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Case study

Accelerated high-frequency repetitive transcranial magnetic stimulation positively influences the behavior, monoaminergic system and cerebral perfusion in anxious aggressive dogs: a case study

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Accelerated high frequency repetitive transcranial magnetic stimulation (aHF-rTMS) was proven to produce fast clinical effects in humans suffering from psychiatric illnesses. Although dogs also frequently present behavioral symptoms similar to mental illness, rTMS treatment was not yet investigated in this species. The aim of this study was to apply an aHF-rTMS treatment over the frontal cortex in an anxious aggressive dog. Since aHF-rTMS is used to treat anxiety and mood disorders in humans and shows changes in neuronal activity and on monoamine concentrations, it was hypothesized that the dog’s behavior would improve after such a treatment. This improvement was expected to be accompanied by alterations in regional cerebral blood flow (rCBF) as well as in monoamine levels in CSF and serum. An aHF-rTMS protocol was applied twice (3 weeks separated) over the left frontal cortex (5 sessions, 20Hz, 110% CMT) in a 5-year-old neutered male Belgian malinois dog showing anxious aggressive behavior. Each protocol was preceded and followed by a behavior assessment and a [\(^{99}\text{Tc}\)]HMPAO-SPECT scan. A Z-score for each volume of interest (VOI) at each time point was obtained, whereby a \(|Z|-score > 3.09\) (P-value of 0.001) indicated significant differences. Monoamines and their metabolites were quantified in cerebrospinal fluid (CSF) and serum using liquid-chromatography coupled to electrochemical detection. An improvement of the dog’s aggressive behavior was detected. At baseline, only a decreased rCBF of the left frontal cortex was noticeable (Z-score = -3.87). Twenty-four hours after the first protocol, the perfusion in the left frontal cortex was normalized and decreased in subcortical region (Z-score = -6.97). Three weeks after each stimulation protocol, no deviations in the rCBF were found. Parallel time-dependent changes of 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in serum and CSF were observed. This case study demonstrates that a single day aHF-rTMS treatment reduces a dog’s anxious/aggressive behavior. This
behavioral change was accompanied by immediate and long-lasting alterations in the rCBF and DOPAC concentration. This study confirms the interaction between the frontal cortex and the subcortical region in behavior in dogs and puts DOPAC forward as possible biomarker.

dog; rTMS; SPECT; dopamine; behavior
Case presentation

A 5-year-old neutered male white coated Malinois dog (32kg) was presented with anxious aggressive behavior.

History and presenting signs

The patient is, together with his 16 siblings, the accidental offspring between his half-brother and his mother. Neither parent showed behavioral problems. The owner purchased the dog at the age of 8 weeks. During the first year of his life, the dog received behavioral training and showed no signs of abnormal behavior. At the age of 1, the dog started to show aggression combined with anxious behavior in several situations (e.g., when meeting unfamiliar people or when separated from the owner). The aggression started out mildly and evolved towards attempting to bite people and dogs without apparent warning signs. Most of the aggressive behavior was seen while walking with the dog. Indoors, no aggression was seen against its owners or the other dog living in the house. Aggressive behavior was first seen towards other dogs, followed by bikes and cars, and finally towards humans. Prior to his aggressive behavior appearing no behavioral signs (snarling, barking, growling), with the exception of fixation toward a close or distant person or object, are seen. Once fixated onto the person or object, the patient would approach this target. The dog sometimes barked at the object or people prior to its aggressive behavior, when he is in the presence of the other dog. The dog also barked at people passing by the owner’s house. Two biting incidents took place, during which physical harm was caused to a person. As a reaction towards his aggressive behavior, the owner trained the dog to bite when asked. After that behavioral training, the dog has been able to release his
bite when asked by the owner. Besides this training, no treatments (behavioral or pharmaceutical) were administered.

Physical evaluation

After thorough clinical examination, no clinical abnormalities were found. The dog’s blood analysis showed no deviations from the norm. A 3T MRI showed no visible structural abnormalities in the dog's brain, whereas a functional \[^{99mTc}\text{HMPAO-SPECT}\] showed a decreased left frontal perfusion (when compared to a control group). The control group consisted of 16 healthy dogs ranging from 1 to 8 years old.

