

# Investigation of behavioral developments of Kangal puppies using open field and barrier tests

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**Summary:** This study aimed to investigate behavioral developments and breed specific characteristics of Kangal puppies. To this aim, Kangal puppies (23 male, 17 female) were weekly exposed to the repeated open field test (OFT) and barrier test between 3 to 8 weeks after birth. It was detected that 3 weeks old Kangal puppies were able to display explorative behaviors. True thigmotactic behaviors and unsteady walking were only observed when the puppies were 3-weeks-old. When the puppies were 4 weeks old, time spent for standing and walking increased ( $p<0.05$ ) and running behavior was first displayed. Sum of durations of passive behaviors increased in general ( $p<0.05$ ) and durations of escape behaviors decreased in the 2nd part of the OFT ( $p<0.05$ ) in 5-weeks-old puppies. Five weeks old Kangal puppies also started to show success in the barrier test. Common body postures of the puppies were recorded as self confident and attendant along test weeks. These findings suggest that Kangal puppies achieved fine motor abilities such as steady walking and running at 4 weeks of age. Five weeks of age, on the other hand, seemed to be important for development of emotional memory, habituation and problem solving in Kangal puppies. Considering activity levels and body postures of the puppies in repeated OFT exposures, Kangal puppies might be classified as bold puppies according to “Shyness-Boldness” axis.

Keywords: Barrier test, behavioral development, Kangal Dog, open field test.

## Kangal yavrularının davranış gelişimlerinin açık alan ve bariyer testleri kullanılarak incelenmesi

**Özet:** Bu çalışmada Kangal yavrularının davranış gelişimleri ve ırka özgü kişilik özelliklerinin incelenmesi amaçlandı. Mevcut çalışmada, Kangal yavrularına (23 erkek, 17 dişi), 3-8 haftalık dönemde haftalık tekrarlı açık alan ve bariyer testleri uygulandı. Üç haftalık Kangal yavrularının keşif davranışlarını sergileme yetisine sahip oldukları tespit edildi. Gerçek tigmotaktik davranışlar ve sallantılı yürüme sadece yavrular 3 haftalıkken gözlemlendi. Kangal yavruları 4 haftalıkken, ayakta durma ve yürüme için harcanan süre arttı ( $p<0,05$ ) ve koşma davranışı ilk kez sergilendi. Beş haftalık yavrularda pasif davranışlar için harcanan süre testin genelinde arttı ( $p<0,05$ ) ve kaçma davranışları için harcanan süre OFT'nin 2. bölümünde azaldı ( $p<0,05$ ). Ayrıca 5 haftalık Kangal yavruları, bariyer testinde başarı göstermeye başladı. Test haftaları boyunca yavruların genel olarak sergiledikleri vücut dili, kendine güvenli ve dikkatli olarak kaydedildi. Bu bulgular, Kangal yavrularının dört haftalık yaşta sallantısız yürüme ve koşma gibi ince motor becerilerini kazandığını göstermektedir. Ayrıca 5 haftalık yaşın Kangal yavrularında duygusal hafıza, alışma ve problem çözme açısından önemli olduğu görülmektedir. Tekrarlı OFT uygulamalarında yavruların aktivite seviyeleri ve vücut dilleri göz önünde bulundurulduğunda, Kangal yavruları “Utangaçlık-cesaret” eksenine göre cesur yavrular olarak sınıflandırılabilir.

Anahtar sözcükler: Açık alan testi, bariyer testi, davranış gelişimi, Kangal köpeği.

