important period is considered to be the first 3 months of life (Scott & Fuller, 1965) but the first few sensitive weeks seem to have the highest impact on the development of social behavior (Battaglia, 2009). The basis of the actual knowledge on the effect of puppies' early rearing environment relies on Scott and Fuller's work, who investigated the development of social behavior of dogs, dividing it in so called "critical periods." The results of their work suggested that dogs, similar to other social species, show a limited period in which the individual is most predisposed to form positive social relationships (Scott, 1962; Scott & Fuller, 1965). Most of the studies mainly concentrated on the effects of social or sensory deprivation/stimulation on puppies' behavior at different ages or on adult behavior (e.g., Fox & Stelzner, 1966, 1967; Freedman et al., 1961; Gazzano et al., 2008; Igel & Calvin, 1960; Pettijohn et al., 1977; Thompson & Heron, 1954). For instance, Fox and Stelzner (1966) investigated the effect of regular handling from birth on the behavior of puppies until the age of 5 weeks and found that puppies from the treated group were generally more sociable with humans and were more dominant in social situations with their peers. The early studies were carried out on laboratory dogs in a highly controlled environment; therefore, despite environmental enrichment and regular social stimulation, the social experiences of these subjects were very limited compared to those of typical family dogs. Consequently, these subjects may have not developed their maximum capacities and may not be fully representative of the family dog population.

Another research line on puppy behavior focuses on the possibility to predict adult behavior for working purposes, by the means of temperament tests mostly designed to select subjects that are promising candidates for becoming guide or service dogs (e.g., Foyer et al., 2013; Goddard & Beilharz, 1983; Sinn et al., 2010; Wilsson & Sundgren, 1997). In these studies, the subjects usually stem from specific breeding facilities that select dogs for working purposes. The puppies are typically group-housed with the mother and their peers until weaning (Foyer et al., 2013; Vaterlaws-Whiteside

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& Hatmann, 2017; Wilsson & Sundgren, 1997). The breeding pairs are selected based on particular traits and around 8 weeks of age puppies are taken to foster homes where specific guidelines are followed for the puppies' handling, so that these puppies cannot also be considered as fully representatives of the family dog population.

The aim of this study was to investigate the effects of early keeping conditions, such as housing and time of separation from the mother, on social behavior toward humans in family dog puppies. The tests were held at the breeder at the age of 8 weeks and were designed to evaluate behavioral responses that indicate tendency to interact socially with humans in various situations. The tests represented typical, everyday situations of dogs living with humans and were carried out before adoption of the subjects. We collected data about the rearing conditions of the puppies from the breeder. We hypothesized that housing conditions such as kennel rearing as opposed to living in the house with the family or being housed with the mother or separated from her before adoption affected puppies' behavior toward humans and their reactions to environmental stimuli. We expected that puppies reared in the house with the family and those that were separated from their mother sooner than 8 weeks of age would spend more time interacting with the experimenter. Because these puppies may have more experience with humans as they had more chance to interact with them from birth, it is also possible that, as an effect of early separation, they are more stressed in novel situations and consequently seek more interaction with humans (Slabbert & Rasa, 1993).

Breed differences in developmental patterns and predispositions to certain behaviors may overshadow the role of environment in the development of various behaviors (Scott & Fuller, 1965). Therefore, we aimed at testing and comparing dogs from only two breeds that are selected for very different purposes: Labrador Retrievers and Czechoslovakian wolf dogs (CSWs). CSW is a recent mix from German Shepherds and Carpathian wolves obtained as a military project to select dogs to guard borders. Labrador Retrievers were originally used as gundogs and later became exceptionally popular as family dogs and guide dogs (Blackwell et al., 2013). In addition, being CSW, a recent mix with wolves, we aimed at investigating whether their behavior is more similar to wolves in terms of being generally more active, exploring more and seeking less contact with humans than Labrador puppies (Gácsi et al., 2005; Rao et al., 2018; Topál et al., 2005).