Behavioral evaluation

The dog’s behavior was assessed using the validated canine behavioral questionnaire completed by the owner (Hsu and Serpell, 2003; Duffy and Serpell, 2012), providing information concerning the dog’s behavior and temperament in 13 scales (Table 1). This questionnaire contains 101 questions grouped into seven sections: training and obedience, aggression, fear and anxiety, separation-related behavior, excitability, attachment and attention seeking and miscellaneous. The responses to the questions were scored with a 5-point frequency scale or a 5-point semantic differential scales. Based on table 1, the dog proved to be highly trained and obedient and showed no aggressive behavior towards the owners or familiar dogs. On the other hand, the dog scored high in the sections stranger-directed aggression and dog-directed aggression/fear.
The owner was asked to complete a second questionnaire (see supplementary files) that allowed insight into the dogs’ reaction towards the owner’s absence (actual and virtual) and noises including (1) thunderstorm, (2) gunshots, (3) fireworks, and (4) other noises (Overall et al., 2001; Overall, 2013; Overall et al., 2016; Scheifele et al., 2016). Responses towards these questions were (1) yes, (2) no, (3) unknown. If “yes” was answered, the owner was asked to estimate the frequency of the dog’s reaction towards the stimulus. The owner could choose among the frequencies (1) 100% of the time, (2) <100% of the time but >60%, (3) 40% to 60% of the time, (4) 0% of the time but <40%. In addition, the owner was asked to specify his dog’s reaction and frequency of exposure to each noise. Possible reactions were salivate, hide, defecate, tremble, urinate, vocalize, destroy, pace, escape, freeze, will not eat food, pupil dilation, and/or pant. Answers to the frequency of exposure were (1) never, (2) occasionally/once a month, (3) regularly/ a few times a month, (4) frequently/multiple times a week. Based on this section questions, a global separation anxiety intensity rank (SAIR) and anxiety intensity rank (AIR) were calculated. These were calculated based on the portrayed behavior multiplied by a weight of 4, 2.5, 1.5, 1 and 0 for the frequency. The maximum score for the SAIR and AIR was 48 and 208 respectively. The second questionnaire included an aggression screen to assess the dog’s reactivity, severity and intensity towards several stimuli (51). Reactivity was defined as the proportion of stimuli to which the dog reacts compared to the total number of listed stimuli. In order to calculate the severity, the owner was asked to tick off his dog’s response towards various stimuli. Possible reactions were (1) no reaction, (2) snarl, (3) lip lift, (4) bark, (5) growl, (6) snap, (7) bite, (8) withdraw or avoid and (9) not applicable. The specific behaviors received a weight factor of 1 (barking and growling), 2 (snarling and lip lifting) or 4 (snapping and biting). The
severity of the dog’s behavior was calculated by dividing the total reaction score (summation of all the weight factors) by total number of possible reactions towards all stimuli (9x51). Intensity was obtained by dividing the total reaction score with the total number of stimuli (51).

**Diagnosis**

Based on the dog’s history, the physical examination, the MRI images, the $^{99}$Tc-HMPAO SPECT (d,l hexamethylpropylene amine oxime single photon emission computed tomography) scan and the questionnaires the dog was diagnosed with anxious aggressive behavior specifically towards non-familiar people and animals.

**Treatment**

**Neuronavigation protocol**

Neuronavigation is a technique whereby three-dimension information about neurological structures enclosed by the skull or the vertebral column is provided. This study focuses on the non-invasive stimulation of the left frontal cortex. Therefore, the left frontal cortex had to be externally located with neuronavigation. In order to obtain this information, a tomographical dataset (MRI) had to be acquired. The neuronavigation and external localization was performed as described by Dockx et al. (2017).