## Introduction

Most parts of the brain of newborn dogs are unmyelinated at birth (24, 29). Dog puppies undergo a gradual maturation in nervous system after birth, which also underlies their behavioral epigenesis (8, 24). Maturing process in nervous system is an important period for dogs since this period determines future behavioral characteristics of an individual. Although effects of genetic factors on maturation of the nervous system, behavioral developments and individual traits have been proved (16, 25), only a limited number of studies investigated breed specific early neurobehavioral development in dogs (11, 28, 29). Most of the data on early behavioral and social developments in dogs based on results of a long term research conducted by Scott and

Fuller (29). However, authors used only five dog breeds, i.e. cocker spaniels, basenjis, beagles, wire-haired terriers and Shetland sheep dogs in that study. Thus, generalization of the results to other dog breeds is questionable. In a recent study, Schoon and Berntsen (28) described neurological and behavioral developments in Belgian Malinois. However, much comparable data on breed specific physiological, behavioral and social developments in dogs are still needed (24).

Learning is one of the basic adaptation mechanisms in dogs. Learning capacities of dog puppies reach adult levels at 4-5 weeks old in accordance with the myelination process in central nervous system (11, 17, 19). Scott and Fuller (29) reported that 4 week old puppy has problem solving ability since it can learn basic

commands. They further stated that earliest age of problem solving in simple problem solving tests such as barrier test is 6 weeks old in dog puppies after vision capacity fully develops.

Barrier test is a simple problem solving test in which a barrier is used in order to separate the dog from any object the dog is motivated to reach. In order to solve the test, the dog in a novel environment had to navigate to the hidden object (29). OFT is a widely used method to evaluate emotional reactivity, activity level, explorative behavior and temperament of animals in response to a novel environment (21, 33). Marchei et al. (22) demonstrated that responses of a growing animal to the repeated OFT showed development of neurological, behavioural and social patterns. Furthermore, in various studies coping style of an individual was determined by using OFT (4, 22, 26). Coping style can be defined as individual variability such as active versus passive, proactive versus reactive and shy versus bold in behavior and underlying physiology to environmental challenge (18). Various studies showed that human five factor, i.e. Big Five which includes the traits such as Extraversion, Neuroticism, Conscientiousness, Agreeableness and Openness can be applied to dogs. Moreover, broad dimensions described in dogs were largely in agreement with the Shyness-Boldness axis (31, 34). Koolhaas et. al. (18), on the other hand, suggested that stable traits in most animals can be determined by using two independent axes, a qualitative coping style axis and a quantitative stress reactivity axis. Accordingly, four extreme characteristics, i.e. shy, docile, panicky and bold can be described in individuals. Shy individuals is characterized by a high stress reactivity combined with reactive coping whereas bold individuals can be defined by low stress reactivity and proactive coping style. Animals having low stress reactivity with reactive coping are labeled as docile while panicky individuals show proactive coping style combined with high stress reactivity.

To our knowledge, no research to date has been conducted on measuring of behavioral developments in a specific dog breed using OFT. The aim of this study is to investigate (1) behavioral developments of Kangal puppies during the first 8 weeks after the birth; (2) to determine breed specific traits considering “Shyness-Boldness” axis; and (3) to observe development of problem solving ability in those dogs. We believe that this is the first effort to evaluate behavioral developments and characteristics of Kangal dogs.

### Materials and Methods

*Subjects:* Forty (23 male and 17 female) purebred Kangal puppies were used in this study. All puppies were diagnosed as healthy after a clinical examination.

Animals were identified according to their mother's name, birth date and sex as well as coat colour. The approval of ethical committee has been obtained for this study.

*Housing:* The experiment was carried out in the Implementation and Research Center of Kangal District Governorate. All puppies lived outdoors in cage environments with their mothers and had free access to water and food.

*Experimental design:* During the first 3 weeks after birth, days of eyes opening and the first balanced standing of the puppies were recorded. To this aim, the animal keeper filled out a time table for each puppy during his daily visit to the cages. Each puppy was first taken to the OFT at 3 weeks of age after subjected to a startle test in which puppy's reaction to a sudden noise was tested. Puppies were only taken to the OFT, in case of opening of eyes, giving startle response to a sound and being able to stand on four legs. OFT was applied to puppies every week on the same day between 3 to 8 weeks after birth.

