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Localisation of cannabinoid and cannabinoid-related receptors in the equine dorsal root ganglia

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1 **Localisation of cannabinoid and cannabinoid-related receptors in the equine dorsal root ganglia**

2

3 Masked for peer review

4

5 **Keywords:** CB1R, CB2R, PPAR α , TRPA1, 5-HT1a receptor

6

7 **Running head:** Cannabinoid receptors in horse sensory ganglia

8

9 **Abstract**

10 **BACKGROUND:** Growing evidence recognises cannabinoid receptors as potential therapeutic targets for
11 pain. Consequently, there is an increasing interest in developing cannabinoid receptor agonists for treating
12 pain. As a general rule, to better understand the actions of a drug, it would be of extreme importance to know
13 the cellular distribution of its specific receptors. The localisation of cannabinoid receptors in the dorsal root
14 ganglia of the horse has not yet been investigated.

15 **OBJECTIVES:** To localise the cellular distribution of canonical and putative cannabinoid receptors in the
16 equine cervical dorsal root ganglia (DRG).

17 **STUDY DESIGN:** Qualitative and quantitative immunohistochemical study.

18 **METHODS:** Cervical (C6-C8) DRG were collected from six horses (1.5 years of age) at the slaughterhouse.
19 The tissues were fixed and processed to obtain cryosections which were used to investigate the
20 immunoreactivity of canonical cannabinoid receptors 1 (CB1R) and 2 (CB2R), and for three putative
21 cannabinoid-related receptors: nuclear peroxisome proliferator-activated receptor alpha (PPAR α), transient
22 receptor potential ankyrin 1 (TRPA1) and serotonin 5-HT1a receptor (5-HT1aR).

23 **RESULTS:** The neurons showed immunoreactivity for CB1R (100%), CB2R (80 \pm 13%), PPAR α (100%),
24 TRPA1 (74 \pm 10%) and 5-HT1aR (84 \pm 6%). The neuronal satellite glial cells showed immunoreactivity for CB2R,
25 PPAR α , TRPA1 and 5-HT1aR.

26 **MAIN LIMITATIONS:** The number of horses included in the study.

27 **CONCLUSIONS:** The present study highlighted the expression of cannabinoid receptors in [the](#) sensory
28 neurons and glial cells of the DRG. These findings could be of particular relevance [for](#) future functional studies
29 assessing the effects of cannabinoids in horses to manage pain.

30

31 **Introduction**

32 The dorsal root ganglia (DRG) reside within the dorsal root of the spinal nerve and [are](#) mainly
33 constituted [of](#) a cluster of primary sensory neurons. Previous research [has](#) described the DRG as passive
34 neural structures [which](#) merely "supported" the physiological communication between the peripheral nervous
35 system and the central nervous system [1]. Nonetheless, novel evidence shows that [the](#) DRG neurons [play a](#)
36 critical role in carrying sensory messages from various receptors, including those for pain and temperature,
37 and transmitting them to the spinal cord [2].

38 The cell bodies of the DRG neurons are separated from each other by an envelope of satellite glial
39 cells (SGCs), which play important roles in both healthy and pathological states. Since [the](#) SGCs carry
40 receptors for numerous neuroactive agents, they have a plethora of roles including receiving signals from other
41 cells and influencing neighboring cells and other DRG neurons. These mechanisms likely influence [signal](#)
42 processing and transmission within the DRG [3] and possibly contribute [to](#) the sensitisation of pain
43 transmission nociceptors [4]. Altogether, this evidence suggests that the study of [the](#) DRG can have significant
44 clinical applications [for](#) pain modulation and novel targeted therapeutic treatment (Hogan, 2010) [5].

45 A growing body of literature demonstrates [that](#) cannabinoid receptors play a critical role in nociception
46 also through peripheral mechanisms (Calignano et al., 1998) [6]. Specifically, upon activation by medical
47 cannabis, beneficial effects have [been recorded on pain perception in humans](#) [7-9].

