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1 Host behavior alteration by its parasite: from brain gene expression to
2 functional test

3

4 Running head: Brain transcriptome of parasitized host

5

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15 ABSTRACT

16 Many parasites with complex life cycles modify their intermediate hosts'
17 behaviour, presumably to increase transmission to their final host. The threespine
18 stickleback (*Gasterosteus aculeatus*) is an intermediate host in the cestode
19 *Schistocephalus solidus* life cycle, which ends in an avian host, and shows
20 increased risky behaviours when infected. We studied brain gene expression
21 profiles of sticklebacks infected with *S.solidus* to determine the proximal causes
22 of these behavioural alterations. We show that infected fish have altered
23 expression levels in genes involved in the inositol pathway. We thus tested the
24 functional implication of this pathway and successfully rescued normal
25 behaviours in infected sticklebacks using lithium exposure. We also show that
26 exposed but uninfected fish have a distinct gene expression profile from both
27 infected fish and control individuals, allowing us to separate gene activity related
28 to parasite exposure from consequences of a successful infection. Finally, we
29 find that Selective Serotonin Reuptake Inhibitor (SSRI)-treated sticklebacks and
30 infected fish do not have similarly altered gene expression, despite their
31 comparable behaviours, suggesting that the serotonin pathway is probably not
32 the main driver of phenotypic changes in infected sticklebacks. Taken together,
33 our results allow us to predict that if *S.solidus* directly manipulates its host, it
34 could target the inositol pathway.

35 Keywords: gene expression, stickleback, parasite, *S. solidus*, brain, fluoxetine,
36 behaviour, IMPase1, inositol, lithium.

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39 **Introduction**

40 Many parasites go through complex life cycles and as they do so alter various
41 aspects of their host's biology, including morphology, physiology, life history, and
42 behaviour [1]. These phenotypic changes can decrease the host's fitness and
43 have been proposed to increase the probability of completion of the parasite's life
44 cycle [2, 3], although in many cases experimental evidence is still needed [1].
45 Host behaviour manipulations can range from slight changes in pre-existing traits
46 to the display of entirely novel behaviours [4]. A striking example is provided by
47 the threespine stickleback (*Gasterosteus aculeatus*) and its tapeworm parasite,
48 *Schistocephalus solidus*. The parasite has three hosts: a copepod, a fish
49 (specifically the threespine stickleback), and a fish-eating bird, the definitive host
50 [5-7]. The presence of *S. solidus* in the body cavity of its host has been reported
51 to have multiple effects on stickleback physiology, including increased oxygen
52 consumption [8], reduced gonad development [9], and decreased energy
53 reserves [10]. Infected sticklebacks lose their anti-predator response and forage
54 under the risk of predation [11-13]. They spend less time swimming within a
55 group than healthy conspecifics [14], tend to swim away from cover, a sign of
56 lower anxiety [18] and tend to swim close to the surface [15] even during the day,
57 which is rarely seen in healthy conspecifics [16].

58

59 Characterizing the mechanistic basis of the interaction between the parasite and
60 its host in the context of behavioural change requires three steps. The first one is
61 to uncover which molecular pathways are altered in parasitized hosts. The
62 second step is to use experimental manipulations to single out which molecular
63 changes are the cause of the changes in behaviour [17, 18]. After confirming the
64 causal role of a molecular pathway in behaviour variation, the third step is to
65 determine which one of these pathways, if any, is directly manipulated by the
66 parasite [19]. Here we present data in the stickleback-*S. solidus* host-parasite
67 system, obtained during experiments pertaining to the two first steps. Despite a

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68 rich literature describing altered host phenotypes, there is comparatively less
69 information on the proximate pathways involved in drastic behavioural changes in
70 most host-parasite pairs [20, 21], including in the stickleback-*S. solidus* system
71 [7]. We can predict that these mechanistic bases include interconnected levels of
72 biological organization: neural circuits, neuroendocrine regulation (potentially
73 including the serotonergic axis [22], see below), gene expression changes, and
74 epigenetic regulation [23]. Because of the multidimensional nature of phenotypic
75 changes in the parasitized sticklebacks that include several types of behaviours,
76 but also physiology [24, 25] and immunity [26, 27], an assumption-free whole-
77 genome approach to characterize gene expression changes in the brain is
78 optimal [28-30]. We can also expect that the host responds to infection [26, 31]
79 and that this will be reflected in the gene expression profiles, as found in innate
80 and adaptive immune system genes of the stickleback's head kidney [32]. The
81 stickleback-*S. solidus* host-parasite system is an excellent model to study the
82 genomic signature of parasitic infection in the host brain, i.e. a group of genes
83 with a characteristic pattern of expression that occurs as a result of a biological
84 process [33-35]. Sticklebacks can be experimentally infected, allowing the control
85 of other environmental variables that could affect control and infected fish [36].