Each week, after OFT was completed, all puppies were subjected to the barrier test. As described by Scott and Fuller (29), target object was placed through the window to the outside of the barrier after puppy showed his/her interest in it. In case that puppy reached the target object in 30 sec by moving around the barrier, he/she was recorded as successful. Barrier test was performed on the following week in case that puppy was not successful in the previous week.

*OFT area:* The test was performed outdoor since puppies were living in outdoor cages. The arena was a hexagon box of 1.80 m diameter with compact foamboard walls at a height of 60 cm. The floor was covered with a nylon sheet divided into 30 cm area squares. The OFT floor was cleaned with alcohol at the end of each test. A “stimulus-rich” installation was used in OFT which contained a novel object as described by Marchei et. al. (22). A toy car with remote control sound effect and movement was used as a novel object. It was placed on the floor of the OFT area close to the wall before the test.

*Barrier test:* A compact foamboard with a height of 90 cm and length of 180 cm was used as a barrier. In the middle of the barrier, a triangle window was opened through which the puppy could see the outside of the barrier. Cheese treats or plush toy was used as a target object depending on puppy's motivation.

*Behavioral data:* For the OFT, each puppy was left gently on the center of the OFT area. Focal animal sampling was used as a sampling method (1). Thus, behaviour of each puppy was observed for a total of 300 s. Novel object was active during the last 60 s of OFT.

During the OFT, durations and frequencies of certain behaviors were recorded for later evaluation by using a video camera (Sony Handycam®) placed outside the arena on a 1.50m high tripod. Accordingly, accumulated times spent in each behaviour were measured as duration of activities (Table 1) and a number of behaviours were measured as frequencies (Table 2) as previously described by Marchei et al. (22).

*Statistical analyses:* All data were analysed using SPSS 18.0 Inc. software program. Mann-Whitney U test was used in order to determine differences between genders considering opening of eyes, the first balanced standing and results of the barrier test. Spearman rank correlation coefficient was used in order to measure relationships between data obtained in the first 3 weeks and physiological data as well as to measure relationships

Table 1. Description of behaviors observed during the OFT measured as durations.

Tablo 1. Açık alan testi sırasında gözlemlenen davranışların süre olarak ifadesi.

Behavior	Description
Walking unsteadily	Unsteady walking using all four legs while head is moving side by side
Walking	Steady walking using all four legs
Running	Running or jumping across the OFT area
Sitting	Sitting back on haunches
Lying	Lying on the floor while its haunch and belly touching the floor
Sleeping	Puppy is in a stationary position while lying on the floor with closed eyes
Standing	Standing up on all four legs
Walking and exploring walls	Walking while sniffing the walls of the OFT area
Walking and exploring floor	Walking while sniffing the floor of the OFT area
Resting and exploring walls	Puppy is in a stationary position (sitting, standing or crouched) and sniffing the walls
Resting and exploring floor	Puppy is in a stationary position (sitting, standing or crouched) and sniffing the floor
Passive exploration of NO	Puppy is in contact with any part of the novel object, sniffing or touching it with nose, tongue or front paws while sitting/standing/lying on the floor
Passive avoidance from NO	Avoiding from NO by turning head away in a sitting/standing/lying position
Active exploration of NO	Puppy is voluntarily approaching NO and sniffing or touching it with nose, tongue or front paws
Active avoidance from NO	Avoiding from NO by walking/running away
Polypne	Rapid breathing, panting
Normal positions of ears	Ears held in normal/ resting position
Lower positions of ears	The pinnae are flat on the head/ backwards for more than half/ turned sideways
Ears directed to stimuli	Each pinnae are directed to source of the stimuli
Lower position of head	Head is held in a low position
Upper position of head	Head is lifted up to form a wide angle with the neck
Lower position of tail	Tail is held lower than neutral position
Tail between legs	Tail is held between the legs
Upper position of tail	Tail is held higher than neutral position
Tail wagging	Wagging movements of the tail
Trembling of head	
Trembling of body	
Circling	Turning 180-360 degree at one point
Circling around test area	Turning 180-360 degree around the test area
Play bow	Chest is lowered to the ground while rear end remains up
Freeze	Stop moving with stiffed body posture
Escape intents	Scratching or climbing the wall/rearing with front paws on the wall
Others	Performing a different behaviour from the described ones
<b>Vocalistions</b>	
Whining	Long, high sound
Yelping	Sudden, short, high sound
Barking	Loud, rough noise
Howling	
Squealing	High-pitch sound