MATERIALS AND METHODS

Subjects

We tested $N = 100$ eight-week-old dog puppies of two breeds: Labrador ($N = 64$ subjects from 9 litters) and CSW ($N = 36$ subjects from 7 litters; Table 1).

Data collection

Besides basic demographic information, we collected data about the housing conditions of the puppies. Assuming that puppies kept in the house had more frequent contact with humans, we classified the puppies into two groups: puppies that were kept in the house where the breeder lived were categorized as raised in a home environment whereas puppies that were kept in a facility (i.e., separated from the house of the breeder) were categorized as raised in a kennel. We also collected information on whether the puppies were housed with their mother or separated from her before the age of 8 weeks (Table 1).

Experimental procedure

The test battery was administered before the puppies were adopted, while still living with the breeder, in his/her facility or house. We used a movable 4 m × 3 m puppy fence to have a standard testing area. The tests were video-recorded using two digital cameras standing on tripods. Whenever dogs

Table 1. Information of the litters participating in the study

Breed	Litter ID	No. of puppies (N; sex ratio as males:females)	Housing condition	Separation from the mother
Czechoslovakian wolf dog	clc1	$N = 6$ (3:3)	House	Separated
	clc2	$N = 5$ (4:1)	House	Separated
	clc3	$N = 4$ (3:1)	House	Separated
	clc4	$N = 7$ (1:6)	House	Separated
	clc5	$N = 4$ (0:4)	Kennel	Separated
	clc6	$N = 4$ (2:2)	House	Separated
	clc7	$N = 6$ (3:3)	House	Not separated
Labrador	lab1	$N = 6$ (4:2)	House	Separated
	lab2	$N = 6$ (2:4)	House	Separated
	lab3	$N = 6$ (5:1)	House	Separated
	lab4	$N = 7$ (4:3)	House	Not separated
	lab5	$N = 8$ (4:4)	Kennel	Not separated
	lab6	$N = 6$ (2:4)	House	Not separated
	lab7	$N = 6$ (3:3)	Kennel	Separated
	lab8	$N = 10$ (6:4)	Kennel	Separated
	lab9	$N = 9$ (7:2)	House	Not separated

(i.e., their owners) were available, we repeated once all tests on a different day (range of time spent between the two repeated tests: 2–4 days) to increase reliability of the measured variables by reducing random, uncontrollable effects (e.g., if a puppy is particularly tired or active on 1 day due to previous free interactions with the littermates).

The test battery consisted of seven tests. After the 4th test – approximately after 30 min – the subjects were given a 1-hr break. Overall, the test battery lasted approximately for 1 hr.

Before the tests were carried out, the puppies interacted briefly with the experimenter who read their microchips and listed their names. Immediately before initiating the tests, the puppy was isolated for 1 min in a crate (dimensions ca. 1 m × 1 m × 1 m) to increase motivation to interact socially with the experimenter during the tests.

The tests were run by a female experimenter and a female helper.

Although this has never occurred, the experimenter would have interrupted the tests if the subject showed excessive signs of stress.

Description of the tests

Preference test. Immediately after the isolation, the helper released the subject in the puppy fence, where the experimenter was sitting motionless on a small stool, 1.5 m from a toy (a knotted rope), and looked at the puppy. The subject's behavior was observed for 3 min from when the helper released the puppy and then immediately the puppy proceeded to the subsequent test.

Play with a toy. Immediately after the preference test, the experimenter tried to invite the dog to play with a toy by pulling it on the floor for 1 min. The test started when the experimenter first tried to take the puppy's attention. If the puppy took the toy in its mouth or played with it, the Experimenter held the toy for 3 s and then left it with the puppy for 20 s before gently taking it away. This procedure was repeated three times.

Social and non-social object permanence test. The test was randomized with half of the puppies in each litter starting with the social and the other half with the non-social object permanence test.