$[^{99}\text{mTc}]$HMPAO-SPECT scan

The patient underwent four $[^{99}\text{mTc}]$HMPAO-SPECT scans. A baseline, one 24 hours and one three weeks after the last aHF-rTMS session was given. Two days after the last SPECT scan the aHF-rTMS treatment was again applied over the left frontal cortex. Three weeks after the last session of the second protocol, the patient received another $[^{99}\text{mTc}]$HMPAO-SPECT scan.
Twenty-four hours prior to each SPECT scan a $^{99m}$Mo generator was eluted and approximately 1.85 GBq $^{99m}$TcO$_4$ was added to the exametazime (hexamethylpropylene amine oxime (HMPAO); Ceretec®, GE Healthcare LTD, UK). The dog was first muzzled and IM premedicated with dexmedetomidine (375 µg/m$^2$ body surface). When sedated, an IV catheter was placed in a cephalic vein and on average 357.27 MBq (SD = 54.62 MBq) of $[^{99m}\text{Tc}]$HMPAO was intravenously injected. After 15-20 minutes, propofol was IV administered to induce general anesthesia and was maintained with isoflurane in oxygen through a rebreathing system. The dog was monitored for respiratory and electrocardiographic function throughout the scan. A triple head gamma camera (Triad, Trionix, Twinsburg, OH, USA), equipped with low energy ultrahigh-resolution parallel hole collimators (tomographic resolution FWHM=9 mm), was used to acquire the data. The camera collected data over a circular 360° rotation in a step-and-shoot mode during 20 minutes (120 steps, 10 sec per step, 3° steps) on a 128~128 matrix. Afterwards, the data were iteratively reconstructed and a Butterworth filter (cut-off 1.4 cycli/cm, order 5) was applied.

After the acquisition of the images, a template containing 11 brain regions (volumes of interest, VOIs) (both frontal, temporal, parietal and occipital lobes, the cerebellum, olfactory bulb and the subcortical area) was fitted onto the dataset using BRASS software (Brain Registration and Automated SPECT Semiquantification, Nuclear diagnostics, Sweden). The regional cerebral blood flow (rCBF; perfusion index (PI)) was semi-quantitatively obtained (normalization of the regional activity to the radioactivity of the entire brain).

A Z-score was obtained for each VOI at each time point with SPECT images of healthy dogs ranging from 1 to 8 years old (control group). The Z-score was
calculated using the following equation: \( Z\)-score = (mean control group – value patient) / (standard deviation control group). The cut-off value was at \(|Z| > 3.09\) (comparable to a \(P\)-value of 0.001) indicating significant differences in the rCBF when compared to the control group.

**Peripheral and central monitoring of the mono-aminergic system**

Immediately following each \([^{99}\text{mTc}]\text{HMPAO-SPECT}\) scan, cerebrospinal fluid (CSF) and serum were acquired. The CSF tap was performed at the cisterna magna using a 19 G needle after the patient was positioned in right lateral recumbency. While still under anesthesia and right lateral recumbency, a 21G needle was used to draw blood from the vena jugularis externa. An anti-oxidative mixture containing 0.1M perchloric acid (Merck, Darmstadt, Germany), 0.05% \(\text{Na}_2\text{EDTA}\) (Sigma Aldrich, Saint Louis, USA) and 0.05% sodium metabisulfite (Merck, Darmstadt, Germany) was made. 900 µl and 25 µl of this mixture were added to 100 µl serum and 100 µl CSF respectively. The diluted samples were immediately frozen (-80 °Celsius) until further analysis. Prior to the analysis, the samples were thawed and centrifuged at 15000 rpm for 15 minutes. The supernatant was transferred and diluted 1/2 (CSF) and 1/5 (serum) with 0.5 M acetic acid (Fisher scientific, Bishop meadow road, UK).