48 [Even though limited empirical research has been carried out concerning the use of medical marijuana for](#)
49 [pain treatment in domestic animals \[10\] and horses \[11\], the business of cannabis products for use in animals](#)
50 [is expanding \[12\]. Of the](#) cannabis products, cannabidiol (CBD), a non-psychoactive compound found in
51 *cannabis sativa*, seems to be one of the most promising therapeutic substances. Due to its numerous health-
52 related benefits, CBD has found multiple clinical applications in the medical field, including analgesic, anti-
53 inflammatory, anti-spasmodic and anti-anxiety uses [13, 14]. For many years, it was assumed that the
54 beneficial effects of the cannabinoids were mediated exclusively by cannabinoid receptors 1 (CB1R) and 2
55 (CB2R). However, it is currently known that phytocannabinoids may act on multiple targets outside the

56 endocannabinoid system, such as other G-protein coupled receptors (GPRs), the transient receptors potential
57 (TRPs) channel, nuclear peroxisome proliferator-activated receptors (PPARs), and serotonin receptors [15].
58 In particular, CBD, which shows indirect interaction with CB1R and CB2R, seems to be involved in the
59 modulation of receptors, such as the serotonergic 5-HT1a receptor (5-HT1aR), and the transient receptors
60 potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1), the latter two being excitatory ion channels expressed
61 by the sensory neurons mediating somatic and visceral pain [7].

62 As a general rule, to better understand the effects exerted by a drug, it would be of extreme importance to
63 know the cellular distribution of its specific receptors. Currently, only a small number of studies have been
64 published regarding the expression of cannabinoid receptors in the DRG of animals [16-19] whereas, to the
65 best of the Authors' knowledge, no analogous studies have yet been carried out involving horses.

66 Thus, the present study was designed to immunohistochemically localise, ~~in the equine DRG~~, two
67 canonical cannabinoid receptors (CB1R and CB2R) and three cannabinoid-related receptors (PPAR α , TRPA1,
68 and 5-HT1aR) in the equine DRG.

69

70 **Material and Methods**

71 ***Animals***

72 The cervical (C6-C8) DRG were collected from the left and the right halves of the carcasses of six
73 horses (4 males and 2 females) which were slaughtered for human food purposes. The horses (1.5 years of
74 age) were of two breeds, 3 Polish and 3 half-breeds. The complete cell blood count (CBC) and routine serum
75 biochemical analyses were carried out with blood samples taken at the time of exsanguination. The horses
76 were considered healthy on the basis of normal results of the CBC count and routine serum biochemical
77 analyses. In addition, the horses did not show lameness of either the thoracic or the pelvic limbs.

78 The tissues were fixed and processed to obtain cryosections, as described elsewhere [20].

79 Since the suppliers of the primary antibodies used in the present study stated that they were rat-
80 specific (CB2R, TRPA1, and 5-HT1aR) or reacted with rat tissues (CB1R, PPAR α), the experiments on the
81 equine DRG were carried out by including rat C6-C8 DRG (authorization no. 112/2018-PR of 12 February
82 2018) as positive controls (Supplementary Item 13). The distribution of the study receptors in the subclasses
83 of the rat sensory neurons was outside the scope of the present study and was therefore not evaluated.

84

85 ***Immunofluorescence***

86 The cryosections (14-16 μm thickness), were hydrated in phosphate-buffered saline (PBS) and
87 processed for immunostaining. To block non-specific bindings, the sections were incubated in a solution
88 containing 20% normal goat serum^a, 0.5% Triton X-100^b and bovine serum albumin (1%) in PBS for 1 h at
89 room temperature (RT) (22-25°C). The cryosections were incubated overnight in a humid chamber at RT with
90 the primary antibodies (Table 1) diluted in 1.8% NaCl in 0.01M PBS containing 0.1% sodium azide. After
91 washing in PBS (3 x 10 min), the sections were incubated for 1 h at RT in a humid chamber with the secondary
92 antibody [goat F(ab)₂ anti-rabbit FITC; ab98430]^c diluted in PBS. After washing in PBS (3 x 10 min), to identify
93 the DRG neurons and the SGCs and to determine the proportion of neurons immunoreactive for each of the
94 markers studied, the sections were counterstained with Blue fluorescent Nissl stain solution (NeuroTrace®, #
95 N-21479, dilution 1:200)^d. The cryosections were then washed in PBS (3 x 10 min) and mounted in buffered
96 glycerol at pH 8.6. A minimum of one hundred Nissl-stained neurons were counted for each marker. Data were
97 collected from preparations obtained from four animals ($n=4$). The percentages of immunopositive neurons
98 were expressed as mean \pm standard deviation.