Non-social object permanence test: The helper held the puppy by its chest while the experimenter stood next to her. They stood 3 m from a barrier made up of a plastic table placed on a side (i.e., turned 90°), to prevent the puppy's view from what was behind it. The experimenter showed the toy (the same toy as in the preference test) to the puppy, attracting its attention on the toy, and then threw it behind a barrier. After the toy disappeared behind the barrier, the helper held the puppy for 10 s and then released it, so that the puppy was free to move for 20 s. This procedure was repeated twice.

Social object permanence: The helper held the puppy while the experimenter stood next to the helper. They stood 3 m from the barrier, facing toward it. The experimenter called the puppy and attracted its attention (by gaze contact, clapping hands gently, and talking with high-pitch voice), while walking in the direction of the barrier. When she reached the barrier, she did not talk anymore and hid behind it. After the experimenter disappeared behind the barrier, the helper held the puppy for 10 more s and then released it and the puppy was free to move for 20 s. This procedure was repeated twice.

Recall test. The helper held the puppy 3.5 m from the experimenter. The experimenter called the puppy (saying "vieni, vieni!" – "come, come!" in Italian – by gently clapping her hands twice) and crouched down. The helper released the puppy. When the puppy approached the experimenter, she praised and petted it for 4 s, then stayed crouched for 20 s without moving or talking. The same procedure was repeated for eight trials.

Part 2. The second part of the test battery started after the puppies had a break of 1 hr, during which they were placed in their housing kennel with their littermates.

Hold and pet test. The experimenter held the puppy in her arms while standing for 1 min (measured from when the Experimenter took up the puppy) petting it gently. The puppy was then placed on the floor and released and the experimenter stood passively for 20 s.

Gazing at the experimenter test. The puppy was first allowed to eat four pieces of food from the hand of the experimenter who lured the puppy's gaze toward her face before giving the treat. Then, the experimenter held the food in her hand extended laterally. The experimenter gave vocal praise ("OK!" with a high-pitch voice) and a piece of food, if the puppy looked at her face. This test phase lasted for 4 min (Gácsi et al., 2005). After 4 min, an extinction phase started, during which no more food and vocal praise were given until the puppy did not look at the experimenter for 2 consecutive min (Bentosela et al., 2016). During this time, the experimenter extended a finger every time the puppy gazed at her face (in order to facilitate subsequent video coding).

Noise test. The puppy was exposed to two different sounds that were played from a smartphone located in a crate, while the experimenter was sitting motionless on a stool in the test area (2.5 m from the crate, facing it). The puppy was free in the test area. The two sounds were a siren and a drill. The order of exposure to the two sounds was semi-randomized with half of the puppies in each litter starting with the siren and the other half with the drill. The first sound started after a minute of silence and the second sound started measured from immediately after the first; each sound lasted for 1 min. After the end of the last sound, the experimenter waited for 20 s seated on the stool before completing the test.

Data analysis

Behavioral coding of video recordings was carried out using Solomon Coder (beta 15.03.15, © András Péter, Budapest, Hungary). Table 2 describes the coded behavioral responses.

Due to experimenter's errors in the experimental procedure or impossibility to carry on a given test for some subjects due to environmental inadequate conditions, some subjects were excluded from various tests. Table 3 describes the final number of analyzed subjects in each test.

We used the R statistical environment (v. 3.2.2; R Development Core Team, 2015) to analyze the behavioral

