Effect of fluoxetine on blood concentrations of serotonin, cortisol and dehydroepiandrosterone in canine aggression

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Canine aggression directed towards people is the most frequent reason for referral to behaviour practices. The serotonergic system and the hypothalamic–pituitary–adrenal (HPA) axis are believed to play an important role in controlling aggression. The selective serotonin reuptake inhibitor fluoxetine is the most commonly used drug in canine aggression. The aim of the present study was to assess the effect of a 30-day-long fluoxetine treatment on the peripheral serotonergic system and the HPA axis in canine aggression. To this end, the concentrations of serum serotonin (5-HT) and plasma cortisol and dehydroepiandrosterone (DHEA) were analysed in a group of aggressive ($n = 22$) and nonaggressive dogs ($n = 9$) during pre- (day 0) and posttreatment (day 30) conditions. Treatment caused a significant decrease in 5-HT concentrations (46% in the aggressive group and 32% in the control group). There was a trend towards a rise of DHEA/cortisol ratio values after treatment both in the aggressive and the control group. The determination of blood 5-HT and the DHEA/cortisol ratio could have important clinical applications in the future for deciding which animals might benefit from a given treatment as well as for monitoring the response. Further large-scale studies with this aim should be carried out to obtain sound conclusions.

INTRODUCTION

Canine aggression directed towards humans, and in particular towards the owners, is the most common diagnosis in referral behavioural practices (Bamberger & Houpt, 2006; Fatjó *et al.*, 2007). To date, no drugs have been approved for the treatment of aggression in dogs. However, most specialists agree that pharmacological support can often facilitate greatly the implementation of a behavioural modification programme (Luescher & Reisner, 2008). In the absence of a scientific basis, opting for pharmacological therapy ultimately relies on the clinician's criterion.

Studies across several species including dogs show an inverse relationship between the serotonergic neurotransmission and aggression as determined by low 5-hydroxyindolaceatic acid (5-HIAA) concentrations in the cerebrospinal fluid (CSF) of aggressive (and impulsive) individuals (Reisner *et al.*, 1996; Stanley *et al.*, 2000; Soderstrom *et al.*, 2001; Howell *et al.*, 2007).

The selective serotonin reuptake inhibitor (SSRI) fluoxetine is the most commonly used drug in canine aggression, although conclusive clinical trials are lacking (Luescher & Reisner, 2008). A crossover study in nine aggressive dogs showed that fluoxetine was effective in reducing aggression towards the owners (Dodman *et al.*, 1996). Similarly, several controlled clinical and experimental trials in humans with a life history of aggression have shown the anti-aggressive properties of SSRIs (Coccaro & Kavoussi, 1997; Cherek *et al.*, 2002).

As a whole, serotonin reuptake inhibitors act by blocking the 5-HT transporter at the presynaptic neurons, which leads to an increase in 5-HT concentrations in the synaptic cleft (Horschitz *et al.*, 2001). Interestingly, it has been shown that serotonergic reuptake inhibitors administered in several psychiatric subpopulations also block the platelet 5-HT uptake site. Considering the similarities in the dynamics of 5-HT (uptake, storage and release mechanisms) in serotonergic neurons and platelets, changes in blood serotonergic parameters have been proposed as peripheral markers of the effect of these drugs in the serotonergic neurotransmission (Figueras *et al.*, 1999; Mück-Seler *et al.*, 2002; Castrogiovanni *et al.*, 2003; Fisar *et al.*, 2008).

In humans, SSRIs are the medication of choice for anxiety and alleviate, among others, clinical signs of anticipatory anxiety (Grillon *et al.*, 2009). Several animal behaviourists have reported that a great proportion of dogs displaying aggression also show signs of stress (Overall, 1997; Bamberger & Houpt, 2006; Reisner *et al.*, 2007). It is suggested that serotonergic neuro-transmission plays an important role in coping with stress by acting on the hypothalamic–pituitary–adrenal (HPA) axis (Lanfumey *et al.*, 2008). It is expected, therefore, that the clinical improvement observed after treatment might be also related to systems other than serotonergic system, such as the HPA axis, as it seems to occur in depression after successful antidepressant therapy (Barden, 2004).