Total noradrenaline (NAD), dopamine (DA), 3,4-dihydroxyphenylacetic (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were measured in CSF and serum based on previously reported methods (El Arfani et al., 2014; Jardi et al., 2018). The samples were injected automatically on a reversed phase liquid chromatography system (autosampler ASI-100 and HPLC pump P680 A HPG/2, Dionex, Amsterdam, The Netherlands) with electrochemical detection (potential= + 700mV) (Amperometric
The separation was achieved using a narrowbore C18 column (Alltech\textsuperscript{TM}, Alltima\textsuperscript{TM}, 5 \(\mu\)m, 150 x 2.1 mm, Grace, Deerfield, IL, USA). The mobile phase buffer contained 0.1 M sodium acetate (Carl Roth GmbH + Co, Karlsruhe, Germany), 20 mM citric acid (Sigma Aldrich, Saint Louis, USA), 1 mM sodium octane sulfonic acid (Carl Roth GmbH + Co, Karlsruhe, Germany), 1 mM dibutylamine (Sigma Aldrich, Saint Louis, USA) and 0.1 mM Na\textsubscript{2}EDTA adjusted to pH 3.7 (mobile phase composition: 97 buffer / 3 methanol (v/v)). The sample concentration was expressed as ng monoamine /100 \(\mu\)l.

\textit{rTMS treatment}

The aHF-rTMS treatment was applied, under general anesthesia, twice (three weeks separated). The dog was muzzled, a cephalic catheter was placed and buthorphanol was IV administered (0.2 mg/kg; Dolorex\textregistered; Intervet Belgium NV). When sedated, the dog was given midazolam IV (0.2 mg/kg; Dormicum\textregistered; Roche Nederland B.V.). This was immediately followed by IV injection of propofol (1-2 mg/kg given to effect) to induce general anesthesia. Since it was chosen to set the stimulation intensity of the treatment based on the excitability of the motor cortex, the motor threshold (CMT) of the left motor cortex was determined. After the induction of the general anesthesia, the spot for determining the MT was identified as the cortical area that provoked the clearest muscular contraction in the right proximal front limb. Once the hotspot was identified, the machine output (Magstim Company Limited) was set at an intensity that provoked 100% of muscular twitches. This output was stepwise decreased with 5%, 2% and 1% until five out of 10 consecutive pulses induced a visible muscular twitch (Rossini et al., 2015).
Once the measurement of the CMT was finished, the center of the left frontal cortex, based on the topographical information from the neuronavigation, was identified with a marker on the fur. The center of a standard figure-of-eight coil was placed over the mark with the handle pointing abaxial. The applied aHF-rTMS protocol consists out of five sessions (frequency = 20Hz, intensity = 110% CMT). A waiting period of 12-15 minutes was set between two sessions. Each session held 40 trains (12 second intertrain interval) during 1.9 seconds each. In total 1560 pulses were given per session. This protocol is also used clinically and experimentally at our medical university hospital (Baeken et al., 2013; Baeken et al., 2015).

Behavioral assessment

After applying each aHF-rTMS treatment, the owner was asked to fill in the canine behavioral questionnaire and the questionnaire focusing on separation anxiety, noise phobia, reactivity and aggression. Since clinical improvement of an rTMS treatment is mostly seen within 2 to 6 weeks, the owner was asked to fill in the questionnaires 3 weeks after the last rTMS session was given (O'Reardon et al., 2007; Feffer et al., 2018).

Follow up

The owner noticed behavioral changes two to three weeks after the first stimulation session. The most prominent reported change was a reduction of the fixation onto persons, dogs or objects. Even more, a reduction in aggressive behavior towards unfamiliar dogs, humans and objects was noticed several weeks after the second aHF-rTMS protocol was administered. The owner reported that the dog did no longer show the urge to approach the person or target it had seen. This was in sharp contrast to its initial behavior. Even more, the dog stopped portraying avoidance
behavior when a human dropped food on the floor, when approached by another dog while eating or playing with a toy and when approached by a human or other dog when sleeping. The C-BARQ revealed a positive improvement of the dog’s aggressive behavior towards strangers and dogs (Table 1). The second questionnaire indicated a decrease in dog’s SAIR, AIR and reactivity (Table 1). No aversive effects were noticed.