99 ***Specificity of the primary antibodies***

100 The choice of the primary antibodies utilised in the study was based on the homology of the
101 aminoacidic sequence between the immunogen of the commercially available antisera and the horse proteins,
102 verified by the “alignment” tool available on the Uniprot database (www.uniprot.org) and the BLAST tool of the
103 National Center for Biotechnology information (NCBI) (www.ncbi.nlm.nih.gov). ~~Details are summarized in~~
104 ~~Supplementary item 1.~~

105 CB1R – The immunogen used to obtain antibody ab23703 was the synthetic peptide
106 MSVSTD TSAEAL, corresponding to carboxy-terminal amino acids 461-472 of Human cannabinoid receptor I.
107 The homology between the full amino acid sequences of Horse (F6SIU9) and Human (P21554) CB1R was
108 97.88%, and the correspondence with the specific sequence of the immunogen was 100%.

109 CB2R - The immunogen used to obtain antibody ab45942 was the synthetic peptide conjugated to
110 keyhole limpet haemocyanin (KLH) derived from within residues 200 - 300 of Rat cannabinoid receptor II. The
111 homology between the full amino acid sequences of Horse (F7CUS7) and Rat (Q9QZN9) CB2R was 80.9%,
112 and the correspondence with the specific sequence of the immunogen was 83.33%.

113 PPAR α - Antibody NB600-636 was prepared from whole rabbit serum produced by repeated
114 immunisations using a synthetic peptide corresponding to amino acids 1 to 18 of Mouse PPAR α . The homology
115 between the full aminoacidic sequences of Horse (F7DSM8) and Mouse (P23204) PPAR α was 90.81%, and
116 the correspondence with the specific sequence of the immunogen was 100%.

117 TRPA1 - The immunogen used to obtain antibody ab58844 was peptide EKQHELIKLIQKME
118 corresponding to amino acids 1060-1075 of Rat TRPA1. The homology between the full amino acid sequences
119 of Horse (F7DXW9) and Rat (Q6RI86) TRPA1 was 82%, and the correspondence with the specific sequence
120 of the immunogen was 100%.

121 Serotonin 5-HT1aR - The immunogen used to obtain antibody ab85615 was the synthetic peptide
122 corresponding to Rat 5HT1aR amino acids 100-200 conjugated to keyhole limpet hemocyanin. The homology
123 between the full amino acid sequences of Horse (Q0EAB6) and Rat (P19327) 5HT1aR was 89.3%, and the
124 correspondence with the specific sequence of the immunogen was 99%.

125 In addition, the specificity of the primary antibodies employed was also tested using Western blot (Wb)
126 analysis.

127 **Specificity of the secondary antibody**

128 The secondary antibody specificity was tested by the lack of signal after omission of the primary antibody on
129 DRG tissues

130 **Fluorescence microscopy**

131 The preparations were analysed by the same observer, and the images were recorded and adjusted
132 as described elsewhere [21].

