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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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Accepted Manuscript

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1 **Case study**

2

3 **Accelerated high-frequency repetitive transcranial magnetic stimulation**

4 **positively influences the behavior, monoaminergic system and cerebral perfusion**

5 **in anxious aggressive dogs: a case study**

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26 Accelerated high frequency repetitive transcranial magnetic stimulation (aHF-rTMS)

27 was proven to produce fast clinical effects in humans suffering from psychiatric

28 illnesses. Although dogs also frequently present behavioral symptoms similar to

29 mental illness, rTMS treatment was not yet investigated in this species. The aim of

30 this study was to apply an aHF-rTMS treatment over the frontal cortex in an anxious

31 aggressive dog. Since aHF-rTMS is used to treat anxiety and mood disorders in

32 humans and shows changes in neuronal activity and on monoamine concentrations, it

33 was hypothesized that the dog's behavior would improve after such a treatment. This

34 improvement was expected to be accompanied by alterations in regional cerebral

35 blood flow (rCBF) as well as in monoamine levels in CSF and serum. An aHF-rTMS

36 protocol was applied twice (3 weeks separated) over the left frontal cortex (5 sessions,

37 20Hz, 110% CMT) in a 5-year-old neutered male Belgian malinois dog showing

38 anxious aggressive behavior. Each protocol was preceded and followed by a behavior

39 assessment and a [^{99m}Tc]HMPAO-SPECT scan. A Z-score for each volume of interest

40 (VOI) at each time point was obtained, whereby a |Z|-score > 3.09 (P-value of 0.001)

41 indicated significant differences. Monoamines and their metabolites were quantified

42 in cerebrospinal fluid (CSF) and serum using liquid-chromatography coupled to

43 electrochemical detection. An improvement of the dog's aggressive behavior was

44 detected. At baseline, only a decreased rCBF of the left frontal cortex was noticeable

45 (Z-score = -3.87). Twenty-four hours after the first protocol, the perfusion in the left

46 frontal cortex was normalized and decreased in subcortical region (Z-score = -

47 6.97). Three weeks after each stimulation protocol, no deviations in the rCBF were

48 found. Parallel time-dependent changes of 3,4-dihydroxyphenylacetic acid (DOPAC)

49 concentrations in serum and CSF were observed. This case study demonstrates that a

50 single day aHF-rTMS treatment reduces a dog's anxious/aggressive behavior. This

51 behavioral change was accompanied by immediate and long-lasting alterations in the

52 rCBF and DOPAC concentration. This study confirms the interaction between the

53 frontal cortex and the subcortical region in behavior in dogs and puts DOPAC

54 forward as possible biomarker.

55

56 dog; rTMS; SPECT; dopamine; behavior

57

58 **Case presentation**

59   A 5-year-old neutered male white coated Malinois dog (32kg) was presented

60 with anxious aggressive behavior.

61

62 **History and presenting signs**

63   The patient is, together with his 16 siblings, the accidental offspring between

64 his half-brother and his mother. Neither parent showed behavioral problems. The

65 owner purchased the dog at the age of 8 weeks. During the first year of his life, the

66 dog received behavioral training and showed no signs of abnormal behavior. At the

67 age of 1, the dog started to show aggression combined with anxious behavior in

68 several situations (e.g., when meeting unfamiliar people or when separated from the

69 owner). The aggression started out mildly and evolved towards attempting to bite

70 people and dogs without apparent warning signs. Most of the aggressive behavior was

71 seen while walking with the dog. Indoors, no aggression was seen against its owners

72 or the other dog living in the house. Aggressive behavior was first seen towards other

73 dogs, followed by bikes and cars, and finally towards humans. Prior to his aggressive

74 behavior appearing no behavioral signs (snarling, barking, growling), with the

75 exception of fixation toward a close or distant person or object, are seen. Once fixated

76 onto the person or object, the patient would approach this target. The dog sometimes

77 barked at the object or people prior to its aggressive behavior, when he is in the

78 presence of the other dog. The dog also barked at people passing by the owner's

79 house. Two biting incidents took place, during which physical harm was caused to a

80 person. As a reaction towards his aggressive behavior, the owner trained the dog to

81 bite when asked. After that behavioral training, the dog has been able to release his

82 bite when asked by the owner. Besides this training, no treatments (behavioral or

83 pharmaceutical) were administered.