At baseline, a significant lower rCBF at the left frontal cortex was noticeable (Z-score = -3.87). Twenty-four hours after the first aHF-rTMS treatment was applied, the rCBF of the left frontal did not differ any longer from the mean rCBF of the control group (Z-score = 0.04). At this time point, the patient’s subcortical region was significantly lower than the mean of the control group (Z-score = -6.97). Three weeks after the first aHF-rTMS treatment was applied, the rCBF left frontal cortex remained comparable the rCBF of the control group (Z-score = -0.58) (Table 2). Three weeks after the second stimulation session, no deviations in the rCBF were found when compared to the control group (Table 2).

The CSF showed mild increases in the concentration of 5-hydroxyindoleacetic acid (5-HIAA) 24 hours after the first aHF-rTMS treatment was given (Table 3). In both CSF and serum 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) increased 24 hours after the aHF-rTMS treatment (4 times baseline value). Three weeks after each treatment, the DOPAC and HVA concentrations decreased again to almost baseline value (Table 3).

**Discussion**

This study showed that an aHF-rTMS treatment applied over the left frontal cortex induced not only changes in the cerebral perfusion at the simulation site but at
remote locations as well. These changes lasted at least 3 weeks and were
accompanied by improvement of the dog’s behavior. Even more, simultaneous
changes of DOPAC concentrations in CSF and serum were observed.

The patient had, at baseline, a hypo-perfused left frontal cortex and showed anxious
aggressive behavior toward people and animals. Hereby confirming Vermeire et al.
(2009), who found a hypo-perfused frontal cortex in anxious dogs. The patient’s
impaired frontal perfusion might have led to a higher emotional state (anxious) and a
loss of drive inhibition (aggressive behavior) (Brutkowski, 1965; Dabrowska, 1971;
Konorski, 1973). After applying aHF-rTMS over the left frontal cortex, the patient’s
rCBF of the frontal cortex normalized, which was accompanied by an improvement of
his aggressive behavior. However, the patient’s subcortical region showed, at
baseline, no perfusion abnormalities. Nonetheless, directly after each stimulation
protocol, the perfusion of the subcortical region (including a large part of the emotion
steering limbic system) decreased significantly (Table 2). This remote effect of the
aHF-rTMS treatment confirms the connectivity between the frontal cortex and the
subcortical region. More specifically, a functional connectivity with the thalamus and
the basal ganglia since the subcortical VOI consisted of these regions. This was to be
expected due to the presence of reciprocal projection fibers between the frontal cortex
and the mediodorsal nucleus of the thalamus and between the thalamus and the basal
ganglia (Narkiewicz and Brutkowski, 1967; Hintzen et al., 2018). These local and
remote changes are in line with the findings in HF-rTMS research in treatment
resistant depression (Catafau et al., 2001; Loo et al., 2003; Knoch et al., 2006; Kito et
al., 2008).

More, rTMS has been shown to induce an endogenous increase of dopamine (DA) in
the striatum dorsal hippocampus, nucleus caudatus and nucleus accumbens (Strafella
et al., 2001; Keck et al., 2002; Pogarell et al., 2006), members of the limbic system. This study demonstrated that rTMS modulated the concentration of the DA metabolites HVA and DOPAC in CSF after each treatment. It has been reported that HVA concentrations in the CSF provide information of the DA turnover in the striatum (You et al., 1998; Kuhar et al., 1999). A reason for the absence of a change in DA concentration itself could be the fact that the detection capacity of the test is suboptimal or a low concentration of DA. In this study, serum and CSF were acquired 24 hours after the last stimulation session, whereas other studies assessed the changes in DA directly after the last stimulation session (Strafella et al., 2001; Keck et al., 2002; Strafella et al., 2003; Kanno et al., 2004; Pogarell et al., 2006). This, combined with the short half-life of DA (Yavich et al., 2007) and the fact that free DA is immediately broken down (MAO, COMT) could have led to the absence of DA changes in the serum and CSF. Nonetheless, since HVA and DOPAC are DA metabolites, an endogenous release of DA, after aHF-rTMS over the left frontal cortex in anxious aggressive dogs can be assumed. This assumption is strengthened by the fact that prefrontal DA also plays a role in the neuronal response to fear, anxiety and stress (Pezze and Feldon, 2004; Riva et al., 2008). Therefore, the neuromodulative action of aHF-rTMS over the left frontal cortex in canine anxious aggressiveness could be facilitated by a release of DA.

