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Learning, memory and exploratory similarities in genetically identical cloned dogs

Chi Won Shin[1,†], Geon A Kim[2,†], Won Jun Park[1,†], Kwan Yong Park[3], Jeong Min Jeon[4], Hyun Ju Oh[2], Min Jung Kim[2], Byeong Chun Lee[2,*]

Departments of [1]Veterinary Medicine, and [2]Theriogenology & Biotechnology, College of Veterinary Medicine, and [4]Department of Statistics, College of Natural Science, Seoul National University, Seoul 08826, Korea

[3]Department of Physics and Astronomy, Dana and David Dornsife College of Letters, Arts and Science, University of Southern California, Los Angeles, CA90089, USA

Somatic cell nuclear transfer allows generation of genetically identical animals using donor cells derived from animals with particular traits. To date, few studies have investigated whether or not these cloned dogs will show identical behavior patterns. To address this question, learning, memory and exploratory patterns were examined using six cloned dogs with identical nuclear genomes. The variance of total incorrect choice number in the Y-maze test among cloned dogs was significantly lower than that of the control dogs. There was also a significant decrease in variance in the level of exploratory activity in the open fields test compared to age-matched control dogs. These results indicate that cloned dogs show similar cognitive and exploratory patterns, suggesting that these behavioral phenotypes are related to the genotypes of the individuals.

Keywords: cloned dogs, exploratory, learning and memory, similarity, somatic cell nuclear transfer

Introduction

It is well known that cloned animals with identical genomes can be produced by somatic cell nuclear transfer (SCNT). SCNT can be performed to preserve endangered species [23], produce transgenic models [12,17] and propagate dogs with elite abilities [22]. For propagation of dogs with elite abilities, seven cloned drug-sniffing dogs were produced using donor cells from a dog possessing a distinct ability to concentrate and identify specific smells among countless other scents [22]. These cloned dogs displayed similar behavioral patterns, achieved a 100% rate in the selection test for becoming a drug detection dog, and obtained higher scores for the selection test than other naturally bred dogs [7]. However, there is little information available regarding behavioral similarities such as learning, memory and exploratory patterns in cloned dogs.

Investigations using identical twins and families were performed in humans to examine the relationships between genes and cognitive abilities [6,21,34]. These studies demonstrated a strong linkage between genetic influence and human cognitive ability [2,3,32]. The intelligence quotient scores of identical twins raised apart were highly similar (nearly the same as those of identical twins raised together), while those of fraternal twins were less similar [35]. Several studies have investigated the heritability of traits in dogs [4,26]. When the heritability of various behaviors of German shepherd puppies bred to be working dogs was investigated, a strong linkage between genetic factors and activity was shown, with the highest heritability score being 0.53 [36]. In addition, there was a high genetic heritability score of 0.90 for aggression expressed towards strangers [33]. However, other studies have reported conflicting results, showing low heritability of activity and cognitive abilities including memory and learning in dogs [18,25]. Therefore, there is currently no direct evidence of linkage relationships between exploratory patterns, cognitive abilities and genotype in dogs.

Cloned dogs with identical genomes have already been produced by SCNT [11,12]. Since they are derived from the same cell donor, we hypothesized that the cloned dogs would show similar behavioral traits such as learning, memory and

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*Corresponding author: Tel: +82-2-880-1269; Fax: +82-2-873-1269; E-mail: bclee@snu.ac.kr

[†]The first three authors contributed equally to this work.

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exploration. To investigate this hypothesis, two behavioral phenotypes were explored using untrained adult cloned dogs whose genome was derived from the same somatic cell donor.