84

85

86 **Physical evaluation**

87 After thorough clinical examination, no clinical abnormalities were found. The

88 dog's blood analysis showed no deviations from the norm. A 3T MRI showed no

89 visible structural abnormalities in the dogs brain, whereas a functional

90 [^{99m}Tc]HMPAO-SPECT showed a decreased left frontal perfusion (when compared to

91 a control group). The control group consisted of 16 healthy dogs ranging from 1 to 8

92 years old.

93

94 **Behavioral evaluation**

95 The dog's behavior was assessed using the validated canine behavioral

96 questionnaire completed by the owner (Hsu and Serpell, 2003; Duffy and Serpell,

97 2012), providing information concerning the dog's behavior and temperament in 13

98 scales (Table 1). This questionnaire contains 101 questions grouped into seven

99 sections: training and obedience, aggression, fear and anxiety, separation-related

100 behavior, excitability, attachment and attention seeking and miscellaneous. The

101 responses to the questions were scored with a 5-point frequency scale or a 5-point

102 semantic differential scales. Based on table 1, the dog proved to be highly trained and

103 obedient and showed no aggressive behavior towards the owners or familiar dogs. On

104 the other hand, the dog scored high in the sections stranger-directed aggression and

105 dog-directed aggression/fear.

106

107   The owner was asked to complete a second questionnaire (see supplementary

108   files) that allowed insight into the dogs' reaction towards the owner's absence (actual

109   and virtual) and noises including (1) thunderstorm, (2) gunshots, (3) fireworks, and

110   (4) other noises (Overall et al., 2001; Overall, 2013; Overall et al., 2016; Scheifele et

111   al., 2016). Responses towards these questions were (1) yes, (2) no, (3) unknown. If

112   "yes" was answered, the owner was asked to estimate the frequency of the dog's

113   reaction towards the stimulus. The owner could choice among the frequencies (1)

114   100% of the time, (2) <100 % of the time but >60%, (3) 40% to 60% of the time, (4)

115   0 % of the time but <40%. In addition, the owner was asked to specify his dog's

116   reaction and frequency of exposure to each noise. Possible reactions were salivate,

117   hide, defecate, tremble, urinate, vocalize, destroy, pace, escape, freeze, will not eat

118   food, pupil dilation, and/or pant. Answers to the frequency of exposure were (1)

119   never, (2) occasionally/once a month, (3) regularly/ a few times a month, (4)

120   frequently/multiple times a week. Based on this section questions, a global separation

121   anxiety intensity rank (SAIR) and anxiety intensity rank (AIR) were calculated. These

122   were calculated based on the portrayed behavior multiplied by a weight of 4, 2.5, 1.5,

123   1 and 0 for the frequency. The maximum score for the SAIR and AIR was 48 and 208

124   respectively. The second questionnaire included an aggression screen to assess the

125   dog's reactivity, severity and intensity towards several stimuli (51). Reactivity was

126   defined as the proportion of stimuli to which the dog reacts compared to the total

127   number of listed stimuli. In order to calculate the severity, the owner was asked to tick

128   off his dog's response towards various stimuli. Possible reactions were (1) no

129   reaction, (2) snarl, (3) lip lift, (4) bark, (5) growl, (6) snap, (7) bite, (8) withdraw or

130   avoid and (9) not applicable. The specific behaviors received a weight factor of 1

131   (barking and growling), 2 (snarling and lip lifting) or 4 (snapping and biting). The

132 severity of the dog's behavior was calculated by dividing the total reaction score

133 (summation of all the weight factors) by total number of possible reactions towards all

134 stimuli (9x51). Intensity was obtained by dividing the total reaction score with the

135 total number of stimuli (51)

136

137 **Diagnosis**

138 Based on the dog's history, the physical examination, the MRI images, the

139 ^{99}Tc-HMPAO SPECT (d,1 hexamethylpropylene amine oxime single photon emission

140 computed tomography) scan and the questionnaires the dog was diagnosed with

141 anxious aggressive behavior specifically towards non-familiar people and animals.