86

87 Changes in gene expression of an infected host compared to a non-infected
88 conspecific might be functionally associated with the behavioural changes
89 observed but could also merely be the consequence of being exposed to a
90 parasite. An important question thus arises: do individuals exposed to a parasite
91 that did not become infected have a similar brain expression profile to control
92 individuals, to infected hosts, or is it unique? Since not all stickleback exposed to
93 *S. solidus* become infected [7], it is possible to also study gene expression
94 profiles of these individuals. While it has been shown that head kidney gene
95 expression patterns do not differ between control and exposed sticklebacks [32],
96 there are no available studies on the brain genomic signatures of exposed
97 individuals to test these contrasting predictions.

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98

99 One approach to address the question of differences in brain genomic signatures
100 is through the study of the host's serotonergic neuroendocrinological pathway,
101 which may be modified indirectly or directly by behaviour-altering parasites.
102 Studies in various systems have shown changes in candidate molecules such as
103 biogenic amines in parasitized individuals (insects: [37, 38], crustaceans: [39],
104 fish: [40]). In stickleback, serotonin activity is higher in *S. solidus*-parasitized wild-
105 caught female sticklebacks compared to healthy females, which has been
106 attributed to the stress of being parasitized [22]. Furthermore, experimental
107 pharmacological manipulation of biogenic amines such as the serotonergic axis
108 in healthy individuals results in behavioural changes typical of infected host (in
109 crustaceans, [17]). In sticklebacks, Selective Serotonin Reuptake Inhibitor
110 (SSRI)-treated non-infected sticklebacks show similar behaviours to *S. solidus*-
111 infected individuals, with a lower tendency to school with conspecifics, and more
112 time spent at the surface, although only in some individuals, while anti-predator
113 response is not affected [18]. Since behavioural changes in parasitized
114 individuals overlap in part with the ones measured in SSRI-treated individuals,
115 they could exhibit similar activity of certain molecular pathways, which can be
116 quantified by comparing their brain gene expression profiles.

117

118 Here, we investigated genome-wide brain gene expression patterns of
119 sticklebacks from four treatments using RNA-seq: healthy controls, infected by *S.*
120 *solidus,* exposed to a *S. solidus* parasite but not infected, and SSRI-treated. First,
121 we analysed the transcriptome of *S. solidus*-infected stickleback. We predicted
122 that they would show changes in expression of genes related to the
123 multidimensional phenotypic changes they exhibit: behaviour, physiological
124 systems and host response to infection. We then performed a follow-up
125 experiment using a pharmacological manipulation, to test the behavioural effects
126 of manipulating a candidate molecule found to be highly expressed in the brain of

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127 infected sticklebacks. Second, we included exposed individuals in which worms
128 did not develop. We predicted that exposed fish would have a gene expression
129 pattern mostly related to the host response to infection, which would match a
130 subset of the expression profile of a successfully infected stickleback. Finally, we
131 used individuals treated with the SSRI fluoxetine. We predicted that if *S. solidus*
132 affects the same molecular pathways as the SSRI, we would detect a high
133 overlap when comparing brain gene expression profiles of SSRI-treated versus
134 infected fish.

135

136 **Materials and Methods**

137 **Exposure of fish host to its parasite or SSRI**

138 Sticklebacks from Llyn Frongoch (UK) were bred and their offspring reared in the
139 laboratory for six months (see [18] and supplementary material). In summary, we
140 created four treatment groups. We exposed individuals to *S.solidus*-infected
141 copepods (see [18] for infection techniques) and waited three months for parasite
142 growth. This treatment resulted in two groups: infected (fish with a parasite) and
143 exposed fish (fish without a parasite). It was not possible to distinguish the
144 exposed and infected individuals prior to dissection. We also exposed individuals
145 to the SSRI fluoxetine for three days at a dose of 1mg / L (Fluoxetine HCl, BML-
146 NS140, Enzo Life Sciences Inc., USA), known to result in behavioural changes
147 similar to those induced by the presence of *S. solidus* (see [18] for details).
148 Control fish that were never exposed to a parasite were kept in the same
149 conditions in parallel. Before fish were euthanized, they were screened for
150 ecologically-relevant behaviours, but the small sample size for infected fish (n=3)
151 prevented meaningful statistical analyses (see [18]). The parasites found in the
152 infected sticklebacks were confirmed to be in the infective stage using their
153 transcriptome profiles [18].