Aggressive dogs have been reported to show a lower level of serum 5-HT than nonaggressive animals (Çakiroglu *et al.*, 2007; Rosado *et al.*, 2010) but the effect of treatment on peripheral serotonergic or neuroendocrine parameters has not been addressed to date. The aim of the present study was to assess the effect of a 30-day-long fluoxetine treatment on the peripheral serotonergic system and the HPA axis in canine aggression. To this end, the concentrations of serum 5-HT and plasma cortisol and dehydroepiandrosterone (DHEA) were analysed in a group of aggressive and nonaggressive dogs during pre- (day 0) and posttreatment (day 30) conditions.

MATERIAL AND METHODS

Aggressive animals

The aggressive group consisted of 22 dogs (14 males and 8 females). The mean age was 4.2 years old (ranging from 9 months to 9 years). The group included dogs of different breeds: English cocker spaniel (4), German shepherd (3), poodle (3), dachshund (2), Catalan sheepdog (1), Yorkshire terrier (1) and crosses (1), West Highland white terrier (1), Great Dane (1) and sharpei (1). The group also included two small-medium mongrels and two specific crosses: dachshund × Yorkshire terrier and German shepherd × Catalan sheepdog.

These dogs were submitted to the behavioural services of two veterinary teaching hospitals (Universities of Zaragoza and Cardenal Herrera-CEU, Valencia) owing to a problem of aggression towards people. Play-related and predatory forms of aggression were not considered for the present study, although the presence of other concomitant behavioural problems did not constitute an exclusion criterion.

Diagnosis of aggression was based on a detailed standard questionnaire regarding the dogs' behaviour and daily routine. Clinical classification of aggression was established in accordance with three main diagnostic criteria: target, context and dog's communicative signals (based on Fatjó *et al.*, 2007). Three main preestablished diagnostic categories were then considered:

a) Social conflict–related aggression directed towards family members. This might occur during status-related interactions and competitive or conflict situations. The dog might show offensive and/or defensive (ambivalent) signals.

b) Defensive aggression towards unfamiliar people. This might occur when approaching or manipulating the dog. The dog might show defensive signals.

c) Offensive aggression towards unfamiliar people. This might occur when approaching or manipulating the dog. The dog might show offensive signals.

Social conflict–related aggression towards the owners was diagnosed in nineteen animals, and defensive and offensive aggression towards unfamiliar people was diagnosed in two and one animals, respectively. A physical examination, complete blood count, serum biochemistry and thyroid hormone measurement was carried out at the time of admission in all the cases. These tests showed that dogs included in the present study did not present any underlying causative nor contributory medical condition to the aggression problem.

The prescription of fluoxetine (1 mg/kg, PO, every 24 h) was established in all the selected animals according to clinical criteria. As well as the pharmacological treatment, all the owners were provided with a series of recommendations in writing, for guidance on how to correctly and safely interact with the dog. In the case of dogs suffering any other behavioural problem, treatment measures for these problems were also included. Dogs were physically and behaviourally reevaluated after of a period of 30 days of fluoxetine treatment. The assessment of clinical improvement was based on the absence or drop in frequency and severity of aggressive episodes according to the owners' reports.

Control animals

The control group was made up of nine Beagles (four males and five females, mean age 3.3 years old, ranging from 1 to 6 years) owned by the University Cardenal Herrera-CEU for research purposes. All these dogs were healthy and lacked any history of aggression towards people and/or other dogs. A laboratory group of beagles instead of privately nonaggressive owned dogs was chosen as a control group for the present study considering the following pharmacological intervention. The beagles group was administered a preestablished fluoxetine treatment (Fluoxetina Rubio® 20 mg) at a dosage of 1 mg/kg, PO, every 24 h for 30 days. The care and use of animals followed the European guidelines (European Union Directive 86/609/EEC, 1986).

Sample collection and biochemical analyses

Blood extractions were carried out on day 0 (pretreatment condition) and on day 30 (posttreatment condition) in the morning from 09.00 to 11.00 h. Blood samples (6 mL) were collected from the jugular or cephalic vein into EDTA and normal plastic tubes and centrifuged at 4500 *g* at 4 °C for 10 min. Aliquots of plasma and serum were frozen and stored at

−30 °C and −80 °C, respectively. A part of serum aliquots was set aside for clinical analysis before freezing.

Serum 5-HT was measured with a commercial EIA technique (Serotonin-ELISA; DLD Dianostika GMBH, Hamburg, Germany). The intra and interassay coefficients of variation were 3.9–5.4% and 6%, respectively. Concentrations were expressed in ng/mL. The variation (%) of 5-HT concentration after treatment was expressed as a ratio between the difference in concentrations [posttreatment − pretreatment] and the pretreatment concentrations.