Materials and Methods

In this study, two female beagles (Cl1, Cl2) 6 years of age were generated by SCNT as previously described [11]. Four more cloned dogs (Cl3, Cl4, Cl5 and Cl6) 5 years of age were also produced by SCNT [12]. The results revealed no clinical symptoms of disease during continuous monitoring throughout the experimental period. Since all six dogs were derived from the same fetal fibroblast donor cell line, they have identical nuclear genotypes. Four healthy, age matched female beagles produced by natural breeding were used as controls. All animals used in this study were reared in the same environments and maintained in accordance with recommendations in The Guide for the Care and Use of Laboratory Animals published by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (approval No. SNU-130619-2). All dogs were fed a constant amount of commercial adult dry food (Natural Balance; Natural Balance Pet Foods, USA) and water daily. All dogs were housed individually in single cages.

Several tasks are commonly used to evaluate cognitive ability of dogs resulting from genetic and environmental factors [8,10,19,20,31]. Among them, a reversal learning task using the Y-maze test was selected for this study to evaluate learning and memory ability [27], with some modifications. The testing apparatus was a plastic T-maze (4 foot wide × 8 foot length × 3 foot height) fence. All dogs could receive the food rewards from only one side of the maze; therefore, they were trained to search only one side as the obligatory preferred site. The time to reach