154

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155 **Gene expression quantification by RNA-seq**

156 Fish were euthanized following authorised protocol and dissected brains were
157 kept in RNALater (Ambion Inc., Austin, TX, USA). We extracted total RNA from
158 the brains of three infected, six exposed, six SSRI-treated, and six control fish (all
159 females) using a standard Trizol reagent protocol (miRNeasy Micro kit, Qiagen)
160 and stored at -80°C after verifying concentration and quality by
161 spectrophotometer (Nanodrop, Thermo scientifics) and a Bioanalyzer (RNA 6000
162 Nano Kit, Agilent Technologies Inc). We produced libraries for these 21
163 individuals using the TruSeq RNA Library Prep Kit v2 (Illumina, Inc., USA) with a
164 unique barcode for each library. Library quality and size was assessed on a
165 Bioanalyzer High Sensitivity DNA Assay (Agilent Technologies). The 21 cDNA
166 libraries were then pooled and sequenced (Illumina HiSeq 2000). See
167 supplementary material for details.

168

169 **Analysis of differential gene expression**

170 The complete RNA-Seq data preparation pipeline is available in details in
171 supplementary material. We used the R packages "edgeR" 3.24.3 [41] and
172 "limma-voom" v.3.7 [42] to filter the dataset and determine differential gene
173 expression. After quality control and data filtering, we used 12,520 annotated
174 transcripts and 20 of the 21 original libraries (one exposed individual was
175 removed because of poor quality, see supplementary figure 1). Absolute read
176 counts were converted into their respective CPM value and log2-transformed
177 using the "voom" function. Each transcript was fitted to an independent linear
178 model using the log2(CPM) values as the response variable and the treatment as
179 the explanatory variable. Each linear model was then analysed through limma's
180 Bayes pipeline. We determined which genes were differentially expressed in
181 each group (infected, exposed, SSRI-treated) compared to healthy controls
182 based on a p-value of $p<0.005$. We did not apply a false discovery rate

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183 correction, as it greatly reduced our dataset, with the caveat that interpretation of
184 changes in expression of a specific gene must be done only as a preliminary
185 result and an additional functional analysis is needed to corroborate our findings
186 (which we did for one candidate gene, see "Functional analysis" section and
187 discussion). The results of statistical comparisons between control individuals
188 and each treatment with associated fold-change and p-value are in
189 supplementary tables S1, S4 and S6. Within differentially expressed genes, we
190 identified genes that are differentially expressed only in that specific treatment vs
191 the control group, to define a genomic signature of that treatment group (ex:
192 significantly more expressed in infected fish compared to controls, but not
193 differentially expressed between exposed fish and controls, or between SSRI-
194 treated fish and controls) [29, 35]. These genes are marked in bold in the
195 corresponding supplementary tables. We performed an enrichment analysis for
196 each genomic signature separately, to test if certain biological functions were
197 significantly overrepresented. GO terms for each gene were based on the
198 published transcriptome of *Gasterosteus aculeatus*. We used the Python
199 package 'goatools' v.0.6.5 [43] to perform Fisher's exact tests using a p-value of
200 $p<0.005$ as a significance threshold.

201

202 Functional analysis in infected sticklebacks: pharmacological rescue of 203 behaviour

204 Several genes coding for molecules involved in the inositol pathway were found
205 to be differentially regulated in the brain of *S. solidus*-infected fish (see Results
206 section). One of them is inositol monophosphatase 1 (IMPA1, table S1). This
207 gene codes for the IMPAse 1 enzyme, which is a central step in the synthesis of
208 myo-inositol [44]. Altered inositol metabolism has been implicated in various
209 human neuropsychiatric and neurological diseases [45]. Lithium chloride is used
210 to diminish behavioural symptoms of these diseases, such as the manic phase
211 symptoms observed in bipolar patients [46], which include sleeplessness,

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212 hallucinations, psychosis, or paranoid rage [47]. One of the most accepted
213 mechanisms of lithium action is the *inositol depletion hypothesis* [44] which
214 suggest that lithium acts by blocking the IMPase 1 enzyme activity, leading to a
215 depletion of inositol in the brain of treated patients [44, 45, 48, 49]. Therefore, we
216 predicted that we could rescue normal behaviour in infected stickleback by
217 modulating the inositol pathway using lithium exposure. We measured two well-
218 characterized behaviours in infected individuals: the tendency to swim near the
219 surface and the response to a simulated bird strike (here the time spent frozen
220 after an attack). Using wild-caught threespine sticklebacks from Lac Témiscouata
221 (QC, Canada), we quantified these two behaviours in *S.solidus*-infected
222 individuals before and after exposure to lithium. One group was exposed to two
223 low doses of lithium (at 2.5 mM and 5mM), and a second group to a high dose (at
224 15 mM). Significant effects on behaviour in treated infected fish were tested for
225 each dose using a linear mixed effects analysis of the relationship between our
226 dependent variable (behaviour) and treatment. See supplementary material for
227 details.