142 **Treatment**

143 *Neuronavigation protocol*

144 Neuronavigation is a technique whereby three-dimension information about

145 neurological structures enclosed by the skull or the vertebral column is provided. This

146 study focuses on the non-invasive stimulation of the left frontal cortex. Therefore, the

147 left frontal cortex had to be externally located with neuronavigation. In order to obtain

148 this information, a tomographical dataset (MRI) had to be acquired. The

149 neuronavigation and external localization was performed as described by Dockx et al.

150 (2017).

151 *[^{99m}Tc]HMPAO-SPECT scan*

152 The patient underwent four [^{99m}Tc]HMPAO-SPECT scans. A baseline, one 24

153 hours and one three weeks after the last aHF-rTMS session was given. Two days after

154 the last SPECT scan the aHF-rTMS treatment was again applied over the left frontal

155 cortex. Three weeks after the last session of the second protocol, the patient received

156 another [^{99m}Tc]HMPAO-SPECT scan.

157   Twenty-four hours prior to each SPECT scan a ^{99}Mo generator was eluted and

158   approximately 1,85 GBq $^{99m}TcO_4$ was added to the exametazime

159   (hexamethylpropylene amine oxime (HMPAO); Ceretec®, GE Healthcare LTD, UK).

160   The dog was first muzzled and IM premedicated with dexmedetomidine ($375\mu g/m^2$

161   body surface). When sedated, an IV catheter was placed in a cephalic vein and on

162   average 357.27 MBq (SD = 54.62 MBq) of [^{99m}Tc]HMPAO was intravenously

163   injected. After 15-20 minutes, propofol was IV administered to induce general

164   anesthesia and was maintained with isoflurane in oxygen through a rebreathing

165   system. The dog was monitored for respiratory and electrocardiographic function

166   throughout the scan. A triple head gamma camera (Triad, Trionix, Twinsburg, OH,

167   USA), equipped with low energy ultrahigh-resolution parallel hole collimators

168   (tomographic resolution FWHM=9 mm), was used to acquire the data. The camera

169   collected data over a circular 360° rotation in a step-and-shoot mode during 20

170   minutes (120 steps, 10 sec per step, 3° steps) on a 128~128 matrix. Afterwards, the

171   data were iteratively reconstructed and a Butterworth filter (cut-off 1.4 cycli/cm, order

172   5) was applied.

173   After the acquisition of the images, a template containing 11 brain regions

174   (volumes of interest, VOIs) (both frontal, temporal, parietal and occipital lobes, the

175   cerebellum, olfactory bulb and the subcortical area) was fitted onto the dataset using

176   BRASS software (Brain Registration and Automated SPECT Semiquantification,

177   Nuclear diagnostics, Sweden). The regional cerebral blood flow (rCBF; perfusion

178   index (PI)) was semi-quantitatively obtained (normalization of the regional activity to

179   the radioactivity of the entire brain).

180   A Z-score was obtained for each VOI at each time point with SPECT images

181   of healthy dogs ranging from 1 to 8 years old (control group). The Z-score was

182 calculated using the following equation: Z-score = ([mean control group] – [value

183 patient])/ (standard deviation control group). The cut-off value was at $|Z| > 3.09$

184 (comparable to a *P*-value of 0.001) indicating significant differences in the rCBF

185 when compared to the control group.

186

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

188 Immediately following each [^{99m}Tc]HMPAO-SPECT scan, cerebrospinal fluid (CSF)

189 and serum were acquired. The CSF tap was performed at the cisterna magna using a

190 19 G needle after the patient was positioned in right lateral recumbency. While still

191 under anesthesia and right lateral recumbency, a 21G needle was used to draw blood

192 from the vena jugularis externa. An anti-oxidative mixture containing 0.1M perchloric

193 acid (Merck, Darmstadt, Germany), 0.05% Na$_2$EDTA (Sigma Aldrich, Saint Louis,

194 USA) and 0.05% sodium metabisulfite (Merck, Darmstadt, Germany) was made. 900

195 µl and 25 µl of this mixture were added to 100 µl serum and 100 µl CSF respectively.