Plasma cortisol and DHEA were determined in duplicate using two home enzyme immunoassay (EIA) techniques (G. Chacón, 2004, dissertation). In the cortisol EIA validation test, the intra and interassay coefficients of variation were 3.5–6% and 3.9–9.9%, respectively. Regarding DHEA, the intra and interassay coefficients of variation were 7.4–8.8% and 8.3–9.05%, respectively. Concentrations were expressed in ng/mL. The DHEA/cortisol ratio was calculated.

Statistical analysis

Serotonin, cortisol, DHEA and DHEA/cortisol ratios were defined as dependent variables. Two factors were considered for statistical analysis: (i) 'disease' (D), which grouped two categories, namely 'aggressive' and 'control', and (ii) 'treatment' (T), which included the 'pretreatment' and 'posttreatment' conditions.

Distribution of 5-HT data was shown by Shapiro–Wilk test to be normal, whereas distributions of cortisol and DHEA were nonnormal. Parametric tests were finally used, and the limitations related with this analysis are dealt in the discussion.

A multifactorial multivariate analysis with repeated measures was used to assess the effect of factors (D) and (T). Interaction between factors (D × T) was also studied. A logarithmic regression was used to assess the relationship between the variation (%) of 5-HT concentrations after treatment and the pretreatment concentrations. Finally, correlations between all parameters were analysed using the Pearson test.

Calculations were carried out using the statistical program SPSS 14.0. for Windows (SPSS, Inc, Chicago, IL, USA). We considered that a P-value of <0.05 denoted statistical significance, and $P < 0.10$ was treated as a trend.

RESULTS

Following treatment, all the owners reported to have noticed an improvement of the situation, which was reflected in the absence/decrease in the number and/or severity of aggressive episodes.

Multifactorial multivariate analysis of variance showed a significant effect of the factors D ($P = 0.002$) and T ($P = 0.001$). A nonsignificant interaction was detected between both factors (D × T). Pre- and posttreatment mean concentrations of all biochemical variables are described in Table 1. Treatment caused a significant decrease in 5-HT concentrations (46% in the aggressive group and 32% in the control group). A negative correlation was found between the variation (%) of serum 5-HT after treatment and the concentration on day 0 ($R^2 = 0.51$; $P < 0.001$). The relation between both parameters is shown in Fig. 1.

Positive correlations were detected between pre- and posttreatment cortisol and pre- and posttreatment DHEA both in the aggressive ($r = 0.502$; $P = 0.02$ and $r = 0.675$; $P = 0.001$, respectively) and in the control groups ($r = 0.741$; $P = 0.022$ and $r = 0.845$; $P = 0.004$, respectively). In the aggressive group, negative correlations were found between pretreatment cortisol and the DHEA/cortisol ratio ($r = -0.571$; $P = 0.026$) and between posttreatment cortisol and the DHEA/cortisol ratio ($r = -0.429$; $P = 0.046$).

DISCUSSION

All the owners reported an improvement of the problem after the 30-day-long fluoxetine treatment, albeit different degree. Recommendations to the owners on how to correctly and safely interact with the dogs could also have had a beneficial effect on clinical outcome. Literature in canine psychopharmacology is scarce although studies to date have shown that a 3-week fluoxetine treatment was effective in reducing aggression (Dodman et al., 1996), whereas a 6-week Clomipramine and 4-week Amytriptilyne administration had no effect compared with placebo and behavioural modification therapy alone, respectively (White et al., 1999; Virga et al., 2001). In humans,

Table 1. Pre and posttreatment mean (standard error) concentrations of biochemical variables in aggressive and control dogs

Variable	Group	Pretreatment Mean (SE)	Posttreatment Mean (SE)	P-value D	T	D × T
Serotonin (ng/mL)	Aggressive	293.7 (32.7)	122.9 (12.3)	0.47	<0.001**	0.26
	Control	287.3 (28.2)	178.5 (30.2)			
Cortisol (ng/mL)	Aggressive	18.1 (3.2)	16.8 (3.7)	0.03*	0.76	0.86
	Control	6.7 (2.3)	6.4 (2.4)			
DHEA (ng/mL)	Aggressive	67.2 (11.9)	65.6 (9.2)	0.01*	0.08	0.06
	Control	168.1 (66.1)	224.7 (79.1)			
DHEA/cortisol ratio	Aggressive	7.6 (1.8)	11.1 (17.0)	0.001**	0.08	0.13
	Control	41.2 (16.5)	95.8 (47.8)			