133 **Western blot: specificity of the primary antibodies**

134 Cervical (C-6-C8) DRG and spinal cord samples were collected, frozen in liquid nitrogen, and stored
135 at - 80°C until sample processing. The primary antibodies were tested in our laboratories according to
136 standardized protocols [21]. The Wb analysis of CB1R (1:500) revealed double bands of ~ 100 and 120 kDa
137 (the theoretical molecular weight of CB1R is 53-60 kDa) (Fig. 1 aSupplementary item 2a), that of CB2R
138 (1:1000) revealed a single band of ~ 90 kDa (the theoretical molecular weight of CB2R is 45 kDa) (Fig. 1
139 bSupplementary item 2b), that of PPAR α (1:2000) revealed a single band of ~ 50 kDa (the theoretical molecular
140 weight of PPAR α is 52 kDa) (Fig. 1 cSupplementary item 2c), that of TRPA1 (1:500) revealed a major band at

141 ~ 100 kDa (the theoretical molecular weight 100 kDa) (Fig. 1 dSupplementary item 2d) and that of the 5-HT1aR
142 (1:3000) revealed a major band at ~ 50 kDa (theoretical molecular weight 46 kDa) (Fig. 1 eSupplementary
143 item 2e). Western blot analysis confirmed the specificity of the anti-PPAR α ,-TRPA1 and-5-HT1aR primary
144 antibodies; the latter finding is consistent with the 100% homology of the amino acid sequence between the
145 immunogen of the antisera and the horse proteins (see above). The CB1R (100% homology) and CB2R (83%
146 homology) showed bands with apparent double molecular weights; it is known that G-protein receptors, such
147 as CB1R, may show dimerisation, and form functionally active homodimer and heterodimer complexes, and
148 that polyclonal antibodies directed against the carboxy-terminal may only recognise the high-molecular weight
149 form of CB1R [22]. ~~We have also observed~~An apparent dimeric form for the other G-protein receptor CB2R
150 was also observed; however, no studies regarding the dimeric form of this receptor are available in the
151 literature. Nevertheless, ~~it is not excluded~~the possibility that the anti-CB2R antibody may recognize only the
152 band in which the receptor is conjugated to another CB2R (or to another protein), as observed for the anti-
153 CB1R antibody, cannot be excluded.

154

155 **Results**

156 **CB1R**

157 Bright cytoplasmic and nucleolar CB1R immunoreactivity (CB1R-IR) was displayed, with different
158 degrees of intensity, by 100% of the neurons (604/604 cells counted, $n=4$) (Fig. 24 a-c); no distinction of CB1R
159 immunolabelling was observed among neurons of different sizes. The nerve processes also showed CB1R-
160 IR, although it was fainter than that observed in the neuronal somata. The SGCs also showed faint CB1R-
161 immunolabeling. This finding is partially consistent with what has been observed in the rat DRG in which
162 neurons and SGCs expressed faint cytoplasmic and nuclear CB1R-IR [19].

163

164 **CB2R**

165 The CB2R-IR was expressed by $80\pm 13\%$ of the neurons (665/799 cells counted, $n=4$) and by the
166 SGCs. In general, there was an inverse correlation between the brightness of CB2R-IR in the neurons and
167 SGCs; small-to-medium-sized neurons showed brighter granular CB2R cytoplasmic immunolabeling in

168 comparison with larger ones which were encircled by brightly labeled SGCs (Fig. 24 d-i). In the rat DRG, CB2R-
169 IR was expressed by the neuronal nuclei [19].

170

171 **TRPA1**

172 The TRPA1-IR was expressed by the cytoplasm (moderate staining) and nucleus (bright staining) of
173 both the neurons ($74\pm 10\%$; 497/698 cells counted, $n=4$) and the SGCs, and by the nerve processes (Fig. 32
174 a-c). Of the nerve processes, thin and unmyelinated nerve fibres showed brighter TRPA1 immunolabelling
175 than large myelinated nerve fibres. This finding is partially consistent with what has been observed in the rat
176 DRG in which only the neuronal cytoplasm expressed TRPA1-IR (Supplementary item 13 a-c).

177

178 **PPAR α**

179 Faint cytoplasmic PPAR α -IR was displayed by all the neurons (100%; 456/456 cells counted, $n=4$)
180 whereas it was brightly displayed by the cytoplasm of the SGCs (Fig. 32 d-fg-i). The PPAR α -IR was also
181 expressed by the endothelial cells of the blood vessels (data not shown). This finding is partially consistent
182 with what has been observed in the rat DRG in which neurons and SGCs expressed PPAR α -IR [19].