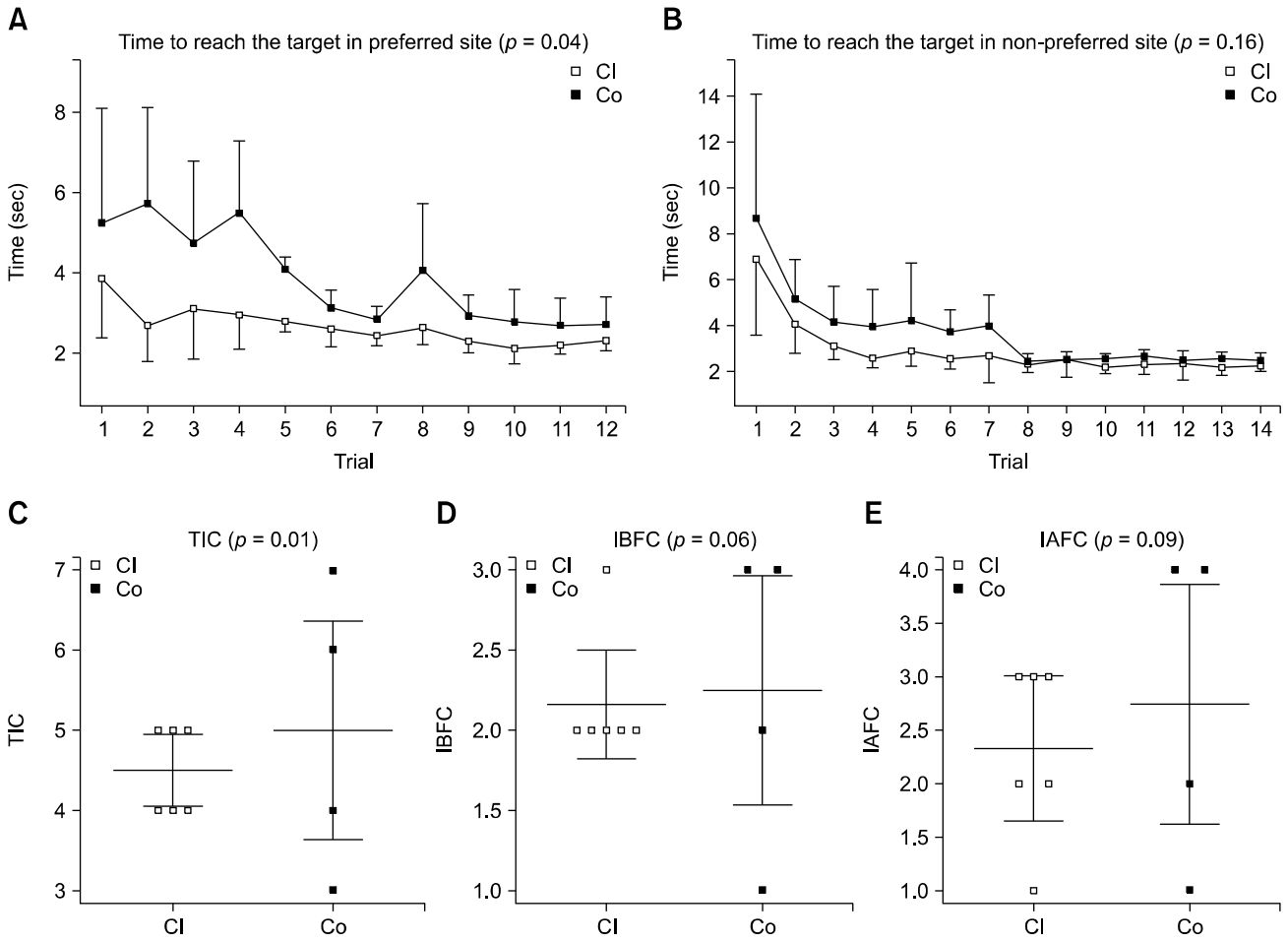

Fig. 1. Cognitive performance among cloned dogs (Cl) and control dogs (Co) based on the Y-maze testing apparatus. (A) Times to reach the obligatory preferred arm of the Y-maze. The variation between Cl and Co was compared using a permutation test. (B) Times to reach the non-preferred site of the Y-maze during reversal learning. The variation between Cl and Co was compared using the permutation test. (C) Total incorrect (TIC) number for Cl and Co. (D) Total incorrect number before the first correct (IBFC) choice during reversal learning. (E) Total incorrect choices after the first correct choice (IAFC) among each dog. $p < 0.05$ indicates statistical significance.

to the obligatory preferred site was recorded and the values were compared between control and cloned dogs.

Following the obligatory preference enforcement periods, the dogs were immediately subjected to a reversal learning task of the Y-maze test. For the reversal learning, the food reward was placed in the obligatory non-preferred site. Once the choice was determined, whether the result was correct or incorrect was recorded. Moreover, the times to reach the non-preferred site were also measured. The total number of incorrect choices (TIC) was calculated for each dog during the reversal learning periods. In addition, the total number of incorrect choices made before (IBFC) and after the first correct choice (IAFC) was evaluated to determine the cognitive performance of dogs. After consuming the reward, the dog was picked up and placed outside in preparation for the next dog. After each dog was removed from the testing area, the floor of the maze was mopped with alcohol to erase any scents left behind by the previous dog.

The open field test was performed to observe any similarities in motor function. Each dog was placed in the testing room (3 × 3 m) for three minutes. Using the Harvard Panlab software, the testing area was divided into three sections (border, periphery and center) and the following three categories of movement pattern were subsequently evaluated: time into zone, distance in zone and mean speed in zone. Each category contains the following four subcategories: border, center and periphery. This experiment was performed three times per day at 3-day intervals.

We used a permutation test for statistical analysis. Specifically, we computed the ratio of the standard deviation of cloned dogs to that of control dogs. Similarly, we calculated the ratios of the standard deviations of each group of six dogs to that of groups with four dogs in all combinations of the group divisions of 10 dogs. The p value was then obtained as a low percentile of the baseline ratio among ratios. Because cloned dogs have much more similarity than control dogs, this method results in the p value becoming small. Therefore, a p value less than 0.05 was taken to indicate that the variance of the cloned dogs is significantly lower than that of control dogs. Analyses were conducted using the R statistical software (ver. 3.2.2; R Foundation).

Results

Learning and memory similarities in cloned dogs

The variation of time to reach the obligatory preferred site in cloned dogs was significantly lower than that in control dogs ($p = 0.04$, panel A in Fig. 1). Although cloned dogs showed no significant similarities when the time to reach the non-obligatory site was analyzed ($p = 0.16$, panel B in Fig. 1), cloned dogs showed a significantly lower variance of TIC ($p = 0.01$) than control dogs (panel C in Fig.1). However, cloned dogs did not show a significantly lower variance in the IBFC and IAFC ($p = 0.06$, 0.09; panels D and E in Fig. 1).

