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Differences in serotonin serum concentration between aggressive English cocker spaniels and aggressive dogs of other breeds

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KEYWORDS: aggression; behavior; dog; English cocker spaniel; serotonin

Abstract Aggression is one of the most common behavioral problems in dogs and may have important negative effects on public health, human–animal bond, and animal welfare. There is ample evidence showing a negative correlation between serum serotonin concentration and aggressive behavior in a variety of species, including the domestic dogs. This negative correlation is particularly pronounced in dogs that show impulsive aggression. Data obtained in some previous studies suggest that the English cocker spaniel (ECS) is more likely to show impulsive aggression than other breeds. Therefore, the aim of this study was to analyze possible differences in serum serotonin levels between aggressive ECS and aggressive dogs of other breeds. Nineteen ECSs presented for aggression at the Animal Behavior Service (School of Veterinary Science, Barcelona, Spain) were evaluated and compared with 20 aggressive dogs of other breeds attended at the same center. Serum serotonin levels were measured using an enzyme-linked immunosorbent assay method. Statistical analysis was done using the SPSS 15.0 for Windows. Aggressive ECSs had significantly \( (P < 0.001) \) lower levels of serum serotonin than aggressive dogs of other breeds (318.6 ± 67.1 and 852.77 ± 100.58 ng/mL, respectively). Variances were not significantly different between ECSs and other breeds (standard deviation 5449.84 ng/mL vs. 292.47 ng/mL, \( P > 0.05 \)). This finding may explain why ECSs are more likely to show impulsive aggression than other breeds, and suggests that the ECS could be a good model to study the neurophysiologic mechanisms underlying impulsive aggression.

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Introduction

Dog aggressive behavior has important negative effects on the welfare of dogs and on public health (Goldstein, 1992; Sacks et al., 1996; Palacio et al., 2005; De Keuster et al., 2006). For instance, in the United States, the treatment of the physical and psychological effects of dog bites costs between 30 and 100 million US dollars each year (Berzon et al., 1972; Matter and Arbeitsgemeinschaft, 1998; Weiss et al., 1998; Borud and Friedman, 2000). In fact, bite accidents are one of the main threats that domestic dogs pose to human health (Overall, 1997; De Keuster et al., 2006). In contrast, behavior problems are one of the main reasons for euthanasia and relinquishment of healthy dogs to animal shelters (Reisner et al., 1994; Edney, 1998; Salman et al., 1998). In addition, because
stress causes an activation of the adrenocortical stress response, most aggression problems have a negative effect on the welfare of dogs (Moberg, 2000; Kruk et al., 2004; Stafford, 2007).

Aggressive behavior can be divided into affective (offensive and defensive aggression) and nonaffective aggression (predatory aggression). Affective aggression is associated with a marked autonomic activation and involves neurotransmitters such as serotonin, dopamine, noradrenaline, acetylcholine, and γ-aminobutyric acid (Dodman and Shuster, 1994), whereas nonaffective aggression is controlled by acetylcholine mainly and does not involve autonomic activation (Beaver, 2006). In addition, the posture of the dog, the brain areas that control the aggressive response, and the function of aggression are different between the 2 types of aggression (Moyer, 1968).

There is strong evidence that breeds differ in their behavior (Takeuchi and Houpt, 2004; Scott and Fuller, 1965; Hart, 1995; Svartberg, 2006; Duffy et al., 2008). For example, Scott and Fuller (1965) found significant differences in all behavior traits under study between 5 breeds that were raised in the same environment. Lately, Hart and Hart compared the behavior of 56 breeds based on expert opinion and concluded that breeds differ in all behavior traits, although the breed effect was more pronounced for some traits than for others (Hart and Hart, 1985). With respect to aggressive behavior, there are many studies that suggest that some breeds may be more prone to show aggression problems than others (Overall, 1997; Sacks et al., 2000; Guy et al., 2001; Fatjó et al., 2007). For instance, English cocker spaniels (ECSs) are presented to the Animal Behavior Service of the School of Veterinary Science in Barcelona (Spain) owing to aggressive behavior more frequently than any other breed and more frequently than expected according to their relative abundance in the population (Fatjó et al., 2007). Other authors have obtained similar results (Lund et al., 1996; Overall, 1997; Bradshaw and Goodwin, 1998; Svartberg, 2006; Takeuchi and Mori, 2006). However, a large individual variation exists within breeds, and preventive programs should be based on individuals rather than breeds themselves (Hart and Hart, 1985).