between data obtained in the first 3 weeks and results of the barrier test. In order to evaluate behavioural trends along time, week was considered as factor and “mother”, “litter size” and “birth time” were considered as covariates in a two way repeated measures ANOVA. Behaviours expressed as counts were analysed with poisson regression model. Results of statistical analysis were considered significant at  $p < 0.05$ .

## Results

*Measurements during the first 3 weeks after the birth:* Dog puppies were only taken to the OFT in case that their eyes and ear channels were open and further that they were able to stand on their fours. Accordingly, the mean age of eyes opening was  $13.62 \pm 0.12$  days from birth in Kangal puppies. The mean age of standing was

Table 2. Description of behaviors observed during the OFT measured as frequency.  
Tablo 2. Açık alan testi sırasında gözlemlenen davranışların frekans olarak ifadesi.

Behavior	Tanım
Line crossing	Crossing one of the lines drawn on the OFT floor with the front paws
Snout licking	Part of the tongue is moved along the upper lip
Yawning	Mouth is opened to apparent fullest extend
Lowering ears	Pinnae are moved to backwards for more than half
Lowering head	Head is changed to a low position
Lowering tail	Tail is changed to a lower position
Tuckling tail between legs	Tail is curled between legs
Lifting tail	Tail is elevated
Lifting front paw	One of the front paws is lifted up
Trembling of head	
Trembling of body	
Circling	Turning 180-360 degree at one point
Circling around test area	Turning 180-360 degree around the test area

Table 3. Mean durations of Kangal puppies for escape intents, lying, standing and passive behaviors.  
Tablo 3. Kangal yavrularının kaçma davranışları, yatma ve pasif davranışlar için harcadıkları ortalama süreler.

Dogs (n=40)	Weeks	Escape intents (sec) $\bar{x} \pm S\bar{x}$	Lying (sec) $\bar{x} \pm S\bar{x}$	Standing (sec) $\bar{x} \pm S\bar{x}$	Passive behaviors (sec) $\bar{x} \pm S\bar{x}$
	1	0.97±0.35 <sup>a</sup>	50.41±7.00 <sup>a</sup>	16.99±3.34 <sup>a</sup>	93.99±5.73 <sup>a</sup>
	2	7.18±1.92 <sup>b</sup>	17.23±6.14 <sup>b</sup>	50.93±5.86 <sup>b</sup>	99.49±7.92 <sup>a</sup>
	3	22.42±4.66 <sup>b</sup>	23.88±9.08 <sup>ab</sup>	67.79±10.64 <sup>b</sup>	138.37±9.52 <sup>b</sup>
	4	81.301±8.71 <sup>cd</sup>	10.18±6.37 <sup>b</sup>	50.31±7.37 <sup>b</sup>	110.64±11.75 <sup>ab</sup>
	5	82.627±8.78 <sup>c</sup>	18.79±8.46 <sup>ab</sup>	40.07±7.68 <sup>ab</sup>	106.93±8.86 <sup>ab</sup>
	6	96.306±9.22 <sup>d</sup>	19.82±7.46 <sup>ab</sup>	35.77±7.12 <sup>ab</sup>	102.99±8.48 <sup>ab</sup>
<i>P values</i>					
<u>Main effect</u>					
Time:		0.04	0.02	0.01	0.01

Table 4. Mean durations of tail positions of Kangal puppies in OFT.  
Tablo 4. Açık alan testindeki Kangal yavrularının sergilediği kuyruk pozisyonlarının ortalama sergilenme süreleri.