The increase of DOPAC in the serum could be twofold. The DOPAC concentration increased in the blood together with its concentration in the CSF coincided with changes in the rCBF and clinical improvement. Therefore, changes in the serum could represent the changes in the CSF. Subsequently, peripheral DA could serve as a valid biomarker to monitor the clinical response of an anxious aggregative patient to an aHF-rTMS treatment. Secondly, DA is also present outside the central nervous system.
(neuronal fibers, adrenal medulla and neuroendocrine cells) and its release is mostly regulated by the sympathetic noradrenergic nerves (Goldstein and Holmes, 2008; Rubi and Maechler, 2010). This combined with the fact that rTMS can influence the autonomic nervous system (Schestatsky et al., 2013), gives rise to the possibility that in this study the serum DOPAC concentration was elevated through activation of the autonomic nervous system. Nonetheless, one would assume a concurrent increase in NAD, which was not noted in this study.

A major limitation of this study is the number of included subjects and the absence of a sham-controlled group. Questionnaires, combined with functional and morphological imaging, were used to assess the patient’s behavior. It has to be kept in mind that in order to evaluate the behavioral improvement more precisely, regular consultations with a small animal behavioral specialist should be minimal requirement, this preferably in a blinded placebo controlled experiment.

To conclude, a single day aHF-rTMS treatment alters the local and remote rCBF and is accompanied by an improvement of the dog’s anxious aggressive behavior. An increase of the DOPAC concentration in the CSF and serum coincides with an improvement of the patient’s pathological behavior. Therefore, DOPAC may be a potential biomarker for treatment effects and also possibly for clinical improvement.

Acknowledgments

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We thank the division of veterinary nuclear medicine (department of medical imaging and small animal orthopedics, university of Ghent) to provide access to their infrastructure.

Ethical considerations
This study was approved by the Ghent University ethical committee (EC 2015-141; 02/03/2016) and the FOD ‘volksgezondheid, veiligheid van de voedselketen en leefmilieu’ (02/05/2016). Written consent was received from the owner. In addition to the approved protocol, the owner requested two diagnostic $[^{99m}Tc]HMPAO$ SPECT scans after the rTMS treatment was applied, this due to positive changes in the patient’s aggressive behavior.

Conflict of interest
The authors have no conflicts of interest relevant to the content of this study.
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Table 1

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1st rTMS treatment</th>
<th>2nd rTMS treatment</th>
</tr>
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<tr>
<td>Training and obedience</td>
<td>3.50</td>
<td>3.13</td>
<td>3.25</td>
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<tr>
<td>Owner-directed aggression</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Stranger-directed aggression</td>
<td><strong>3.20</strong></td>
<td><strong>2.70</strong></td>
<td><strong>2.80</strong></td>
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<td>Dog-directed aggression/fear</td>
<td><strong>2.63</strong></td>
<td><strong>2.13</strong></td>
<td><strong>2.25</strong></td>
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<tr>
<td>Familiar dog aggression</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Chasing</td>
<td>2.50</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Stranger-directed fear</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Nonsocial fear</td>
<td>0.67</td>
<td>0.83</td>
<td>0.17</td>
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<td>Separation-related problems</td>
<td>0.88</td>
<td>0.13</td>
<td>0.5</td>
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<tr>
<td>Touch sensitivity</td>
<td>0.50</td>
<td>0.75</td>
<td>0.75</td>
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<tr>
<td>Excitability</td>
<td>1.83</td>
<td>1.83</td>
<td>0.83</td>
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<tr>
<td>SAIR^A</td>
<td>2</td>
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</tr>
<tr>
<td>AIR^B</td>
<td>8</td>
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<td>2.5</td>
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<tr>
<td>Reactivity</td>
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<tr>
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<td>0.12</td>
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<tr>
<td>Intensity</td>
<td>4.33</td>
<td>3</td>
<td>4.75</td>
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</table>