Exploratory similarities in cloned dogs

The permutation test (Table 1) revealed that the cloned dogs showed significantly less variation in the following categories of open field test: time in the center and periphery and mean speed in the center ($p < 0.05$). However, no significant variations were found in the remaining categories of movement patterns: time in the border, distance and mean speed in the border and periphery region.

Discussion

In the present study, we investigated whether dogs cloned from one cell donor behave similarly as age-matched control dogs. As expected, cloned dogs showed similar learning and memory behaviors, as well as exploratory activity, while the control dogs showed more variability amongst themselves than the cloned dogs in their learning and memory behaviors, as well as their exploratory activity. These results indicate that cloned dogs have similar learning and memory behaviors and exploratory activity, which supports the hypothesis that these six cloned dogs with identical genetics behave more similarly to each other than age-matched control dogs. These findings are concordant with those of studies of cloned mice that also showed similar learning, memory and exploratory activity compared to controls [29,30]. These results also demonstrate a strong linkage between learning, memory, exploratory behaviors and genetics because control dogs and cloned dogs were reared under the same conditions.

Exploratory behavior is also considered a high level aspect of sensory processing involved in investigating novel stimuli rather than an instinctive behavior [14]. Exploratory behavior

Table 1. *P* values obtained through the permutation test for all parameters measured in the open field test

Parameters	Region in open field	*p* value
Time (sec)	Border	> 0.05
	Center	< 0.01
	Periphery	< 0.01
Distance (m)	Border	> 0.05
	Center	> 0.05
	Periphery	> 0.05
Mean speed (m/sec)	Border	> 0.05
	Center	< 0.05
	Periphery	> 0.05

These values represent comparisons made between cloned dogs and control dogs. $p < 0.05$ indicates statistical significance.

partially depends on motor and spatial capabilities and on the motivation to explore [5]. In this study, an open field test was used to assess exploration in dogs [1,24,28]. Each test was 3 min in duration, whereas previous open field tests in dogs used sessions lasting 10 min [1,28]. Recently, one paper reported that 3 min short durations in open fields yield valid behavioral measures and would reduce biased results related to individual variations in temporal activity patterns [24].

In the present study, the distance, speed displayed in certain areas of the testing room and time spent in these areas were studied as measures of locomotor or exploratory behavior. In agreement with the measurement approach of previous studies [9,28], these factors were used as markers of locomotion and exploration in the open field. Furthermore, spontaneous activity including locomotion, exploratory behavior and social responsiveness is related to the aging in dogs [9,24]. Because they are at similar age, none of the parameters measured in dogs showed significant differences. However, the mean speed in the center as well as the time in the center and periphery region showed less variation among cloned dogs than control dogs. It can be assumed that the lower variation of mean speed and time in certain regions in cloned dogs was due to the genetic similarities among them; therefore, genetic factors, which may control the neural circuitry of the brain, also appear to influence exploratory patterns in cloned dogs.

It is already known that cloned dogs derived from one genetic source have completely identical genetic information [11,12]. However, the mitochondrial DNA of cloned dogs originated from the source of recipient cytoplasts [13,16,23]. To determine the effects of the mitochondrial DNA (mtDNA) genotype on behavior, it is essential to produce cloned dogs with the same mtDNA genotype. In the present study, because only two cloned dogs shared the same mtDNA [15], an exact relationship between learning, memory behavior and mtDNA genotype could not be demonstrated. Although only six cloned dogs were used in the present study, this number is relatively large considering that they have completely identical nuclear genomes. However, since this study was performed with cloned dogs derived from a single genetic source, further and more conclusive studies using various breeds and greater numbers of cloned dogs are necessary.

SCNT enables propagation of dogs with certain cognitive performance. The results presented herein will help address national security concerns by expanding and accelerating the available pool of trained detection dogs. Furthermore, this study may lead to interest among dog owners who wish to clone their pets through SCNT.

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Conflict of Interest

There is no conflict of interest.

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