Table 2. Definitions of the measured variables in the tests

Preference test	
Latency of being in proximity to E	The probability of getting within 50 cm from E
Duration of being proximity to E	Time spent close to the experimenter (maximum 50 cm) – including also physical contact with E
Latency of being in proximity to toy	The probability of getting within 50 cm from toy
Duration of being proximity to toy	Time spent close to the toy (maximum 50 cm) – including also physical contact with E
Latency of interacting with E	The probability of being physical contact with E
Duration of interacting with E	Time spent in physical contact with E
Latency of interacting with toy	The probability of being physical contact with toy
Latency of exploration	The probability of exploration (walking, sniffing, chewing, and manipulating the fence or objects in the testing area)
Duration of exploration	The time spent on exploration (walking, sniffing, chewing, manipulating the fence or objects in the testing area)
Object permanence test	
Latency of seeing toy	The probability of being the puppy's head behind the line of the barrier (it can see the toy)
Latency of seeing E	The probability of being the puppy's head behind the line of the barrier (it can see the E)
Recall test	
Latency of being in proximity to E	The probability of getting within 50 cm from E
Duration of being proximity to E	Time spent close to the experimenter (maximum 50 cm) – including also physical contact with E
Latency of interacting with E	The probability of being physical contact with E
Duration of interacting with E	Time spent in physical contact with E
Hold and pet test	
Duration of being proximity to E	Time spent close to the experimenter (max. 50 cm) – including also physical contact with E
Gaze test	
Frequency of receiving a treat	N times when the experimenter gives a treat to the puppy
Latency of gazing E	The probability of gazing at the experimenter (the E indicates this by raising her finger)
Duration of gazing at the E	Time spent gazing at the experimenter (the E indicates this by raising her finger)
Total time of extinction	The puppy does not look at the experimenter for 2 consecutive min
Noise test	
Latency of being proximity to cage	The probability of getting within 50 cm from cage
Time spent being proximity to cage	Time spent close to the cage (maximum 50 cm) – including also physical contact with the cage
Latency of being in proximity to E	The probability of getting within 50 cm from E
Duration of being proximity to E	Time spent close to the experimenter (maximum 50 cm) – including also physical contact with E
Latency of gazing E	The probability of being the puppy's head oriented toward the E
Duration of gazing E	Time spent gazing at the E
Latency of gazing cage	The probability of being the puppy's head oriented toward the cage
Duration of gazing cage	Time spent gazing at the cage
Frequency of gaze shifts	Number of gaze shifts between the E and the cage

responses. For all tests and response variables, we analyzed in separate models whether any of the keeping conditions (housing and separation from the mother) or breed had an effect on frequency, latency, or duration of the behaviors described in Table 2 and the initial models included day and trial number as fixed factors. Latencies were analyzed in Cox Mixed Models (R package "coxme;" Therneau, 2015) with occurrence of getting close to the stimuli, interacting with those and exploring as terminal events, in respective models. Dogs that did not approach the stimuli interacted with those or explored were treated as censored observations. Model selection was based on Akaike information criterion values, and the effects of explanatory variables were analyzed using likelihood ratio tests (LRTs): we provide χ^2 and p values of LRTs of models with and without the explanatory variable. Durations and frequencies were analyzed in separate generalized linear mixed models (GLMMs). Durations were analyzed as log-transformed durations or logit-transformed proportion of time spent with the given activity (e.g., being close to the experimenter, being close to the toy, interacting with those, and exploring them). Frequencies were analyzed in GLMMs with Poisson distribution. All Cox mixed models and GLMMs included litter and dog ID (nested in litter) as random terms.

RESULTS

We found significant breed differences in the following tests: preference test, gaze test, recall test, hold and pet test, and noise test. Housing conditions influenced behavioral responses in the preference test, gaze test, and recall test, whereas early separation from the mother influenced responses of puppies in the preference and noise tests.

Table 3. The analyzed final subject numbers for each test after exclusion

	Gaze test		Hold and pet test		Noise test		Object permanence test		Preference test		Recall test	
Litter ID	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Clc1	0	6	0	6	0	6	0	0	0	6	0	0
Clc2	1	4	1	5	1	3	1	0	1	4	1	0
Clc3	4	3	4	3	4	3	4	4	4	4	4	4
Clc4	7	7	7	7	7	7	7	7	7	7	7	7
Clc5	4	0	4	0	4	0	4	0	4	0	4	0
Clc6	4	4	4	4	4	4	0	0	4	4	0	0
Clc7	6	0	5	0	4	0	0	0	6	0	0	0
Lab1	6	6	6	6	6	5	6	6	6	6	6	6
Lab2	5	0	5	0	5	0	6	0	6	0	6	0
Lab3	6	6	6	6	6	6	6	6	6	6	6	6
Lab4	7	7	7	7	7	7	6	7	7	7	6	7
Lab5	8	7	8	7	8	6	8	8	8	8	8	8
Lab6	4	5	4	5	6	6	6	6	6	6	6	6
Lab7	5	6	5	6	5	6	6	6	6	6	6	6
Lab8	9	0	9	0	9	0	9	5	9	5	9	4
Lab9	7	9	7	9	7	4	0	0	9	8	0	0
Σ	90	76	89	77	90	70	75	62	96	84	75	61