Dogs (n=40)	Weeks	Tail normal $\bar{x} \pm S\bar{x}$	Tail low $\bar{x} \pm S\bar{x}$	Tail between legs $\bar{x} \pm S\bar{x}$
	1	1.06±0.32 <sup>a</sup>	239.13±0.31 <sup>a</sup>	0.00±0.00
	2	140.73±13.42 <sup>b</sup>	47.33±11.50 <sup>b</sup>	5.59±4.10
	3	148.12±10.26 <sup>b</sup>	17.11±7.96 <sup>bc</sup>	1.72±2.26
	4	154.63±11.22 <sup>bc</sup>	27.41±8.84 <sup>bc</sup>	1.08±1.0
	5	161.57±11.55 <sup>bc</sup>	22.78±7.72 <sup>bc</sup>	4.44±3.90
	6	184.37±11.81 <sup>c</sup>	20.10±7.66 <sup>c</sup>	0.00±0.00
<i>P values</i>				
<u>Main effect</u>				
Time:		0.02	0.01	0.76

19.45±0.25 days from birth. All puppies gave startle response to auditory stimuli when they were 3 weeks old.

*Measurements during the OFT:* Behaviors expressed before and after the activation of novel object were recorded separately. Accordingly, OFT was divided into 2 periods, i.e. the 1st period of OFT and the 2nd period of OFT. The behaviors observed in the 1st period of the OFT were evaluated as reactions of puppies to OFT situation, whereas behaviors observed in the 2nd period of the OFT were assessed as reactions to novel object.

*Activity scores:* The 1st period of OFT: The longest durations of thigmotactic behaviors (circling around test area) were detected in the 1st week (24.75±7.62 sec). Unsteady walking was only observed in the 1st test week (72.88±8.18 sec). Durations of unsteady walking in female puppies were significantly longer than in male puppies ( $p<0.05$ ).

Duration of standing significantly increased in the 2nd test week ( $p<0.05$ ) (Table 3). Running and play-bow were first displayed by the puppies in the 2nd week. Durations of escape intents significantly increased across test weeks ( $p<0.05$ ) (Table 3). Frequency of line crossing significantly decreased across test weeks ( $p<0.05$ ). Frequency of circling behavior showed significant differences between males (2.77±0.05) and females (1.68±0.14) ( $p<0.05$ ).

Sum of durations of standing, sitting, lying and freezing behaviors were recorded as durations of passive behaviors. Duration of lying significantly decreased at the 2nd test week ( $p<0.05$ ). At the 3rd test week, total durations of passive behaviors significantly increased ( $p<0.05$ ) (Table 3).

*The 2nd period of OFT:* Sum of durations of activity levels increased between 2nd to 4th test weeks while it started to decrease as of the 4th week of OFT. Sum of durations of escape intents significantly decreased in the 4th week ( $p<0.05$ ). Sum of durations of passive behaviors significantly increased in the 6th test week ( $p<0.05$ ).

*Exploratory behaviors:* Sum of durations of walking and exploring floors, resting and exploring floors, walking and exploring walls, resting and exploring walls, passive exploration of novel object and active exploration of novel object were recorded as exploratory behaviors. Exploratory behaviors were displayed by puppies as of the 1st test week. Significant decrease in duration of exploratory behaviors was observed in the 1st period of the OFT in the 3rd week as well as in the 2nd period of OFT in the 4th week ( $p<0.05$ ).

*Vocalisations:* It was detected that female puppies (80.32±8.46 sec) vocalize more often than male puppies (54.13±7.20 sec). Crying was the predominant vocalization across the first 5 test weeks. Barking and

yelping were first detected in the 2nd week. Yelping was the predominant vocalisation at the last week.