183

184 **5-HT1aR**

185 Moderate cytoplasmic 5-HT1aR-IR was expressed by $84\pm 6\%$ of the neurons (462/547 cells counted,
186 $n=4$); faint to moderate 5-HT1aR-IR was also expressed by the SGCs (Fig. 3 g-i) and the Schwann cells (Fig.
187 3 j-l). This finding is partially consistent with what has been observed in the rat DRG in which only the neuronal
188 cytoplasm expressed 5-HT1aR-IR (Supplementary item 13 d-f).

189 The results of the cellular distribution and intensity of the immunolabeling in the equine DRG are
190 summarised in semiquantitative Table 2.

191

192 **Discussion**

193 This is the first study aimed at investigating the expression and localisation of cannabinoid receptors
194 (CB1R and CB2R) and cannabinoid-related receptors (PPARalpha, TRPA1, 5-HT1aR) in the equine DRG.

195 Cannabinoid CB1R is widely expressed throughout the nociceptive system and its activation by
196 endogenous or exogenous cannabinoids modulates neurotransmitter release. Previous studies carried out on
197 humans and rodents have shown CB1R-IR expression limited to the sensory neurons [16,19,23] whereas the
198 present study showed that CB1R-IR is localised in both the equine sensory neurons and SGCs in line with
199 data recently reported for dogs [18,19].

200 In humans, the CB2R-IR has been localised exclusively in nociceptive DRG neurons [23]. In the
201 present study, CB2R-IR was observed either in the equine DRG neurons and SGCs, as previously observed
202 in rats [17]. The CB2R-IR has recently been observed in the neuronal nuclei of rats and dogs as well as in the
203 Schwann cells of dogs [19]. It has been shown that the application of CB2R agonists functionally inhibits
204 nociceptive signalling in human DRG neurons [24]. Based on the Authors' findings, it can be hypothesised
205 that, in horses, the same agonists could be useful for the peripheral modulation and treatment of painful
206 sensation [24].

207 ~~Beside of~~In addition to canonical cannabinoid receptors, multiple targets can be influenced by
208 phytocannabinoids unravelling the complexity of this endocannabinoid system. For instance, PPARs belong
209 to the family of intranuclear receptors which act as transcription factors, modulating different physiological
210 functions. Once activated by their ligand, PPARs induce the expression of hundreds of genes in each cell type
211 [25]. However, their activation has also been shown to result in rapid cellular changes which do not require
212 transcription, including reduction of inflammation [26, 27]. Recent studies have shown that cannabinoids
213 activated PPARs [15, 27, 28], and that this activation is associated with some of the pain-relieving, anti-
214 inflammatory and neuroprotective properties of cannabinoids.

215 In the present study, PPARα-IR expression was observed in both the sensory neurons and the SGCs
216 as has previously been shown in rats [19] whereas ~~in mice and dogs~~, PPARα was detected exclusively in the
217 neurons or in the SGCs, respectively in mice and dogs [19, 29]. ~~In the present study~~, PPARα-IR was also
218 observed in the endothelial cells of the DRG blood vessels. Recent evidence has shown that
219 endocannabinoids exert a pro-homeostatic function on vascular biology by means of complex mechanisms
220 involving canonical as well as putative cannabinoid receptors [30]. Specifically, different cannabinoid receptors

221 can actively influence vasodilation at different cellular sites, i.e., nerves, endothelial cells, vascular smooth
222 muscle cells or pericytes [31].

223 The TRPA1 is required for normal mechano- and chemo-sensory functions in specific subsets of vagal,
224 splanchnic and pelvic afferents [32]. The TRPA1 also mediates somatic and visceral pain in response to
225 stimulation of a chemical, mechanical or thermal origin [33, 34], and can be desensitised by different
226 mechanisms [35]. Data from the present study demonstrated that TRPA1 was expressed by the sensory
227 neurons and by the SGCs, with bright immunolabelling in thin unmyelinated nerve fibres. This is partially in
228 line with previous studies carried out on humans and rodents showing that the TRPA1-IR sensory neurons are
229 involved in nociception [36]. As a consequence, the analgesic effects of CBD might be due, in part, to its ability
230 to activate and desensitise the TRPA1 [37].