Breed differences

In the preference test, CSW puppies were more likely to approach [Cox mixed models, effect of breed: $\chi^2_1 = 18.88$, $p < .001$; CSW → labr: exp(β) = 0.12 (0.06, 0.24), $z = -6.03$, $p < .001$] and interact with the toy [$\chi^2_1 = 11.79$, $p < .001$; CSW → labr: exp(β) = 0.14 (0.05, 0.36), $z = -4.06$, $p < .001$; Fig. 1], and spent more time close to it [$\chi^2_1 = 10.45$, $p = .001$; CSW → labr: exp(β) = 0.38 (0.23, 0.63), $t = -3.71$] than Labrador puppies. They were also more likely to explore the area [$\chi^2_1 = 6.74$, $p = .009$; CSW → labr: exp(β) = 0.29 (0.13, 0.66), $z = -2.97$, $p = .003$] than Labrador puppies.

In the recall test, CSW were more likely to approach the experimenter [$\chi^2_1 = 12.47$, $p < .001$; CSW → labr: exp(β) = 0.37 (0.24, 0.56), $z = -4.65$, $p < .001$] and spent more time close to her than Labrador puppies [$\chi^2_1 = 7.76$, $p = .005$; CSW → labr: exp(β) = 0.40 (0.21, 0.75), $t = -2.86$]. They were also more likely to interact with the experimenter [$\chi^2_1 = 14.32$, $p < .001$; CSW → labr: exp(β) = 0.35 (0.23, 0.53), $z = -4.92$, $p < .001$] and spent more time with her [GLMM of proportion of time spent interacting with Experimenter, effect of breed: $\chi^2_1 = 15.08$, $p < .001$; CSW → labr: exp(β) = 0.35 (0.22, 0.55), $t = -4.47$].

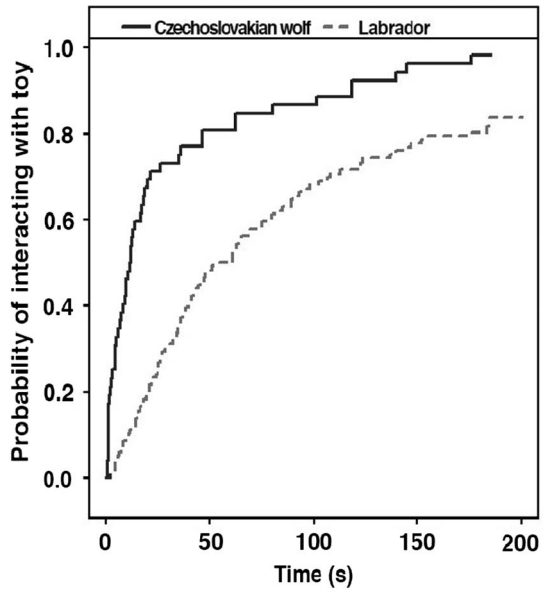

Fig. 1. Breed differences in the probability of interacting with the toy in the preference test