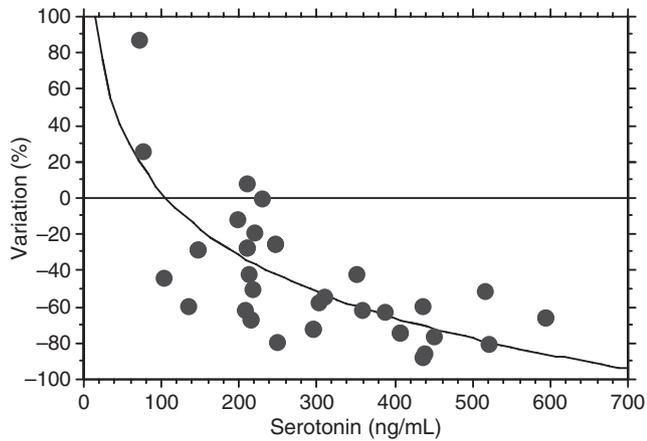D, Disease; T, Treatment; DHEA, dehydroepiandrosterone. *P < 0.05, **P < 0.01.

Fig. 1. Regression plot of the relationship between the variation of serotonin concentrations after treatment (%) and pretreatment serotonin concentration in aggressive and control dogs.

a decrease in aggressiveness, impulsiveness, hostility and irritability following SSRIs administration has been reported (Coccaro & Kavoussi, 1997; Cherek *et al.*, 2002; Kamarck *et al.*, 2009).

Pretreatment concentration of serum 5-HT was similar in the aggressive and control groups. This finding contrasts with a previous one by the authors (Rosado *et al.*, 2010) and other published results where aggressive dogs were characterized by lower 5-HT concentrations in serum (Çakiroglu *et al.*, 2007) as well as lower 5-HIAA concentrations in CSF (Reisner *et al.*, 1996). One possible reason to account for the difference with the previous results by the authors may be the different population used as control group. The control population in the previous studies was made up of a heterogeneous population of household dogs who lived with their owners, whereas in the current study, it was made of a homogeneous group of beagles living under experimental conditions. An experimental population of dogs, however, is required for pharmacological intervention according to ethical research criteria.

Overall, fluoxetine treatment markedly reduced serum 5-HT in the aggressive and control dogs. Similarly, previous studies on depression have shown a significant decrease in platelet 5-HT content following SSRIs administration (Alvarez *et al.*, 1999a; Figueras *et al.*, 1999; Mück-Seler *et al.*, 2002; Maurer-Spurej *et al.*, 2004). In particular, changes on serum 5-HT after treatment seemed to occur in a nonlinear manner: those animals with very low and high concentrations on day 0 tended to show the higher variation (%) of 5-HT concentrations on day 30. This finding may be explained as a regulation phenomenon of peripheral 5-HT turnover after blocking the platelet 5-HT uptake place by fluoxetine.

The strong correlation found between the variation of 5-HT level on day 30 and that of day 0 may suggest that the effect of treatment on serum 5-HT was largely (50%) conditioned by the pretreatment condition. This finding might in turn suggest a predictor value of pretreatment 5-HT levels for posttreatment levels. However, such pretreatment levels cannot be used as a

predictor of the clinical outcome. In this regard, several studies on human platelets suggest that a high pretreatment platelet 5-HT content is associated with a later poor therapeutic outcome to antidepressants (Figueras *et al.*, 1999; Mück-Seler *et al.*, 2002; Castrogiovanni *et al.*, 2003). In addition, some authors suggest that plasma 5-HT levels after 1 day of fluoxetine treatment may predict the antidepressant response, as responders showed significantly lower concentrations than nonresponders (Alvarez *et al.*, 1999b). Whether these findings are true for fluoxetine-treated aggressive dogs was not addressed in the present study but it might represent an interesting question to assess in future studies. Anecdotally, it is worth mentioning that the three aggressive animals with the highest 5-HT pretreatment concentrations showed a poorer improvement of the problem than did other cases.