There are several studies showing an association between low levels of serotonin and aggressiveness in different species (Brown et al., 1979; Da Prada et al., 1988; Mehlman et al., 1995; Virkkunen et al., 1995; Reisner et al., 1996; Berman and Coccaro, 1998 (revision); Peremans et al., 2003; Miczek and Fish, 2006; Cakiroglu et al., 2007; Rosado et al., 2010). For instance, in rodents, the reduction of brain serotonin activity increases some forms of aggression (Vergnes et al., 1986), whereas pharmacological manipulations that increase serotonin activity reduce aggression (Nelson and Chiavegatto, 2001). Nevertheless, other authors observed a positive correlation between cerebrospinal fluid levels of 5-hydroxyindoleacetic acid (5-HIAA, the main serotonin metabolite) and aggression (Van der Vegte et al., 2003). In nonhuman primates, some studies indicate a negative correlation between cerebrospinal fluid 5-HIAA levels and aggression (Higley et al., 1992; Westergaard et al., 2003). A similar link between serotonin levels and aggression has been reported in dogs. For example, in one study, dogs with dominance-type aggression had lower levels of the main metabolite of serotonin in the central nervous system than nonaggressive dogs (Reisner et al., 1996). Similarly, a group of dogs with owner-directed aggression had lower levels of serotonin than a control group without aggression problems (Cakiroglu et al., 2007; Rosado et al., 2010). In addition, treatment with drugs that increase the serotonin levels reduces aggressive behavior (Coccaro et al., 1990; Fava et al., 1996; Ferris et al., 1999; Leon et al., 2006), and diets with low levels of tryptophan, which is the precursor of serotonin, cause an increase in aggressive behavior (Young, 1991).

The aim of this study was to ascertain whether the differences in aggressive behavior between ECSs and other breeds could be related to lower levels of serotonin.

**Material and methods**

Nineteen ECSs attended for aggression (toward family members, unfamiliar people, and other dogs) at the Animal Behavior Service of the School of Veterinary Science in Barcelona (Spain) were compared with 20 aggressive dogs of other breeds presented at the same center. All dogs were considered healthy based on physical examination, serum biochemistry, Thyroxine (T4) and Thyroid-stimulating hormone (TSH) evaluation, and complete blood count. Dogs included in the study were not receiving pharmacological treatment. A 5-mL blood sample was collected from the jugular vein of each dog into anticoagulant-free tubes. Samples were centrifuged at 4,500 × g for 15 minutes. Serum was frozen and stored at −80°C until its analysis. Serotonin ELISA kit (DLD Diagnostika GMBH, Hamburg, Germany) was used to measure serotonin levels in serum (Chauveau et al., 1991).

A χ² test was used to assess the association between experimental groups and serotonin levels. A value of P < 0.05 was considered significant for all analyses. Data were analyzed using statistical software for Windows (SPSS version 15.0, SPSS Inc., Chicago, IL, December 18, 2006).

**Results**

In the ECS group, there were 7 male (85.71% of which were intact) and 12 female dogs (50% of which were intact). The average age was 9.5 years.

In the non-ECS group, there were 13 male (84.61% of which were intact) and 7 female dogs (all were intact). In this group, we included 11 purebred and 9 crossbred dogs.
Breeds included in this group are detailed in the Table. The average age of the non-ECS dogs was 5 years. Regarding the type, all were diagnosed with affective aggression. The serum serotonin levels are given in the Table. The mean serum serotonin level in the ECSs was 318.6 (standard error = 567.1) ng/mL. The mean serum serotonin level in the non-ECS dogs was 852.77 (standard error = 100.58) ng/mL. Thus, aggressive ECSs had significantly lower levels of serum serotonin than aggressive dogs of other breeds ($P < 0.05$). The standard deviations in the ECS group and in the non-ECS group were 292.47 and 449.84 ng/mL, respectively, and variances were not significantly different between both groups ($P > 0.05$).

### Discussion

Dog behavior is a complex trait influenced by genetic, epigenetic, and environmental factors (Brain, 2000; Nelson, 2006), and all these may be partly responsible for the differences found in our study.

It has been suggested that aggressive behavior in humans could be related to polymorphisms in genes involved in the transport, synthesis, release, or metabolism of serotonin (Courtet et al., 2001; Sukonick et al., 2001). For instance, a polymorphism in one of the most important enzymes in the metabolism of serotonin (monoamine oxidase A) is associated with abnormal impulsive behavior,
including aggression (Brunner et al., 1993). In laboratory animals, the absence of 5-hydroxytryptamine receptors 1B (5-HT1B receptors), one of the main receptors involved in aggression behavior, is linked to impulsiveness (Bouwknegt et al., 2001). In dogs, recent studies have identified polymorphisms that could explain behavioral differences between breeds (Niimi et al., 1999; Takeuchi et al., 2005), although thus far, the polymorphisms studied are not related to serotonin, but to other neurotransmitters such as dopamine (Niimi et al., 1999). However, the possibility exists that putative differences in serotonin levels between breeds, as shown in our study, are caused by genetic factors.