In the hold and pet test and in the noise test, Labradors spent more time close to the experimenter [GLMM and effect of breed in the two tests, respectively: $\chi^2_1 = 7.44$, $p = .006$; CSW → labr: exp(β) = 5.61 (1.80, 17.47), $t = 2.98$; Fig. 2; and $\chi^2_1 = 4.90$, $p = .027$; CSW → labr: exp(β) = 2.52 (1.16, 5.49), $t = 2.33$]. Labrador puppies were also more likely to gaze at the experimenter during the noise test [Cox mixed models, effect of breed: $\chi^2_1 = 15.06$, $p < .001$; CSW → labr: exp(β) = 0.48 (0.35, 0.64), $z = -4.91$, $p < .001$], whereas CSW puppies were more likely to approach the source of the noise [$\chi^2_1 = 13.99$, $p < .001$; CSW → labr: exp(β) = 0.26 (0.15, 0.47), $z = -4.54$, $p < .001$] and spent more time close to it [GLMM, effect of breed: $\chi^2_1 = 17.65$, $p < .001$; CSW → Labrador: exp(β) = 0.30 (0.18, 0.49), $t = -4.76$]. CSW puppies also shifted more often their gaze between the experimenter and the cage [GLMM, effect of breed: $\chi^2_1 = 20.68$, $p < .001$; CSW → labr: exp(β) = 0.42 (0.32, 0.55), $z = -6.46$, $p < .001$].

During the gaze test, CSWs were more resistant to extinction than Labradors [total time to extinction was longer for CSW puppies than for Labrador puppies: GLMM, effect of breed: $\chi^2_1 = 5.18$, $p = .023$; CSW → labr: exp(β) = 1.39 (1.06, 1.81), $t = 2.41$].

Separation from the mother

Puppies in the "separated" group (Table 1) were separated from their mother for 49.22 ± 6.63 days [mean ± standard

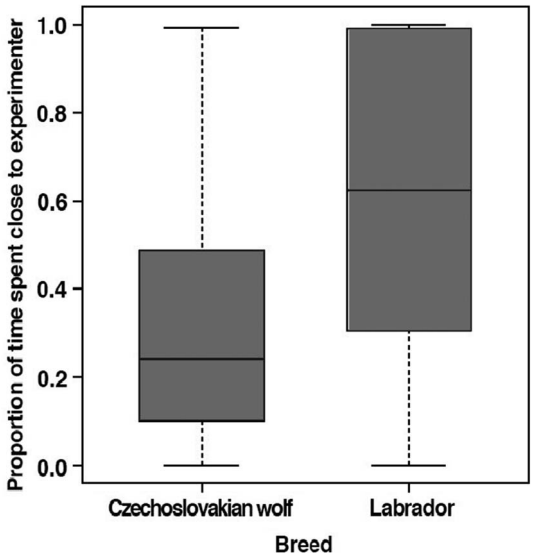

Fig. 2. Breed differences in the proportion of time spent close to the Experimenter in the hold and pet test

deviation (*SD*)]. During the preference test, puppies that were housed separately from the mother spent more time interacting with the toy [GLMM, effect of separation: $\chi^2_1 = 4.88$, $p = .027$; not separated → separated: exp(β) = 2.39 (1.18, 4.86), $t = 2.41$; Fig. 3] than puppies that were still housed with their mother.

We also found significant effect of early separation from the mother in the noise test; puppies separated from the mother spent more time gazing at the experimenter than puppies that were kept with their mother [GLMM, effect of separation: $\chi^2_1 = 6.88$, $p = .009$; not separated → separated: exp(β) = 1.84 (1.23, 2.73), $t = 2.99$].

Housing condition

In the preference test, puppies raised in a kennel spent more time close to the experimenter than puppies raised at home

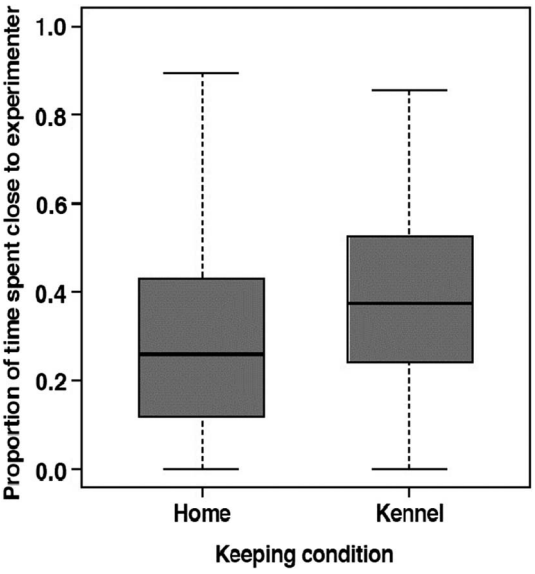

Fig. 3. Effect of housing condition on the proportion of time spent close to the Experimenter in the preference test