Regarding neuroendocrine parameters, pretreatment concentration of plasma cortisol was found to be significantly higher in the aggressive group than in the control group, suggesting some degree of stress in the former. This result is in agreement with previous results by the authors (Rosado *et al.*, 2010) and studies on humans (Soderstrom *et al.*, 2004; van Bokhoven *et al.*, 2005), where high concentrations of glucocorticoids have been related to affective forms of aggression. It moreover reflects the clinical finding that a great proportion of aggressive dogs also show anxiety and other signs of stress (Overall, 1997; Bamberger & Houpt, 2006; Reisner *et al.*, 2007).

Pretreatment concentration of plasma DHEA and the DHEA/cortisol ratio value, on the other hand, appeared to be significantly higher in the control group than in the aggressive group. In humans, a high level of the sulphated form of DHEA (DHEAS) has been related to aggression in boys with conduct disorder (van Goozen *et al.*, 1998; Dmitrieva *et al.*, 2001; Golubchik *et al.*, 2009), although DHEA was not related to aggression according to the latter study (Golubchik *et al.*, 2009). This finding stresses the need for an assessment of the role of both the sulphated and nonsulphated forms of DHEA in aggression. In this regard, it is considered that DHEAS is a GABA$_A$ antagonist (Baulieu & Robel, 1998; Park-Chung *et al.*, 1999), whereas DHEA metabolites display GABA$_A$ agonistic properties (van Broekhoven & Verkes, 2003). Therefore, disequilibrium between both forms could alter aggressive behaviour, as it has been experimentally observed in mice (Nicolas *et al.*, 2001).

The treatment did not induce any significant change on cortisol concentrations in any of the groups studied. Correlations between pre- and posttreatment cortisol values in both groups suggest that posttreatment levels largely depend on the starting conditions. It has been suggested that the normalization of the hyperactive HPA system occurs during successful antidepressant pharmacotherapy of depressive illness (Barden, 2004). Regarding the present results, it is possible that the regulatory role of the serotonergic system on cortisol secretion, which seems to be mediated by the 5-HT1A y 5-HT2C receptors (Lanfumey *et al.*, 2008), may have required a more extended period of treatment.

On the other hand, treatment induced an upward trend on plasma DHEA concentrations only in the control group. Again,

the correlation between pre- and posttreatment DHEA values in both groups suggests that starting levels are important for the outcome. Interestingly, there was a trend towards a rise of DHEA/cortisol ratio values after treatment both in the aggressive and in the control group. Cortisol and DHEA co-regulate each other and hence co-elevations or imbalances determine net effect on tissues. Considering this, it has been suggested that it may be important to consider the ratio of both steroids in addition to their absolute concentrations (Maninger *et al.*, 2009). In the present study, the ratio DHEA/cortisol, rather than concentrations of either hormone alone, more accurately discriminated changes in HPA axis after treatment in both groups.

Considering the antiglucocorticoid properties of DHEA(S), it has been suggested that a high circulating DHEA(S)/cortisol ratio may indicate the degree to which an individual counteracts the centrally mediated, negative effects of stress (Morgan *et al.*, 2004). It may be hypothesized that fluoxetine, a drug with both anxiolytic and anti-aggressive properties, induced an improvement of well-being of the dogs studied, as indicated by an increased ratio value. Several studies in depressed patients have shown that concentrations of the neurosteroid allopregnanolone, a positive allosteric modulator of the GABA$_A$ receptors, increased following effective SSRIs treatment (Uzunova *et al.*, 1998; Ströhle *et al.*, 2000). To the authors' best knowledge, there are no published studies focusing on the effect of serotonergic drugs on DHEA concentrations.

Finally, the increase in DHEA concentration (control group) and DHEA/cortisol ratio value (control and aggressive group) following treatment could coincide to a certain extent with the results of studies that show DHEA administration to improve signs of depression and anxiety, mood, sense of well-being, activity and energy in several human subpopulations (Wolkowitz *et al.*, 1999; Johnson *et al.*, 2002; Strous *et al.*, 2003; Schmidt *et al.*, 2005; Rabkin *et al.*, 2006). Apart from the antiglucocorticoid properties, it has been suggested that the beneficial effect of DHEA administration might result from its sigma 1 receptor-mediated enhancement of noradrenaline and 5-HT neurotransmission (van Broekhoven & Verkes, 2003). To date, there is no scientific evidence for intervention in dogs in this sense.

One limitation to the present work is the use of parametric tests for variables showing a nonnormal distribution (i.e. cortisol and DHEA). This may limit the generalization of the results on these variables. However, the authors found the multivariable multifactorial analysis more robust than univariable unifactorial analyses. Further studies considering larger and more homogenous groups would allow a better assessment of individual differences.