228

229 **Results and discussion**

230 **<u>Altered molecular pathways in the brain following an infection by *S. solidus*</u>**

231 There were 105 differentially expressed genes between infected and control
232 sticklebacks: 92 up-regulated and 13 down-regulated in infected, with a median
233 log2 fold change of 0.60 (range: 0.26 to 2.76) and - 0.40 (range: -0.82 to -0.28)
234 respectively (table S1). A total of 45 out of the 105 differentially expressed genes
235 were differentially expressed only in the infected-control comparison and are thus
236 considered as a genomic signature of infection (37 up-regulated, 8 down-
237 regulated), table S1). The 45 genes forming the infected genomic signature were
238 significantly enriched for categories associated with behaviour alterations
239 (aromatic amino acid transport, thyroid hormone transport), host response to

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240 infection (catalase activity, oligosaccharyl transferase activity), and cellular
241 growth (thymidylate kinase activity, dTDP biosynthetic process) (table S2).

242

243 i) Molecular changes associated with behavioural alteration

244 Aromatic amino acids include all the precursors to biogenic amines (dopamine,
245 serotonin, and epinephrine), melatonin, and thyroid hormone. Several biogenic
246 amines are related to behaviour variation [50] and this result is in accordance
247 with the altered serotonin metabolism found in the brain of infected fish [22]. The
248 thyroid hormone transport function was also overrepresented in genes
249 differentially expressed in infected fish. Administration of thyroid hormone
250 (thyroxine) in the *Schistosoma mansoni* host increased worm numbers and lead
251 to the development of giant worms [51]. Thyroid hormone can also affect
252 behaviour : treatments with thyroid hormones cause salmons to move to open
253 water in daytime [52] and to change from a territorial phase to schooling phase
254 during smelting [53]. Thus, increase of thyroid hormone transport might be
255 beneficial for *S. solidus* growth and the completion of its cycle.

256 IMPA 1 (inositol monophosphatase 1) was among the up-regulated genes in the
257 brain of infected fish that is associated with behaviour (figure 1, table S1). This
258 gene encodes IMPase 1, a central enzyme in the inositol pathway [48], which is
259 implicated in a diverse range of responses in the central nervous system [44, 54].
260 Alterations to this signalling pathway could be the cause of behaviour changes in
261 infected sticklebacks. We tested the functional link between an increase in
262 IMPA1 expression and behaviour by pharmacologically blocking IMPase 1
263 activity with lithium, which is used to treat symptoms of bipolar disorder by
264 targeting IMPase activity [45, 49]. We attempted to rescue two behaviours that
265 are altered in *S.solidus*-infected individuals: the tendency to swim closer to the
266 surface and the lack of response to predator attacks. Infected sticklebacks
267 exposed to low doses of lithium chloride (2.5 and 5 mM) did not reduce the

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268 proportion of time they spent swimming in the upper part of the aquarium
269 compared to the control week (2.5 mM, t-ratio = 0.123, p = 0.99, n = 17; 5 mM, t-
270 ratio = -0.607, p = 0.82, n = 17, figure 2a). However, infected sticklebacks treated
271 with lithium chloride at a dose of 15 mM spent significantly less time in the top of
272 the aquarium than before treatment (figure 2a) (t-ratio = 5.69, p < 0.001, n = 5).
273 Infected sticklebacks treated with both low doses of lithium chloride spent almost
274 no time frozen after a simulated bird strike, which was not significantly different
275 from their behaviour before treatment (figure 2b) (2.5 mM of lithium, t-ratio =
276 0.742, p = 0.74, n = 17; 5 mM of lithium, t-ratio = -0.021, p = 0.99, n = 17).
277 However, infected fish treated with lithium chloride at a dose of 15 mM spent
278 significantly more time frozen after a simulated bird strike (figure 2b) (t-ratio = -
279 2.803, p = 0.003, n = 5). These results suggest that lithium can block the IMPase
280 1 enzyme activity in the infected stickleback brain and alter their behaviour,
281 making them respond more like healthy fish. Indeed, fish treated with high doses
282 of lithium spent on average 7 % (sd = 7 %) of time near the surface and 85
283 seconds (sd = 62 seconds) frozen after a simulated bird strike, while non-infected
284 sticklebacks studied in the same conditions in a separate study spent 6 % (sd = 6
285 %) of time near the surface and stayed frozen 34 sec (sd = 65 sec) (Alves and
286 Aubin-Horth, unpublished). Such observations imply that alterations in the inositol
287 pathway could be the direct cause, at least in part, of the striking behavioural
288 alterations observed in this host-parasite model. To our knowledge, our results
289 are one of the first examples of a pharmacological rescue of the behaviour of a
290 host infected with a putative manipulative parasite, along with findings in the
291 *Toxoplasma*-rodent system. Indeed, the risky behaviour of *Toxoplasma*-infected
292 rodents towards predators has been successfully returned to cautiousness using
293 antipsychotic drugs used to treat symptoms of schizophrenia [55].