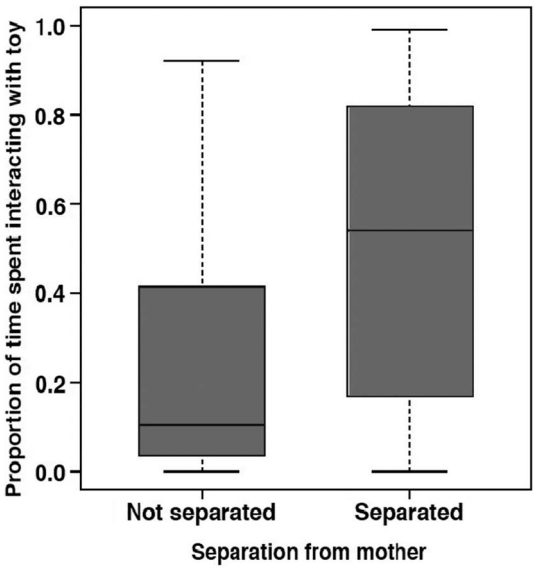

Fig. 4. Effect of early separation from the mother on the proportion of time spent interacting with the toy in the object choice test

[GLMM, effect of housing: $\chi^2_1 = 7.53$, $p = .006$; home → kennel: exp(β) = 1.83 (1.24, 2.70), $t = 3.04$; Fig. 4].

In the gaze test, puppies raised at home were more resistant to extinction of looking at the experimenter than puppies raised in a kennel [i.e., total time of extinction was longer: GLMM, effect of housing: $\chi^2_1 = 8.43$, $p = .004$; home → kennel: exp(β) = 1.68 (1.25, 2.25), $t = 3.47$].

In the recall test, puppies raised at home spent more time interacting with the experimenter than puppies raised in a kennel [GLMM, effect of housing: $\chi^2_1 = 10.53$, $p = .001$; home → kennel: exp(β) = 0.46 (0.31, 0.68), $t = -3.86$].

DISCUSSION

The main aim of this study was to investigate the effect of environmental variables on social behavior of dog puppies toward humans. In support of our expectations, housing condition and early separation from the mother affected the puppies' behavior in various situations. In addition, many test situations also revealed breed differences. Based on our results, CSW puppies were typically more active than Labradors, while the tendency of the two breeds to interact with humans and to look at them was context-dependent.

In the preference test, CSWs explored and interacted more with the toy compared to Labradors. The higher activity level of CSW puppies might be the result of the crossbreed between dogs and wolfs. In line with this interpretation, Moretti et al. (2015) showed that wolves approach a novel object sooner and explore it more than dogs. Consistently, we found that, in the noise test, CSW puppies spent more time close to the novel object than Labradors. Labradors, in contrast, spent more time in the proximity of the experimenter.

The results of the recall test seem to be in contrast with the results of the hold and pet test, because CSWs, in the recall test, interacted more with the experimenter than Labradors, while Labradors spent more time close to the

experimenter after being held. However, the situations presented in these two tests were rather different and the results of the hold and pet test may indicate greater tolerance of Labradors for being restrained, as suggested by their tendency to remain close to the experimenter after being released. This interpretation is also supported by the findings of Svartberg (2006) showing that Labrador puppies obtained higher scores in sociability compared to German Shepherd puppies. Based on the origin of the breed, it is reasonable to assume that CSW puppies behave more similarly to German Shepherds than Labrador puppies during social interactions.