196 The diluted samples were immediately frozen (-80 °Celsius) until further analysis.

197 Prior to the analysis, the samples were thawed and centrifuged at 15000 rpm for 15

198 minutes. The supernatant was transferred and diluted 1/2 (CSF) and 1/5 (serum) with

199 0.5 M acetic acid (Fisher scientific, Bishop meadow road, UK).

200 Total noradrenaline (NAD), dopamine (DA), 3,4-dihydroxyphenylacetic (DOPAC),

201 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), serotonin (5-HT)

202 and 5-hydroxyindoleacetic acid (5-HIAA) were measured in CSF and serum based on

203 previously reported methods (El Arfani et al., 2014; Jardi et al., 2018). The samples

204 were injected automatically on a reversed phase liquid chromatography system

205 (autosampler ASI-100 and HPLC pump P680 A HPG/2, Dionex, Amsterdam, The

206 Netherlands) with electrochemical detection (potential= + 700mV) (Amperometric

9

207 Detector LC-4C, BAS, Indiana, USA). The separation was achieved using a

208 narrowbore C18 column (Alltech®, Alltima™, 5 µm, 150 x 2.1mm, Grace, Deerfield,

209 IL, USA). The mobile phase buffer contained 0.1 M sodium acetate (Carl Roth GmbH

210 + Co, Karlsruhe, Germany), 20 mM citric acid (Sigma Aldrich, Saint Louis, USA), 1

211 mM sodium octane sulfonic acid (Carl Roth GmbH + Co, Karlsruhe, Germany), 1

212 mM dibutylamine (Sigma Aldrich, Saint Louis, USA) and 0.1 mM Na_2EDTA

213 adjusted to pH 3.7 (mobile phase composition: 97 buffer / 3 methanol (v/v)). The

214 sample concentration was expressed as ng monoamine /100 µl.

215

216   *rTMS treatment*

217   The aHF-rTMS treatment was applied, under general anesthesia, twice (three

218 weeks separated). The dog was muzzled, a cephalic catheter was placed and

219 buthorphanol was IV administered (0.2 mg/kg; Dolorex®; Intervet Belgium NV).

220 When sedated, the dog was given midazolam IV (0.2 mg/kg; Dormicum®; Roche

221 Nederland B.V.). This was immediately followed by IV injection of propofol (1-2

222 mg/kg given to effect) to induce general anesthesia. Since it was chosen to set the

223 stimulation intensity of the treatment based on the excitability of the motor cortex, the

224 motor threshold (CMT) of the left motor cortex was determined. After the induction

225 of the general anesthesia, the spot for determining the MT was identified as the

226 cortical area that provoked the clearest muscular contraction in the right proximal

227 front limb. Once the hotspot was identified, the machine output (Magstim Company

228 Limited) was set at an intensity that provoked 100% of muscular twitches. This output

229 was stepwise decreased with 5%, 2% and 1% until five out of 10 consecutive pulses

230 induced a visible muscular twitch (Rossini et al., 2015).

231   Once the measurement of the CMT was finished, the center of the left frontal

232   cortex, based on the topographical information from the neuronavigation, was

233   identified with a marker on the fur. The center of a standard figure-of-eight coil was

234   placed over the mark with the handle pointing abaxial. The applied aHF-rTMS

235   protocol consists out of five sessions (frequency = 20Hz, intensity = 110% CMT). A

236   waiting period of 12-15 minutes was set between two sessions. Each session held 40

237   trains (12 second intertrain interval) during 1.9 seconds each. In total 1560 pulses

238   were given per session. This protocol is also used clinically and experimentally at our

239   medical university hospital (Baeken et al., 2013; Baeken et al., 2015).