231 Another finding which emerged from this study was the expression of 5-HT1aR in sensory neurons,
232 SGCs and Schwann cells. It is well established that 5-HT exerts a pivotal role in sensory information processing
233 [38]. At the level of the spinal cord, 5-HT is primarily released by the descending bulbospinal serotonergic
234 neurons and causes analgesia by inhibiting dorsal horn neuronal responses to noxious stimuli by means of
235 the activation of the 5-HT1aR [39]. In addition, the activation of the 5-HT1aR inhibits glutamate release from
236 the sensory neurons, reducing pain transmission [40]. Functional studies have suggested the presence of the
237 5-HT1aR in the DRG and its role in nociception [41]. In the present study, the 5-HT1aR-IR was observed in
238 the sensory neurons and the SGCs of horses, and in the neurons of rats. Therefore, it is plausible to consider
239 that the 5-HT1aR might play a role in pain modulation. We-The Authors also observed the 5-HT1aR in Schwann
240 cells, a finding which is consistent with the expression of other serotonergic receptors on Schwann cells in
241 rodents [42].

242 In summary, this study showed a notable distribution of cannabinoid and cannabinoid-related receptors
243 in the equine DRG.

244 The reduced number of animals and ganglia considered, as well as the specificity of the anti-CB2R
245 antiserum, represent the limitations of the present study. Additional biomolecular studies as well as the
246 neurochemical characterisation of the immunoreactive DRG neurons are needed to reinforce the data in the
247 present. Nonetheless, these findings represent novel insight regarding the complex processing of nociceptive
248 input in the equine peripheral nervous system and will hopefully encourage new studies regarding the role of
249 cannabinoid-related receptors in the equine DRG and their interactions with non-psychotic cannabis-derived

250 molecules. In addition, cannabinoids, specifically CBD, has been proposed as an opioid alternative in human
251 medicine, having a comparable efficacy with a better safety profile [43].

252 Therefore, considering the present results, it would be appropriate to also test them in horses with
253 preclinical and clinical studies to verify safety, dosages, the kinetics of the substance and whether similar
254 beneficial effects also occur in equines with the possible presence or absence of side effects.

255

256 **Conclusions and relevance**

257 Cannabinoid and cannabinoid-related receptors had a wide distribution in the sensory neurons and
258 SGCs of the equine DRG. -These findings represented an important anatomical basis upon which it would be
259 possible to continue with other preclinical and clinical studies aimed at investigating and possibly supporting
260 the specific therapeutic uses of non-psychotropic cannabinoid agonists against noxious stimulation in horses.

261

262 **Manufacturers' addresses**

263 ^a Colorado Serum Co., Denver, CO, USA

264 ^b Sigma Aldrich, Milan, Italy, Europe

265 ^c abcam, Cambridge, UK

266 ^d Molecular Probes, Eugene, OR

267

268

269 **Figure legends**

270 **Fig. 1:** Western blot (WB) analysis showing the specificity of the following primary antibodies utilised: a) rabbit
271 anti-cannabinoid receptor 1 (CB1); b) rabbit anti-cannabinoid receptor 2 (CB2); c) rabbit anti-nuclear
272 peroxisome proliferator-activated receptor alpha (PPAR α); d) rabbit anti-transient receptor potential ankyrin 1
273 (TRPA1) and e) rabbit anti-serotonin receptor 5-HT1a (5-HT1a).