*Other behaviors:* Body trembling was first detected in the 1st week and head trembling was first detected in the 3rd week. Body trembling and yawning were displayed at the highest frequency in the 3rd week. Mouth licking, polipne and paw lifting reached their highest frequencies in the 5th week. Eliminative behaviors were only detected in the 1st period of the OFT across test weeks. Time spent for elimination significantly increased in the 2nd test week.

*Body language and temperament:* Body expressions such as lowering ears, lowering tail, directing ears to the stimuli were first detected in the 2nd week. Sum of durations of normal tail position significantly increased in the 2nd test week ( $p<0.05$ ) (Table 4). Self confident body posture, i.e. normal head, ear and tail position was dominant in Kangal puppies in repeated OFT exposures.

*Barrier test:* In total, 36 dogs (90 %) showed success in the barrier test. Puppies started to show success in the 3rd test week ( $n=9$ ; 25%). Most of the puppies ( $n=11$ ; 31 %) showed success in the 5th test week.

*Correlations:* Positive correlations were found between opening of eyes and standing ( $\rho=0.68$ ;  $p<0.001$ ). Barrier test success was found to have a positive correlation with opening of eyes ( $\rho=0.33$ ;  $p<0.05$ ).

## Discussion and Conclusion

*Measurements during the first 3 weeks after the birth:* In the current literature, the first balanced standing for dog puppies is reported as 12-14 days after the birth (17, 19). In the present study, however, mean age of the first balanced standing was detected as 19 days in Kangal puppies. Different studies reported that large breeds tend to develop motor abilities later than small breeds in different species (17, 22, 29). Accordingly, it can be suggested that Kangal puppies which belong to giant breed puppies develop motor abilities more slowly than normal breeds. However, there is still lack of comparable data for development of early motor abilities in dog breeds of different sizes. Thus, future studies should be conducted in order to investigate development of motor abilities in different dog breeds. In this study, it was also shown that puppies stand earlier in case that their eyes open earlier. This finding supports the hypothesis that vision is important on improving motor abilities in dog puppies (17).

*Measurements during the OFT:* Different authors stated that thigmotactic behaviors may reflect high emotional reactivity in animals and, further that in case of high emotional reactivity, animals tend to spend more time close to the OFT walls (6, 20). In this study, longest

durations of circling around test area and wall-seeking behaviors were detected in the 1st test week. Explorative behaviors were displayed by Kangal puppies as of the 1st test week. Thus, it can be suggested that puppies were able to react to the OFT at 3 weeks old age which supports the hypothesis that myelination in thalamocortical way in dog puppies completed at 3-4 weeks old age (12). Since hippocampus and dorsal striatum are important for spatial organisation, lesions on those areas increase thigmotactic behaviors in animals (9). Therefore, limbic system development seems to link to lower thigmotactic behavior in repeated OFT exposures (22). In our study, it was also observed that circling around test area in the following weeks is more likely to be looking for a way of escape rather than true thigmotactic behavior which is accompanied with anxiety and fear.

In the present study, “shaky walking” (walking unsteadily) was only detected in the 1st test week. In the 2nd test week, time spent for walking significantly increased and running behavior appeared. Moreover, during the 2nd test week, time spent for lying significantly decreased whereas time spent for standing significantly increased. Marchei et al. (22) stated that the rise in activity and standing position in kittens across weeks might be linked to progressive maturation of corticospinal pathways. Accordingly, one may suggest that progressive myelination of corticospinal pathway results in rise in time spent for steady walking, standing and running in Kangal puppies in the 2nd OFT week. Significant sex differences were detected considering shaky walking. Mean time of shaky walking was significantly higher in females than those in males. Several studies showed that development of brain differs in boys and girls (2, 5). Moreover, a cross-sectional diffusion tensor imaging study which investigated development of white matter microstructure in children showed greater mean diffusivity in the corticospinal tract of boys in comparison to of girls (27). Thus, our finding may indicate that maturation of corticospinal tract differs in male and female puppies.