240   *Behavioral assessment*

241   After applying each aHF-rTMS treatment, the owner was asked to fill in the

242   canine behavioral questionnaire and the questionnaire focusing on separation anxiety,

243   noise phobia, reactivity and aggression. Since clinical improvement of an rTMS

244   treatment is mostly seen within 2 to 6 weeks, the owner was asked to fill in the

245   questionnaires 3 weeks after the last rTMS session was given (O'Reardon et al., 2007;

246   Feffer et al., 2018).

247

248   **Follow up**

249   The owner noticed behavioral changes two to three weeks after the first

250   stimulation session. The most prominent reported change was a reduction of the

251   fixation onto persons, dogs or objects. Even more, a reduction in aggressive behavior

252   towards unfamiliar dogs, humans and objects was noticed several weeks after the

253   second aHF-rTMS protocol was administered. The owner reported that the dog did no

254   longer show the urge to approach the person or target it had seen. This was in sharp

255   contrast to its initial behavior. Even more, the dog stopped portraying avoidance

11

256 behavior when a human dropped food on the floor, when approached by another dog

257 while eating or playing with a toy and when approached by a human or other dog

258 when sleeping. The C-BARQ revealed a positive improvement of the dog's

259 aggressive behavior towards strangers and dogs (Table 1). The second questionnaire

260 indicated a decrease in dog's SAIR, AIR and reactivity (Table 1). No aversive effects

261 were noticed.

262   At baseline, a significant lower rCBF at the left frontal cortex was noticeable

263 (Z-score = -3.87). Twenty-four hours after the first aHF-rTMS treatment was applied,

264 the rCBF of the left frontal did not differ any longer from the mean rCBF of the

265 control group (Z-score = 0.04). At this time point, the patient's subcortical region was

266 significantly lower than the mean of the control group (Z-score = -6.97). Three weeks

267 after the first aHF-rTMS treatment was applied, the rCBF left frontal cortex remained

268 comparable the rCBF of the control group (Z-score = -0.58) (Table 2). Three weeks

269 after the second stimulation session, no deviations in the rCBF were found when

270 compared to the control group (Table 2).

271

272   The CSF showed mild increases in the concentration of 5-hydroxyindoleacetic

273 acid (5-HIAA) 24 hours after the first aHF-rTMS treatment was given (Table 3). In

274 both CSF and serum 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid

275 (HVA) increased 24 hours after the aHF-rTMS treatment (4 times baseline value).

276 Three weeks after each treatment, the DOPAC and HVA concentrations decreased

277 again to almost baseline value (Table 3).

278 **Discussion**

279   This study showed that an aHF-rTMS treatment applied over the left frontal

280 cortex induced not only changes in the cerebral perfusion at the simulation site but at

281 remote locations as well. These changes lasted at least 3 weeks and were

282 accompanied by improvement of the dog's behavior. Even more, simultaneous

283 changes of DOPAC concentrations in CSF and serum were observed.

284 The patient had, at baseline, a hypo-perfused left frontal cortex and showed anxious

285 aggressive behavior toward people and animals. Hereby confirming Vermeire et al.

286 (2009), who found a hypo-perfused frontal cortex in anxious dogs. The patient's

287 impaired frontal perfusion might have led to a higher emotional state (anxious) and a

288 loss of drive inhibition (aggressive behavior) (Brutkowski, 1965; Dabrowska, 1971;

289 Konorski, 1973). After applying aHF-rTMS over the left frontal cortex, the patient's

290 rCBF of the frontal cortex normalized, which was accompanied by an improvement of

291 his aggressive behavior. However, the patient's subcortical region showed, at

292 baseline, no perfusion abnormalities. Nonetheless, directly after each stimulation

293 protocol, the perfusion of the subcortical region (including a large part of the emotion

294 steering limbic system) decreased significantly (Table 2). This remote effect of the

295 aHF-rTMS treatment confirms the connectivity between the frontal cortex and the

296 subcortical region. More specifically, a functional connectivity with the thalamus and

297 the basal ganglia since the subcortical VOI consisted of these regions. This was to be

298 expected due to the presence of reciprocal projection fibers between the frontal cortex

299 and the mediodorsal nucleus of the thalamus and between the thalamus and the basal

300 ganglia (Narkiewicz and Brutkowski, 1967; Hintzen et al., 2018). These local and

301 remote changes are in line with the findings in HF-rTMS research in treatment

302 resistant depression (Catafau et al., 2001; Loo et al., 2003; Knoch et al., 2006; Kito et

303 al., 2008).