294 In the *Schistocephalus*-stickleback system, it remains to be tested whether the
295 alteration of the inositol pathway is a side-effect of a host response or if it is the
296 result of a direct manipulation by the parasite. To test these different hypotheses,
297 a combination of approaches will be needed to gather indirect and direct

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298 evidence [19]. Such approaches include characterizing which molecules are
299 secreted/excreted by the parasite (termed “manipulation factors” [19], if these
300 manipulation factors alter the host behaviour [56], and through which molecular
301 mechanisms in the host.

302

303 ii) Molecular changes associated with the host response

304 Enrichment for certain biological functions in infected fish brains suggested that a
305 host response to infection could be at play. The over representation of catalase
306 activity, an important enzyme protecting the cell from oxidative damage by
307 reactive oxygen species (ROS) [57] might be explained as a consequence of
308 infection, since ROS production is increased in head kidney leucocytes in contact
309 with *S. solidus* extracts *in vitro* [26] and appears to play an important role in
310 stickleback defence against *S. solidus* [32]. However, the over-representation in
311 catalase activity might also indicate a way by which the parasite can manipulate
312 its host in order to eliminate an oxidative stress that would otherwise compromise
313 parasite survival [58]. This over-representation could also indicate a reaction from
314 the host aimed at decreasing the oxidative stress caused by the parasite. Genes
315 whose function was associated with oligosaccharyl transferase activity were also
316 over-represented in infected fish. This transferase is implicated in post-
317 translational modifications of proteins by glycosylation, which determines the
318 localization and function of these proteins [59]. Again, those modifications might
319 be a global host response to infection at the protein level that can have major
320 effects on cellular activity, among other wide-ranging effects. Finally, thymidylate
321 kinase activity and dTDP biosynthetic process are biological functions enriched in
322 infected fish brains that are related to cellular growth. The over representation of
323 the thymidylate kinase activity, an important enzyme that assists biosynthesis of
324 mitochondrial DNA, might also be explained as a consequence of infection, since
325 a thymidylate kinase-like gene was found up-regulated in infected salmon and
326 may be linked to the innate response to infection by a monogenean parasite [60].

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327 An interesting feature of the *S. solidus*-stickleback system is that the hypothesis
328 of a global host response could be verified by a gene expression study in the first
329 intermediate host of *S. solidus*, the copepod. Determining if the biological
330 functions of genes differentially expressed in infected copepod mirror the ones
331 found in infected fish would allow us to determine the degree of overlap in the
332 molecular response of both hosts and at the same time learn about the specificity
333 of interactions at each life stage of the parasite [20, 61].

334

335 Our results come with limitations. Because of a small sample size, high biological
336 variation within a group and small fold changes associated with the use of whole
337 brain sampling, using a false discovery rate (FDR) resulted in little or no
338 significant differentially expressed genes depending on the comparison. Genes
339 found to be up- or down-regulated in INF fish will therefore each necessitate
340 further functional validations, as for all gene expression studies that show an
341 association between a phenotype and expression changes (rather than a causal
342 link). Our test of a causal link between a disruption of the inositol pathway and
343 behaviours typical of infected sticklebacks using a pharmacological treatment
344 supports the notion that some of the genes differentially expressed in the brain of
345 infected individuals are indeed associated with behaviour modification following
346 infection. However, it is crucial to underscore that it is highly probable that most
347 changes in gene expression do not affect behaviour. Some of them may control
348 other changes observed in infected individuals at the physiological level, others
349 may be related to a general host response to infection, while some of the
350 changes in gene expression may be a side-product of the presence of the
351 parasite, or simply be false positive. Once we confirm the causal role of a
352 molecular pathway in behaviour variation, the next step would be to test which
353 one, if any, of these pathways are directly manipulated by the parasite. In this
354 host-parasite system, a next step would be to determine if the increase in IMPA1
355 activity is a direct manipulation by the parasite, an indirect effect of its presence,
356 or an active response from the host [19].