In conclusion, this is the first study that assesses the effect of a 30-day-long fluoxetine treatment on several peripheral biochemical parameters in control and aggressive dogs. Treatment caused a drop in serum 5-HT concentrations in both groups, supporting the hypothesis that the peripheral serotonergic system in canine species is sensitive to drugs that work upon the central serotonergic system. If a clear correlation between blood and brain 5-HT is confirmed, the determination of serum 5-HT could have important clinical applications in the future. For example, it could be used for deciding which animals might benefit from a given pharmacological treatment as well as for monitoring the response. In addition, a trend was found towards an increase in DHEA concentrations (control group) and in DHEA/cortisol ratio values (control and aggressive group) following fluoxetine treatment. The role of DHEA in mediating canine aggression has yet to be fully determined but this finding gives a new insight into the management of stress-related canine behaviour problems. Further large-scale studies with this aim should be carried out to obtain sound conclusions.

REFERENCES

Alvarez, J.C., Gluck, N., Arnulf, I., Quintin, P., Leboyer, M., Pecquery, R., Launay, J.M., Perez-Diaz, F. & Spreux-Varoquaux, O. (1999a) Decreased platelet serotonin transporter sites and increased platelet inositol triphosphate levels in patients with unipolar depression: effects of clomipramine and fluoxetine. *Clinical Pharmacology and Therapeutics*, **66**, 617–624.

Alvarez, J.C., Gluck, N., Fallet, A., Gregoire, A., Chevalier, J.F., Advenier, C. & Spreux-Varoquaux, O. (1999b) Plasma serotonin level after 1 day of fluoxetine treatment: a biological predictor for antidepressant response? *Psychopharmacology (Berl)*, **143**, 97–701.

Bamberger, M. & Houpt, K.A. (2006) Signalment factors, comorbidity, and trends in behavior diagnoses in dogs: 1,644 cases (1991–2001). *Journal of the American Veterinary Medical Association*, **229**, 1591–1601.

Barden, N. (2004) Implication of the hypothalamic–pituitary–adrenal axis in the physiopathology of depression. *Journal of Psychiatry & Neuroscience*, **29**, 185–193.

Baulieu, E.E. & Robel, P. (1998) Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) as neuroactive neurosteroids. *Proceedings of the National Academy of Science of the United States of America*, **95**, 4089–4091.

van Bokhoven, I., Van Goozen, S.H., van Engeland, H., Schaal, B.L., Arseneault, L., Séguin, J.R., Nagin, D.S., Vitaro, F. & Tremblay, R.E. (2005) Salivary cortisol and aggression in a population-based longitudinal study of adolescent males. *Journal of Neural Transmission*, **112**, 1083–1096.

van Broekhoven, F. & Verkes, R.J. (2003) Neurosteroids in depression: a review. *Psychopharmacology (Berl)*, **165**, 97–110.

Çakiroglu, D., Meral, Y., Sancak, A.A. & Cifti, G. (2007) Relationship between the serum concentrations of serotonin and lipids and aggression in dogs. *The Veterinary Record*, **161**, 59–61.

Castrogiovanni, P., Blardi, P., De Lalla, A., Dell'Erba, A. & Auteri, A. (2003) Can serotonin and fluoxetine levels in plasma and platelets predict clinical response in depression? *Psychopharmacology Bulletin*, **37**, 102–108.

Cherek, D.R., Lane, S.D., Pietras, C.J. & Steinberg, J.L. (2002) Effects of chronic paroxetine administration on measures of aggressive and impulsive responses of adult males with a history of conduct disorder. *Psychopharmacology (Berl)*, **159**, 266–274.

Coccaro, E.F. & Kavoussi, R.J. (1997) Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. *Archives of General Psychiatry*, **54**, 1081–1088.

Dmitrieva, T.N., Oades, R.D., Hauffa, B.P. & Eggers, C. (2001) Dehydroepiandrosterone sulphate and corticotropin levels are high in young male patients with conduct disorder: comparisons for growth factors, thyroid and gonadal hormones. *Neuropsychobiology*, **43**, 134–140.

Dodman, N.H., Donnelly, R., Shuster, L., Mertens, P., Rand, W. & Miczek, K. (1996) Use of fluoxetine to treat dominance aggression in dogs. *Journal of the American Veterinary Medical Association*, **209**, 1585–1587.