Dogs' tendency to look at humans is a product of domestication (Miklósi et al., 2003), but it is also shaped by several other factors (Barrera et al., 2011; Bentosela et al., 2008; Jakovcevic et al., 2010). In this study, the difference between the gazing behavior of the puppies belonging to the two breeds was context-dependent. While Labrador puppies spent more time looking at the experimenter during the gaze test, CSW puppies more often shifted their gaze between the experimenter and the cage during the noise test. This result may reflect the tendency of CSWs to be more active and explorative in general and spending more time investigating the novel object (the cage). As a consequence, they may have shifted their gaze between the cage and the experimenter more often, looking for information about the object through social referencing (Fugazza et al., 2018).

In the gaze test, CSW puppies kept looking at the experimenter for a longer time after she did not give any more food. Resistance to extinction may be connected with persistence (Jakovcevic et al., 2010) and CSW might be more persistent as, during the selection of this breed, endurance was a desirable trait that might be connected to it. Consistent with our result, it has also been suggested that wolves are more persistent than dogs in problem-solving tasks (Marshall-Pescini et al., 2017; Udell, 2015).

It is known that the quality and the quantity of early maternal care affect puppies' social behavior (Guardini et al., 2016, 2017) and, during the socialization period, puppies' behavior is shaped by learning from their mother and littermates (e.g., Fugazza et al., 2018; Slabbert & Rasa, 1997). Early maternal separation results in high levels of stress in puppies and may cause several behavioral problems such as fearfulness or destructive behavior (Pierantoni et al., 2011; Slabbert & Rasa, 1993). In the noise test, increased gazing toward the experimenter shown by the puppies housed separately from their mother may indicate that the presence of a novel stimulus (the noise) was more stressful for them than for the non-separated puppies. The relatively big *SD* of the age of separation from the mother of the separated subjects in our sample indicates that, in some cases, there was no big difference between puppies in the separated group and in the non-separated group. Accordingly, a strong impact of this condition on puppies' behavior should not be expected. Although early separation from the mother poses ethical concerns, future studies may investigate on how separation at different ages affects the behavior of puppies.

In the preference test, puppies raised in a kennel spent more time with the experimenter, whereas in the recall test, home-raised dogs interacted more with her. The preference test was the first test of the battery, whereas the recall test was the last of the first part. A possible explanation is that kennel-reared puppies were more interested in the human at the beginning due to the relatively higher degree of deprivation from human contact and more monotonous environment, although they lost their interest earlier, perhaps due to the (relative) lack of social experience. Experience with humans may also play a role in resistance to extinction of gazing toward humans. Puppies raised at home were more resistant to extinction in our gaze test. In line with this, it was shown that adult kennel dogs gaze less toward humans than pet dogs during an unsolvable task (D'aniello & Scandurra, 2016). Our results suggest that this ontogenetic difference can emerge at a very early age, further emphasizing the importance of early interactions with humans.

Finally, we note that although our intention was to collect equal sample for each combination of experimental groups, we did not succeed because of the real-life practice of breeding these puppies. For instance, except for one litter, we could not recruit CSW pups that were kept together with their mother. Similarly, almost all CSW pups were kept in houses. Therefore, we acknowledge that our experimental design was unbalanced in terms of separation from the mother and housing conditions and low sample size in certain combinations of experimental groups hindered detecting low effects. At the same time, this also outlines the significance of separation and housing condition in the tests where we reported the effects of these keeping conditions.

CONCLUSIONS

This study provides evidence for early keeping conditions, such as housing and separation from the mother, and breed to influence social behavior of dog puppies toward humans. The puppies' behavior appears to be shaped by rearing conditions but also by genetic (breed) differences in a complex and context-specific way. The tendency to interact with humans differs between CSW and Labrador puppies in a situation-specific manner. CSW puppies also seem generally more active. Whether these differences can be attributed to different developmental patterns and whether they last throughout the dogs' lives remains to be explored.

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