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357

358 **Overlap of exposed and infected fish transcriptomes**

359 i) Overlap between exposed and infected fish

360 Contrary to our prediction, infected and exposed fish did not have similar brain
361 expression profiles. Only nine genes were differentially expressed both in
362 exposed and infected fish compared to controls (table S3). Interestingly, fold
363 changes for these nine genes were very similar in amplitude between infected
364 and exposed fish and were all in the same direction (see supp. Fig 2). Four of
365 those nine genes were found as differentially expressed only in the comparison
366 of each of these two treatments compared to control individuals: a solute carrier
367 family protein, a myosin light chain, a lipase gastric, and a spermine
368 acetyltransferase. On the other hand, only one gene was significantly
369 differentially expressed between infected fish and exposed fish: gdpd5a, a
370 glycerophosphodiester phosphodiesterase domain containing 5a, which was
371 down-regulated in infected fish (FC=-0.418, p=0.002). This gene codes for a
372 protein proposed (by similarity) to be involved in neurite formation, the regulation
373 of the metabolite glycerophosphocholine (which can act as an osmolyte,
374 https://hmdb.ca/metabolites/HMDB0000086, [62]), and in the cleavage of the GPI
375 anchor of RECK, which in turns is involved in Wnt7-specific actions in the brain
376 (https://www.uniprot.org/uniprot/Q8WTR4, [63]). Interestingly, a genomic study
377 on *S. solidus* [64] has shown the existence of mimicry proteins (similar to the
378 vertebrate host protein), with one of them belonging to the Wnt protein family and
379 being the same protein found to be over-expressed in the head of orthopterans
380 infected by a behaviour-altering hairworm [65]. Based on these similarities
381 between two manipulating parasites and the observed change in the host, it
382 could be proposed that a general disruption of cell-to-cell communication leading
383 to various changes in behaviour may be at play [66].

384

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385 ii) Exposed fish also have a distinct gene expression profile

386 Interestingly, the brains of exposed fish are also very different from the ones of
387 healthy control fish. 153 genes were differentially expressed in exposed fish
388 compared to control individuals (table S4): 48 up- and 105 down-regulated, with a
389 median fold change of 0.34 (range: 0.22 to 1.43) and -0.47 (range: -1.16 to -
390 0.22), respectively. More than 85% of these genes were specific to that exposed
391 vs control comparison, thus forming an "exposed" genomic signature, including
392 95 of the 105 down-regulated genes. The enrichment analysis (table S2)
393 performed on the exposed-specific genes showed no significant enrichment.
394 Whether this unique expression profile of exposed fish is a cause of the failure of
395 the parasite to successfully infect these fish and was already present before
396 infection, or if it is a long-lasting consequence of exposure (or both) is unknown.
397 Indeed, we cannot exclude the fact that individuals that became infected and the
398 ones that did not were already different before the experimental infection, as
399 interaction between the genotype of the host and the parasite has been
400 previously shown [67]. However, this is unlikely in our case, as all individuals
401 come from the same crosses and laboratory environment. One way to test that
402 these differences in expression existed before exposure and are the cause of the
403 resistance to the parasite would be to redo a brain transcriptome analysis on a
404 much higher number of control sticklebacks to detect difference in expression in
405 the genes assigned to the exposed genomic signature in the present study. If so,
406 it would suggest that the genotype of a proportion of individuals is the cause of
407 differential gene expression rather than exposure to the parasite. On the other
408 hand, being exposed is known to modify the immune system of the host.
409 Sticklebacks resistant to parasitic eye flukes have a higher basic
410 immunocompetence than more susceptible host [68]. A transcriptomic study of
411 changes in gene expression in the head kidney following exposure to *S.solidus* in
412 sticklebacks found that ROS production and recycling, B cell activation and
413 targeting, and fibrosis appear to play important roles in defence against cestodes
414 [32], but did not find significant differences in gene expression between exposed

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415 and control fish. Similarly, exposed but non-infected gilthead sea bream (*Sparus*
416 *aurata*) appear closer to the unexposed fish than the infected fish in their gene
417 expression [69].

418

419 It is worth noting that while successful infection resulted in the up-regulation of
420 genes in the brain, exposure without infection resulted mostly in lower expression
421 of genes in the brain, suggesting that they reflect different processes. The
422 transcriptome response to pathogen lines of different virulence also show this
423 opposite response in a *Daphnia* host, with the infective line resulting in more
424 down-regulated genes, while the non-infective strain resulted in up-regulation of
425 genes, with little overlap between the genes affected [70]. It thus appears that
426 exposure could have a significant and distinct effect in the brain of the host, even
427 when infection ultimately fails, and that this effect is carried over several weeks.

428

429 **<u>Overlap of SSRI-treated and infected fish transcriptomes</u>**

430 The analysis of the overlap between SSRI and infected fish brain expression
431 profiles revealed only four genes in common (table S5). These four genes are
432 related to cellular organization and transport or have unknown functions. In
433 comparison, changes in gene expression specific to the SSRI treatment (table
434 S6) include genes that have functions related to neurotransmission. Drugs in the
435 SSRI family are designed to target the serotonin axis, which has its own range of
436 effects on behaviour and physiology. The small overlap between genes affected
437 in infected individuals and in SSRI-treated ones may thus be explained in part by
438 the fact that SSRIs are specifically designed for a unique target, while *S. solidus*
439 affects several traits in addition to behaviour. Infections have other major impacts
440 on host sticklebacks that include reduced body condition, changes in metabolism,
441 nutrient balance, and reproduction [7]. A small overlap does not suggest

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442 necessarily that *S. solidus* and SSRI target different molecular pathways, only
443 that these similarities are masked by multiple molecular differences.