274 The antibody anti-CB1 receptor showed double bands of ~ 100 and 120 kDa (the theoretical molecular
275 weight of the CB1 receptor is 53-60 kDa), more evident in the spinal cord. The antibody anti-CB2 receptor

276 showed a double band (only in the DRG) of ~ 90 kDa (the theoretical molecular weight of the CB2 receptor is
277 45 kDa). The antibody anti-PPAR α showed a single band of ~ 50 kDa (the theoretical molecular weight of
278 PPAR α is 52 kDa). The antibody anti-TRPA1 showed a major band at ~ 100 kDa (theoretical molecular weight
279 100 kDa). The antibody anti-5-HT1A receptor showed a double band (only in the spinal cord) at ~ 50 kDa
280 (theoretical molecular weight 46 kDa). The numbers on the DRG and the spinal cord lines indicate the
281 molecular weights. The images of the immunoblots were slightly adjusted in brightness and contrast to match
282 their backgrounds.

283

284 **Fig. 24:** Photomicrographs of cryosections of an equine cervical (C8) dorsal root ganglion showing
285 cannabinoid receptor 1 (CB1R) (a-c) and cannabinoid receptor 2 (CB2R) (d-i) immunoreactivity (IR). a-c)
286 CB1R-IR was expressed by the sensory neurons whereas the satellite glial cells, the nuclei of which are
287 indicated by arrows, were CB1R-negative. d-i) The arrows indicate the satellite glial cells which were CB2R
288 immunoreactive. Sensory neurons, in particular the smallest ones, showed very faint granular CB2R
289 immunolabelling.

290 Scale bar = 50 μ m

291

292 **Fig. 32.** Photomicrographs of cryosections of an equine cervical (C8) dorsal root ganglion showing TRPA1-
293 (a-c), TRPV1 (d-f), PPAR α - (d-fg-i) and 5-HT1a receptor- (g-i) PPAR γ - (j-l) immunoreactivity (IR). a-c) Sensory
294 neurons and satellite glial cells (small arrows) expressed TRPA1-IR. Large arrows indicate groups of myelinated
295 sensory fibres which showed very bright TRPA1-IR whereas the nerve fibres with a larger diameter (stars)
296 showed weaker immunostaining. d-e) Only the sensory neurons were TRPV1 immunoreactive whereas the
297 satellite glial cells (arrows) were TRPV1 negative. d-fg-i) Weak PPAR α -IR was expressed by the cytoplasm of
298 all the neurons whereas the satellite glial cells (arrows) showed bright PPAR α -IR. j-l) Bright PPAR γ -IR was
299 expressed by neuronal nuclei (large arrows) whereas the nuclei of the glial cells (small arrows) showed fainter
300 immunolabelling. g-i) Sensory neurons expressed bright 5-HT1a receptor-IR; satellite glial cells (arrows) also
301 showed moderate 5-HT1a receptor-IR. j-l) The Schwann cells (arrows) showed 5-HT1a receptor-IR.

302 Scale bar = 50 μ m

303

304 **Fig. 3.** Photomicrographs of cryosections of an equine cervical (C8) dorsal root ganglion showing GPR3 (a-
305 e), GPR55 (d-f) and 5-HT1a receptor (d-i) immunoreactivity (IR). a-c) The three arrows indicate the sensory
306 neurons expressing weak GPR3-IR. The star indicates the nucleus of one large sensory neuron expressing
307 moderate GPR3-IR. d-f) The white stars indicate the sensory neurons expressing bright GPR55-IR whereas
308 the open stars indicate the neurons with weaker immunolabeling. The arrows indicate some SGCs showing
309 GPR55-IR. g-i) Sensory neurons expressed bright 5-HT1a receptor-IR; satellite glial cells (arrows) also showed
310 moderate 5-HT1a receptor-IR. j-l) The Schwann cells (arrows) showed 5-HT1a receptor-IR.

311 Scale bar = 50 μ m

312

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419 **Supplementary Information Items**

420 ~~Supplementary item 1—Homology of the aminoacidic sequence between the immunogen of the commercially~~
421 ~~antisera employed in the study and the horse proteins.~~

422 ~~Supplementary item 2—Specificity of the primary antibodies tested with Western blot analysis.~~

423 Supplementary item 13 – Immunohistochemistry on rat dorsal root ganglia (positive control).

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