European Union Directive 86/609/EEC (1986) Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities Serie*, **358**, 132.

Fatjó, J., Amat, M., Mariotti, V., Ruiz De La Torre, J.L. & Manteca, X. (2007) Analysis of 1040 cases of canine aggression in a referral practice in Spain. *Journal of Veterinary Behavior: Clinical Applications and Research*, **2**, 158–165.

Figueras, G., Pérez, V., San Martino, O., Alvarez, E. & Artigas, F. (1999) Pretreatment platelet 5-HT concentration predicts the short-term response to paroxetine in major depression. *Biological Psychiatry*, **46**, 518–524.

Fisar, Z., Kalisová, L., Paclt, I., Anders, M. & Vevera, J. (2008) Platelet serotonin uptake in drug-naïve depressive patients before and after treatment with citalopram. *Psychiatry Research*, **161**, 185–194.

Golubchik, P., Mozes, T., Maayan, R. & Weizman, A. (2009) Neurosteroid blood levels in delinquent adolescent boys with conduct disorder. *European Neuropsychopharmacology*, **19**, 49–52.

van Goozen, S.H., Matthys, W., Cohen-Kettenis, P.T., Thijssen, J.H. & van Engeland, H. (1998) Adrenal androgens and aggression in conduct disorder prepubertal boys and normal controls. *Biological Psychiatry*, **43**, 156–158.

Grillon, C., Chavis, C., Conington, M.F. & Pine, D.S. (2009) Two-week treatment with the selective serotonin reuptake inhibitor citalopram reduces contextual anxiety but not cued fear in healthy volunteers: a fear-potentiated startle study. *Neuropsychopharmacology*, **34**, 964–971.

Horschitz, S., Hummerich, R. & Schloss, P. (2001) Structure, function and regulation of the 5-hydroxytryptamine (serotonin) transporter. *Biochemical Society Transactions*, **29**, 728–732.

Howell, S., Westergaard, G., Hoos, B., Chavanne, T.J., Shoaf, S.E., Clevelan, A., Snoy, P.J., Suomi, S.J. & Dee Higley, J. (2007) Serotonergic influences on life-history outcomes in free-ranging mal rhesus macaques. *American Journal of Primatology*, **69**, 851–865.

Johnson, M.D., Bebb, R.A. & Sirrs, S.M. (2002) Uses of DHEA in aging and other disease states. *Ageing Research Reviews*, **1**, 29–41.

Kamarck, T.W., Haskett, R.F., Muldoon, M., Flory, J.D., Anderson, B., Bies, R., Pollock, B. & Manuck, S.B. (2009) Citalopram intervention for hostility: results of a randomized clinical trial. *Journal of Consulting and Clinical Psychology*, **77**, 174–187.

Lanfumey, L., Mongeau, R., Cohen-Salmon, R. & Hamon, M. (2008) Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neurosciences and Biobehavioral Reviews*, **32**, 1174–1184.

Luescher, A.U. & Reisner, I.R. (2008) Canine aggression toward familiar people: a new look at an old problem. *Veterinary Clinics of North America, Small Animal Practice*, **38**, 1107–1130.

Maninger, N., Wolkowitz, O.M., Reus, V.I., Epel, E.S. & Mellon, S.H. (2009) Neurobiological and neurpsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS). *Frontiers in Neuroendocrinology*, **30**, 65–91.

Maurer-Spurej, E., Pittendreigh, C. & Solomon, K. (2004) The influence of selective serotonin reuptake inhibitors on human platelet serotonin. *Thrombosis and Haemostasis*, **91**, 119–128.

Morgan, C.A. III, Southwick, S., Hazlett, G., Rasmusson, A., Hoytz, G., Zimolo, Z. & Charney, D. (2004) Relationships among plasma dehydroepiandrosterone sulphate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. *Archives of General Psychiatry*, **61**, 819–825.

Mück-Seler, D., Pivac, N., Sagud, M., Jakovljevic, M. & Mihaljevic-Peles, A. (2002) The effects of paroxetine and tianeptine on peripheral biochemical markers in major depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, **26**, 1235–1243.

Nicolas, L.B., Pinoteau, W., Papot, S., Routier, S., Guillaumet, G. & Mortaud, S. (2001) Aggressive behavior induced by the steroid sulfatase inhibitor COUMATE and by DHEAS in CBA/H mice. *Brain Research*, **922**, 216–222.