444

445 **Conclusion**

446 Parasitic alteration of animal behaviour is predicted to be caused by, and to result
447 in, multiple physiological changes in the host. Our study aimed at determining the
448 brain gene expression profiles of parasitised fish to identify a genomic signature
449 that distinguishes a *S.solidus*-infected stickleback from a control fish. Obtaining a
450 general portrait of molecular pathways affected in an infected host is a valuable
451 step to help determine if these hosts are manipulated by their parasite. Indeed,
452 once it is known what changes in the brain of a host, one can manipulate these
453 molecular pathways to recreate the infected host phenotype (or parts of it) in
454 healthy individuals and ultimately test evolutionary predictions about effects on
455 the parasite's fitness of host's behavioural differences, as done in *Gammarus*
456 [71]. Our results allow us to predict that if *S.solidus* directly manipulates its fish
457 host, its manipulations factors could target the inositol pathway. A next step
458 would thus be to determine if *S. solidus* directly alters the behaviour of its host by
459 affecting the inositol pathway, and if it does, to test if sticklebacks with an
460 experimentally-altered inositol pathway consequently exhibit altered behaviours
461 that increase the probability of being predated by the final avian host of
462 *Schistocephalus solidus*.

463

464 **Acknowledgments**

465 We thank the IBIS genomics analysis platform for help with RNA extraction and
466 library preparation, L. Benestan for the specific subsetting idea, and J. Turgeon,
467 C. R. Landry and J. Le Luyer for discussion. Funding for this project was
468 provided by the Fonds de Recherche du Québec Nature et Technologie (FRQ-

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NT) through a Team project grant to NAH, the Natural Sciences and Engineering Research Council of Canada Discovery grant to NAH, and a NSERC Vanier fellowship to FOH.

Authors' contributions

L.G. designed the study with the input of N.A.-H. and I.B. L.G. extracted RNA from the brains. L.G. and F.-O.H. performed transcriptomic and statistical analyses. VAA designed and performed the lithium pharmacological manipulation experiments. L.G. and N.A.-H. drafted the manuscript with input from F.-O.H., VAA, and I.B. All authors reviewed and gave input on the final version of the manuscript.

Ethics

The experimental exposure to *S. solidus* parasites and / or SSRIs was undertaken at the University of Leicester, UK, under the authority of a UK Home Office project license (PPL 70/8148, held by I.B.). The lithium exposure was performed at Université Laval under the CPAUL certificate number 2017085-2. The project was authorised by the Comité de Protection des Animaux de l'Université Laval (2014069-1).

Data availability

The RNA-seq dataset consists of raw read counts for the 17417 transcripts that passed quality control and filters. Behaviour data from the lithium treatment is presented for all individuals.

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493

494 **Electronic supplementary material**

495 A) Supplementary tables. Results of statistical analyses of differential gene
496 expression, overlap of differentially expressed genes between two treatments,
497 and results of enrichment analyses.

498 B) Supplementary figures.

499 C) Supplementary material and methods.

500

501

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502 FIGURES

503 **Figure 1. The IMPA 1 gene is significantly more expressed in the brain of**
504 ***S.solidus*-infected sticklebacks compared to exposed, SSRI-treated and**
505 **control fish.** Box plots with median, 25th and 75th percentiles, vertical bars
506 representing the largest value no further than 1.5 times the interquartile range,
507 means (red triangles), and individual data (black dots). The four treatment groups
508 are: CON = healthy controls, EXP = fish that were exposed to the parasite but did
509 not become infected, INF = infected fish, SSRI = SSRI-treated fish, sample size
510 in parentheses.

511

512 **Figure 2. Lithium chloride exposure at a dose of 15mM rescues normal**
513 **behaviours in *S.solidus*-infected sticklebacks.** A) Infected sticklebacks
514 treated with lithium chloride at a dose of 15 mM spent less time in the top of the
515 aquarium compared to the control week (15 mM, t-ratio = 5.69, p = 0.0007, n =
516 5), while infected sticklebacks treated with lower doses of lithium chloride did not
517 change their vertical preference after treatment (2.5 mM, t-ratio = 0.123, p =
518 0.99, n = 17; 5 mM, t-ratio = -0.607, p = 0.82, n = 17). B) Infected sticklebacks
519 treated with lithium chloride at a dose of 15 mM spent more time frozen after a
520 simulated bird strike in comparison to their control week (15 mM, t-ratio = -2.803,
521 p = 0.003, n = 5), while infected sticklebacks treated with lower doses of lithium
522 chloride did not change the time they spent frozen following a simulated bird
523 strike after treatment (2.5 mM, t-ratio = 0.742, p = 0.74, n = 17; 5 mM, t-ratio = -
524 0.021, p = 0.99, n = 17). Box plots with median, 25th and 75th percentiles,
525 vertical bars representing the largest value no further than 1.5 times the
526 interquartile range, means (red triangles), and individual data (black dots). The
527 four treatment groups are: INF CON = infected control, INF LIT = infected fish
528 treated with lithium chloride, with the number representing the dose in mM,
529 sample size in parentheses .