Sum of durations of passive behaviors significantly increased in the 3rd test week. One possible explanation could be habituation and emotional conditioning as a result of progressive development of hypothalamus (15, 22). Another explanation might be emergence of fear behaviors as a result of development of amygdala (32). However, since body languages of dog puppies were mostly evaluated as self-confident in the 3rd test week, first hypothesis, i.e. habituation is the most likely.

During the first period of the OFT, activity level was measured above 50 % as of 4th week of the OFT. However, significant decrease in frequency of line crossing has been detected across weeks. Although these

results seem to contradict each other, one possible explanation is that increase in escape attempts, i.e. scratching or climbing the wall/rearing with front paws on the wall is the main factor of increase of activity level whereas it decreases the number of line crossing. Interestingly, it was observed that in the 2nd period of OFT, escape intents decrease across weeks as of the 3rd weeks of repeated OFT exposures. This result may be explained by that puppies were able to associate novel object with the end of the test as a result of development of hippocampus and medial lobe which associate with associative learning (23, 30). Thus, they learned to lower their activity and waited for the end of the test with the activity of novel object.

In this study, significant differences were found in durations of vocalisations and circling behaviors between male and female puppies. In general, female puppies displayed circling behavior and vocalisations more often than male puppies. Circling behavior in OFT situations can be classified as displacement behavior. Displacement behaviors are behaviors expressed by animals under acute stress in case of that they are not able to escape from or remove the source of stress (7, 13). Thus, one may suggest that female Kangal puppies are more sensitive to stress in comparison to male Kangal puppies as in humans (3). Another explanation might be that male and female Kangal puppies chose different coping strategies in case of stress.

It was observed that types of vocalisation have been changed across test weeks. In parallel with findings of Fredericson (14), yelping was the predominant stress vocalisation in 8 week old Kangal puppies in OFT.

In this study, it was detected that passive stress reactions such as head and body trembling as well as yawning were predominant reactions in the 3rd test week whereas active stress related behaviors such as escape intents and vocalisations were predominant reactions in the 5th week in Kangal puppies. Different stress reactions in different weeks in Kangal puppies may be linked to progressive development of central nervous system and behavior. Elimination peak was observed in the 2nd week of age. Since it was the time in which Kangal puppies started to display fear related body postures, one may assume that stress related body postures and behaviors started to appear as a result of progressive development of amygdala in 4 weeks old Kangal puppies (10).

*Body posture and breed specific characteristics:* In the present study, escape attempts of the puppies significantly increased along test weeks. Moreover, percentage of activity time over the first period of OFT was above 50%. Body postures of the puppies were detected as self-confident and attendant, in general. Considering above mentioned results, Kangal puppies

might be classified as bold and active puppies according to “Shyness-Boldness” axis (18).

Since Kangal puppies started to display fear related body posture in the 2nd test week, one may suggest that it is possible to evaluate emotional body expression in 4 weeks old Kangal puppies.

**Barrier test:** Although Scott and Fuller (29) stated that 6 weeks old age was the earliest age of problem solving behavior in dog puppies, 5 weeks old Kangal puppies started to show success in the barrier test. Since in this week escape behavior significantly decreased in the 2nd part of OFT, this outcome also might show that progressive developments of brain parts associated with associative learning and problem solving in 5 weeks old Kangal puppies.

Findings of this study showed that 4 weeks of age is important for development of fine motor abilities such as steady walking and running in Kangal puppies. Five weeks of age, on the other hand, was determined as significant period for development of emotional memory (amygdala), habituation (hippocampus), learning and problem solving (amygdala, medial lob and prefrontal cortex). In general, characteristics of Kangal puppies can be classified as “bold” according to “shyness-boldness axis”.

As a result, this study demonstrated that although development of motor and physiological abilities is seemly slow during the first 3 weeks after birth in Kangal puppies, behavioral development is considerably faster in the following weeks.

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