Table 1: Results obtained by the both questionnaire. A decrease in stranger- and dog-directed aggression, SAIR, AIR, reactivity and severity was noticeable. A: SAIR = separation anxiety intensity ranks. B: AIR = anxiety intensity rank.
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>24 hours</th>
<th>3 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left temporal</td>
<td>-0.65</td>
<td>0.29</td>
<td>-1.63</td>
<td>-1.05</td>
</tr>
<tr>
<td>Right temporal</td>
<td>-1.00</td>
<td>0.40</td>
<td>-1.40</td>
<td>1.16</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-0.57</td>
<td>-0.79</td>
<td>-0.75</td>
<td>-1.70</td>
</tr>
<tr>
<td>Subcortical</td>
<td>0.24</td>
<td>-6.97*</td>
<td>0.54</td>
<td>-0.77</td>
</tr>
<tr>
<td>Bulbus olfactorius</td>
<td>0.56</td>
<td>0.88</td>
<td>0.21</td>
<td>1.26</td>
</tr>
<tr>
<td>Left frontal</td>
<td>-3.87*</td>
<td>0.04</td>
<td>-0.58</td>
<td>-1.86</td>
</tr>
<tr>
<td>Right frontal</td>
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<td>-0.55</td>
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<td>0.11</td>
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<td>2.95</td>
<td>0.82</td>
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<td>1.77</td>
<td>1.23</td>
<td>3.69*</td>
<td>2.14</td>
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* = |Z-value| > 3.06 (equivalent P-value of 0.001).
Table 3

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<th>Baseline</th>
<th>24 hours</th>
<th>3 weeks</th>
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<td>NAD</td>
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<tr>
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<td><strong>122.41</strong></td>
<td><strong>33.067</strong></td>
<td><strong>27.90</strong></td>
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<td>HVA</td>
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<td><strong>42.86</strong></td>
<td><strong>24.72</strong></td>
<td><strong>29.79</strong></td>
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<td>5-HT</td>
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<td>18.62</td>
<td>29.97</td>
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<tr>
<td>5-HIAA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td><strong>CSF</strong></td>
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<tr>
<td>DA</td>
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<td>Na</td>
<td>Na</td>
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<tr>
<td>DOPAC</td>
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<td><strong>0.25</strong></td>
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<td><strong>3.70</strong></td>
<td><strong>2.35</strong></td>
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Table 3: Concentrations (ng/100 µl) of the investigated monoamines and their metabolites measured with liquid chromatography – electrochemical detection. NAD = Norepinephrine; DA = Dopamine; DOPAC = 3,4-Dihydroxyphenylacetic acid; HVA = Homovanillic acid; 5-HT = Serotonin; 5-HIAA = 5-Hydroxyindoleacetic acid; ND = not detected; - = missing value due to with blood contaminated CSF.
• Accelerated high frequency repetitive transcranial magnetic stimulation (aHF-rTMS)
• aHF-rTMS over the left frontal cortex in an anxious aggressive dog induced local and remote changes in the regional cerebral perfusion
• The changes were long lasting and accompanied by improvement of aggressive behavior
• Increases in DOPAC concentrations in CSF and serum were observed after aHF-rTMS
The *Journal of Veterinary Behavior: Clinical Applications and Research* encourages submission of multi-author papers and those with acknowledgments that accurately reflect help received in the preparation of the manuscript or in the research and analysis.

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  - Submission of the same paper to more than one journal while a decision from another journal on that same paper is still pending
- Repetitive (Redundant) submission
  - Reporting the same results or methodologies in somewhat different form
- Improper authorship
  - Crediting individuals who did NOT provide a substantive contribution to the research and the analysis presented in the paper
  - Lack of credit to individuals who DID provide a substantive contribution
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We thank you for your understanding of and compliance with this necessary detail.

Sincerely,

Karen L. Overall
Editor-in-Chief

I am the senior author and understand and will comply with the above policy.

Name: Robrecht Dockx
Signature
Date: 24/09/2018

<table>
<thead>
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<th>e-mail addresses of coauthors:</th>
<th>e-mail addresses of those mentioned in the acknowledgments</th>
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