304 More, rTMS has been shown to induce an endogenous increase of dopamine (DA) in

305 the striatum dorsal hippocampus, nucleus caudatus and nucleus accumbens (Strafella

306 et al., 2001; Keck et al., 2002; Pogarell et al., 2006), members of the limbic system.

307 This study demonstrated that rTMS modulated the concentration of the DA

308 metabolites HVA and DOPAC in CSF after each treatment. It has been reported that

309 HVA concentrations in the CSF provide information of the DA turnover in the

310 striatum (You et al., 1998; Kuhar et al., 1999). A reason for the absence of a change

311 in DA concentration itself could be the fact that the detection capacity of the test is

312 suboptimal or a low concentration of DA. In this study, serum and CSF were acquired

313 24 hours after the last stimulation session, whereas other studies assessed the changes

314 in DA directly after the last stimulation session (Strafella et al., 2001; Keck et al.,

315 2002; Strafella et al., 2003; Kanno et al., 2004; Pogarell et al., 2006). This, combined

316 with the short half-life of DA (Yavich et al., 2007) and the fact that free DA is

317 immediately broken down (MAO, COMT) could have led to the absence of DA

318 changes in the serum and CSF. Nonetheless, since HVA and DOPAC are DA

319 metabolites, an endogenous release of DA, after aHF-rTMS over the left frontal

320 cortex in anxious aggressive dogs can be assumed. This assumption is strengthened

321 by the fact that prefrontal DA also plays a role in the neuronal response to fear,

322 anxiety and stress (Pezze and Feldon, 2004; Riva et al., 2008). Therefore, the

323 neuromodulative action of aHF-rTMS over the left frontal cortex in canine anxious

324 aggressiveness could be facilitated by a release of DA.

325 The increase of DOPAC in the serum could be twofold. The DOPAC concentration

326 increased in the blood together with its concentration in the CSF coincided with

327 changes in the rCBF and clinical improvement. Therefore, changes in the serum could

328 represent the changes in the CSF. Subsequently, peripheral DA could serve as a valid

329 biomarker to monitor the clinical response of an anxious aggressive patient to an aHF-

330 rTMS treatment. Secondly, DA is also present outside the central nervous system

331 (neuronal fibers, adrenal medulla and neuroendocrine cells) and its release is mostly

332 regulated by the sympathetic noradrenergic nerves (Goldstein and Holmes, 2008;

333 Rubi and Maechler, 2010). This combined with the fact that rTMS can influence the

334 autonomic nervous system (Schestatsky et al., 2013), gives rise to the possibility that

335 in this study the serum DOPAC concentration was elevated through activation of the

336 autonomic nervous system. Nonetheless, one would assume a concurrent increase in

337 NAD, which was not noted in this study.

338 A major limitation of this study is the number of included subjects and the absence of

339 a sham-controlled group. Questionnaires, combined with functional and

340 morphological imaging, were used to assess the patient's behavior. It has to be kept in

341 mind that in order to evaluate the behavioral improvement more precisely, regular

342 consultations with a small animal behavioral specialist should be minimal

343 requirement, this preferably in a blinded placebo controlled experiment.

344 To conclude, a single day aHF-rTMS treatment alters the local and remote rCBF and

345 is accompanied by an improvement of the dog's anxious aggressive behavior. An

346 increase of the DOPAC concentration in the CSF and serum coincides with an

347 improvement of the patient's pathological behavior. Therefore, DOPAC may be a

348 potential biomarker for treatment effects and also possibly for clinical improvement.

349

356 We thank the division of veterinary nuclear medicine (department of medical imaging

357 and small animal orthopedics, university of Ghent) to provide access to their

358 infrastructure.