Overall, K.L. (1997) *Clinical Behavioral Medicine for Small Animals*. Mosby-Year Book, St Louis.

Park-Chung, M., Malayev, A., Purdy, R.H., Gibbs, T.T. & Farb, D.H. (1999) Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function trough distinct sites. *Brain Research*, **830**, 72–87.

Rabkin, J.G., McElhiney, M.C., Rabkin, R., McGrath, P.J. & Ferrando, S.J. (2006) Placebo-controlled trial of dehydroepiandrosterone (DHEA) for treatment of nonmajor depression in patients with HIV/AIDS. *American Journal of Psychiatry*, **163**, 59–66.

Reisner, I.R., Mann, J.J., Stanley, M., Huang, Y.Y. & Houpt, K.A. (1996) Comparison of cerebrospinal fluid monoamine metabolite levels in dominant-aggressive and non-aggressive dogs. *Brain Research*, **714**, 57–64.

Reisner, I.R., Shofer, F.S. & Nance, M.L. (2007) Behavioral assessment of child-directed canine aggression. *Injury Prevention*, **13**, 348–351.

Rosado, B., García-Belenguer, S., León, M., Chacón, G., Villegas, A. & Palacio, J. (2010) Blood concentrations of serotonin, cortisol and dehydroepiandrosterone in aggressive dogs. *Applied Animal Behaviour Science*, **123**, 124–130.

Schmidt, P.J., Daly, R.C., Bloch, M., Smith, M.J., Danaceau, M.A., St Clair, L.S., Murphy, J.H., Haq, N. & Rubinow, D.R. (2005) Dehydroepiandrosterone monotherapy in midlife-onset major and minor depression. *Archives of General Psychiatry*, **62**, 154–162.

Soderstrom, H., Blennow, K., Manhem, A. & Forsman, A. (2001) CSF studies in violent offenders. I. 5-HIAA as a negative and HVA as a positive predictor of psychopathy. *Journal of Neural Transmission*, **108**, 869–878.

Soderstrom, H., Blennnow, K., Forsman, A., Liesivuori, J., Pennanen, S. & Tiihonen, J. (2004) A controlled study of tryptophan and cortisol in violent offenders. *Journal of Neural Transmission*, **111**, 1605–1610.

Stanley, B., Molcho, A., Stanley, M., Winchel, R., Gameroff, M.J., Parsons, B. & Mann, J.J. (2000) Association of aggressive behavior with altered serotonergic function in patients who are not suicidal. *The American Journal of Psychiatry*, **157**, 609–614.

Ströhle, A., Pasini, A., Romeo, E., Hermann, B., Spalletta, G., di Michele, F., Holsboer, F. & Rupprecht, R. (2000) Fluoxetine decreases concentrations of 3 alpha, 5- alpha-tetrahydrodeoxy-corticosterone (THDOC) in major depression. *Journal of Psychiatric Research*, **34**, 183–186.

Strous, R.D., Maayan, R., Lapidus, R., Stryjer, R., Lusting, M., Kotler, M. & Weizman, A. (2003) Dehydroepiandrosterone augmentation in the management of negative, depressive, and anxiety symptoms in schizophrenia. *Archives of General Psychiatry*, **60**, 133–141.

Uzunova, V., Sheline, Y., Davis, J.M., Rasmusson, A., Uzunov, D.P., Costa, E. & Guidotti, A. (1998) Increase in the cerebrospinal fluid

content of neurosteroids inpatients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proceedings of the National Academy of Sciences of United States of America*, **95**, 3239–3244.

Virga, V., Houpt, K.A. & Scarlett, J.M. (2001) Efficacy of amitriptyline as a pharmacological adjunct to behavioral modification in the management of aggressive behaviors in dogs. *Journal of the American Animal Hospital Association*, **37**, 325–330.

White, M.M., Neilson, J.C., Hart, B.L. & Cliff, K.D. (1999) Effects of clomipramine hydrochloride on dominance-related aggression in dogs. *Journal of the American Veterinary Medical Association*, **215**, 1288–1291.

Wolkowitz, O.M., Reus, V.I., Keebler, A., Nelson, N., Friedland, M., Brizendine, L. & Roberts, E. (1999) Double-blind treatment of major depression with dehydroepiandrosterone. *American Journal of Psychiatry*, **156**, 646–649.