Epigenetic factors may also be involved in neurophysiological differences within a given species. For instance, several studies in laboratory rodents have found that the tactile stimulation provided by the mother during the first few days after birth caused by maternal licking causes an increase in glucocorticoid receptor expression in the hippocampus (Liu et al., 1997; Meaney, 2001; Weaver et al., 2004; Holmes et al., 2005) and an increase in serotonin levels in the central nervous system owing to epigenetic effects (Meaney et al., 2000; Matthews et al., 2001). Therefore, different mothering styles within a given species could cause differences in serotonin levels in the offspring. Although further research is needed to see whether dog breeds, in general, and ECSs, in particular, differ in their maternal behavior, breed differences in the expression of maternal behavior has been observed in other species (Dwyer and Lawrence, 1998).

Breed differences could also be due to the fact that dogs of the same breed share a similar environment, including owner personality, husbandry, or training. In fact, some authors have observed some resemblance in the owner personality of aggressive ECSs (Peachey, 1993; Podbersek and Serpell, 1997). Additionally, some handling aspects might have an important influence on dog behavior. For example, dog training methods based on punishment increase aggression problems (Roll and Unshelm, 1997; Hiby et al., 2004; Blackwell et al., 2007; Herron et al., 2009). Also, owners of smaller dogs are more inconsistent than owners of larger dogs in the sense that they reward some behaviors in some cases but not always (Arhant et al., 2010). Because the stress response is influenced by the predictability and controllability of events (Weiss, 1970; Beerda et al., 1998; Schalke et al., 2007) and there is a clear link between stress and aggression (Kruk et al., 2004), inconsistent handling could be related to increased aggressive behavior (Arhant et al., 2008). Therefore, it would be helpful to investigate whether the owners of ECSs are more inconsistent and use punishment training methods more frequently than the owners of other breeds.

Possible differences between ECSs and other breeds in the amount of physical exercise and their diet could also explain differences in serotonin levels, although studies on this particular issue are lacking. For example, some studies in laboratory rodents and humans suggest that physical exercise increases serotonin levels (Chaouloff, 1997) and that low levels of exercise correlate with aggressive behavior (Jagoe and Serpell, 1996; Tsatsoulis and Fountoulakis, 2006); however, other studies have failed to find such an effect (Acworth et al., 1986; Hoffmann et al., 1994). Preliminary findings suggest that aggressive dogs exercise less than nonaggressive dogs (Mariotti et al., 2009). Low-protein and high-carbohydrate diets increase the amount of tryptophan that enters the brain and, consequently, the synthesis of serotonin (Benton, 2002). Further studies evaluating the possible differences in physical exercise and diet between breeds could be useful.

ECSs are obtained more frequently from pet shops that dogs of other breeds (Amat et al., 2009), perhaps owing to the popularity of this breed (Serpell and Jagoe, 1995). Dogs obtained from pet shops have more behavioral problems than those obtained from other sources, probably owing to the early weaning and a stressful environment in the pet shop (Serpell and Jagoe, 1995).

Regarding the neutering status, all female dogs included in the non-ECS group were intact. Nevertheless, although female sex hormones may have an effect on some types of affective aggression (Kim et al., 2006), there is no report on any link between female sex hormones and serotonin level or impulsivity.

Finally, an effect of age cannot be ruled out because in our study, the average ages of the dogs in the ECS group and the non-ECS group were different. In humans, the levels of serotonin increase during development in children and decline before puberty (Chugani et al., 2001), and there is an inverse correlation between serotonin levels and age (Seifert et al., 2004). Therefore, it would be useful to analyze whether the serotonin levels of ECSs change during their development.

Low levels of serotonin are correlated with impulsive aggression (Soubrié, 1986; Virkkunen and Linnoila, 1993; Mehlman et al., 1994; Higley et al., 1996; Kavoussi et al., 1997; Peremans et al., 2003). Studies in human and nonhuman primates show that low levels of serotonin are correlated with impulsive aggression rather than with overall aggression (Coccaro, 1989; Virkkunen and Linnoila, 1993; Higley, 2003). The term “impulsiveness” has been defined as a reduction or complete lack of warning signals previous to an attack (Peremans et al., 2003) or as the tendency to respond prematurely without adequate foresight (Robbins and Crockett, 2010). Studies in laboratory rats indicate that decreasing serotonergic activity intensifies impulsiveness, whereas increasing serotonergic activity enhances the preferences for delayed rewards (Manuck et al., 2003). A previous study found that ECSs show impulsiveness more frequently than other purebred dogs (Amat et al., 2009), and impulsive aggression is one of the main risk factors for euthanasia of aggressive dogs (Reisner et al., 1994). Violent impulsive humans have lower levels of 5-HIAA in cerebrospinal fluid than violent individuals who had committed a crime.
with premeditation (Virkkunen et al., 1995). In dogs, impulsive individuals have lower levels of 5-HIAA in cerebrospinal fluid than nonimpulsive ones (Reisner et al., 1996; Quadros et al., 2010), and there is a lower activity of serotonin receptors in some areas of brain in impulsive dogs compared with nonimpulsive ones (Peremans et al., 2003; Vermeire et al., 2011).

Conclusions

According to these results, lower levels of serotonin could influence aggressive behavior. However, further research is needed to find out the precise role of serotonin on aggressive behavior in this particular breed.

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