359

360 **Ethical considerations**

361 This study was approved by the Ghent University ethical committee (EC 2015-141;

362 02/03/2016) and the FOD 'volksgezondheid, veiligheid van de voedselketen en

363 leefmilieu' (02/05/2016). Written consent was received from the owner. In addition to

364 the approved protocol, the owner requested two diagnostic [^{99m}Tc]HMPAO SPECT

365 scans after the rTMS treatment was applied, this due to positive changes in the

366 patient's aggressive behavior.

367

368 **Conflict of interest**

369 The authors have no conflicts of interest relevant to the content of this study.

370

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542

543 **Table 1**

	Baseline	1st rTMS treatment	2nd rTMS treatment
Traning and obedience	3.50	3.13	3.25
Owner-directed aggression	0.00	0.00	0.00
Stranger-directed aggression	**3.20**	**2.70**	**2.80**
Dog-directed aggression/fear	**2.63**	**2.13**	**2.25**
Familiar dog aggression	0.00	0.00	0.00
Chasing	2.50	2.00	2.00
Stranger-directed fear	0.75	0.75	0.75
Nonsocial fear	0.67	0.83	0.17
Separation-related problems	0.88	0.13	0.5
Touch sensitivity	0.50	0.75	0.75
Excitability	1.83	1.83	0.83
SAIR[A]	**2**	**0**	**1**
AIR[B]	**8**	**0**	**2.5**
Reactivity	**0.33**	**0.18**	**0.24**
Severity	**0.11**	**0.06**	**0.12**
Intensity	4.33	3	4.75

544 **Table 1: Results obtained by the both questionnaire. A decrease in stranger- and dog-directed**
545 **aggression, SAIR, AIR, reactivity and severity was noticeable. A: SAIR = separation anxiety intensity**
546 **ranks. B: AIR = anxiety intensity rank.**

547

548   **Table 2**

	Baseline	*24 hours*	*3 weeks*	*6 weeks*
Left temporal	-0,65	0,29	-1,63	-1,05
Right temporal	-1,00	0,40	-1,40	1,16
Cerebellum	-0,57	-0,79	-0,75	-1,70
Subcortical	0,24	-6,97*	0,54	-0,77
Bulbus olfactorius	0,56	0,88	0,21	1,26
Left frontal	-3,87*	0,04	-0,58	-1,86
Right frontal	0,94	-0,55	0,17	0,17
Left occipital	0,68	0,40	0,03	-0,41
Right occipital	1,18	0,11	-0,70	-0,22
Left parietal	-0,39	0,34	2,95	0,82
Right parietal	1,77	1,23	3,69*	2,14

549   **Table 2: Z-values for each VOI at each time point. * = |Z-value| > 3.06 (equivalent *P*-value of 0.001).**

550

22

551 **Table 3**

		Baseline	*24 hours*	*3 weeks*	*6 weeks*
Serum					
	NAD	3.62	4.55	4.16	6.23
	DA[a]	ND	ND	ND	ND
	DOPAC	**26.39**	**122.41**	**33.067**	**27.90**
	HVA	**30.63**	**42.86**	**24.72**	**29.79**
	5-HT	21.59	18.62	29.97	25.17
	5-HIAA[a]	ND	ND	ND	ND
CSF					
	NAD	1.45	1.63	1.54	-
	DA[a]	Na	Na	Na	-
	DOPAC	**0.16**	**0.49**	**0.25**	-
	HVA	**12.57**	**19.40**	**12.70**	-
	5-HT	ND	ND	ND	-
	5-HIAA	**2.20**	**3.70**	**2.35**	-

552 Table 3: Concentrations (ng/100 µl) of the investigated monoamines and their metabolites
553 measured with liquid chromatography – electrochemical detection. NAD = Norepinephrine; DA =
554 Dopamine; DOPAC = 3,4-Dihydroxyphenylacetic acid; HVA = Homovanillic acid; 5-HT = Serotonin; 5-
555 HIAA = 5-Hydroxyindoleacetic acid; ND = not detected; -= missing value due to with blood
556 contaminated CSF.

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23

- 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

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