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530 # REFERENCES
531

532 1. Poulin R. Parasite manipulation of host behavior: an update and frequently asked
533 questions. Advances in the Study of Behavior. 2010;41:151-86.
534 2. Seppälä O, Karvonen, A., Valtonen, E. T. Susceptibility of eye fluke-infected fish
535 to predation by bird hosts. Parasitology. 2006; 132(04):pp.575-9.
536 3. Lagrue C, Kaldonski N, Perrot-Minnot MJ, Motreuil S, Bollache L. Modification
537 of Hosts' Behavior by a Parasite: Field Evidence for Adaptive Manipulation. Ecology.
538 2007;88(11):2839-47.
539 4. Poulin R. “Adaptive” changes in the behaviour of parasitized animals: a critical
540 review. International journal for parasitology. 1995;25(12):1371-83.
541 5. Smyth J. Studies on Tapeworm Physiology. Journal of Experimental Biology.
542 1946;23(1):47-70.
543 6. Clarke A. Studies on the life cycle of the pseudophyllidean cestode
544 *Schistocephalus solidus*. Proceedings of the Zoological Society of London.
545 1954;124(2):257-302.
546 7. Barber I, Scharsack J. The three-spined stickleback-*Schistocephalus solidus*
547 system: an experimental model for investigating host-parasite interactions in fish.
548 Parasitology. 2010;137(03):411-24.
549 8. Meakins R, Walkey M. The effects of parasitism by the plerocercoid of
550 *Schistocephalus solidus* Muller 1776 (Pseudophyllidea) on the respiration of the
551 three□spined stickleback *Gasterosteus aculeatu*s L. Journal of Fish Biology.
552 1975;7(6):817-24.
553 9. Heins DC, Singer SS, Baker JA. Virulence of the cestode *Schistocephalus solidus*
554 and reproduction in infected threespine stickleback, *Gasterosteus aculeatus*. Canadian
555 Journal of Zoology. 2000;77(12):1967-74.
556 10. Tierney J, Huntingford F, Crompton D. Body condition and reproductive status in
557 sticklebacks exposed to a single wave of *Schistocephalus solidus* infection. Journal of
558 Fish Biology. 1996;49(3):483-93.
559 11. Milinski M. Risk of predation of parasitized sticklebacks (*Gasterosteus aculeatus*
560 L.) under competition for food. Behaviour. 1985;93(1):203-16.
561 12. Giles N. Predation risk and reduced foraging activity in fish: experiments with
562 parasitized and non□parasitized three□spined sticklebacks, *Gasterosteus aculeatus* L.
563 Journal of Fish Biology. 1987;31(1):37-44.
564 13. Tierney JF, Huntingford FA, Crompton DW. The relationship between infectivity
565 of *Schistocephalus solidus* (Cestoda) and anti-predator behaviour of its intermediate host,
566 the three-spined stickleback, *Gasterosteus aculeatus*. Animal Behaviour. 1993;46(3):603-
567 5.
568 14. Barber B, Huntingford F, Crompton D. The effect of hunger and cestode
569 parasitism on the shoaling decisions of small freshwater fish. Journal of Fish Biology.
570 1995;47(3):524-36.
571 15. Demandt N, Saus B, Kurvers RH, Krause J, Kurtz J, Scharsack JP. Parasite-
572 infected sticklebacks increase the risk-taking behaviour of uninfected group members.
573 Proceedings of the Royal Society B: Biological Sciences. 2018;285(1881):20180956.
574 16. Quinn T, Kendall N, Rich Jr H, Chasco B. Diel vertical movements, and effects of
575 infection by the cestode *Schistocephalus solidus* on daytime proximity of three-spined

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576 sticklebacks *Gasterosteus aculeatus* to the surface of a large Alaskan lake. Oecologia.
577 2012;168(1):43-51.
578 17. Perrot-Minnot M-J, Sanchez-Thirion K, Cézilly F. Multidimensionality in host
579 manipulation mimicked by serotonin injection. Proceedings of the Royal Society of
580 London B: Biological Sciences. 2014;281(1796):20141915.
581 18. Grécias L, Hébert FO, Berger CS, Barber I, Aubin-Horth N. Can the behaviour of
582 threespine stickleback parasitized with *Schistocephalus solidus* be replicated by
583 manipulating host physiology? The Journal of Experimental Biology. 2017;220(2):237-
584 46.
585 19. Herbison R, Lagrue C, Poulin R. The missing link in parasite manipulation of host
586 behaviour. Parasites & vectors. 2018;11(1):222.
587 20. Hébert FO, Aubin-Horth N. Ecological genomics of host behavior manipulation
588 by parasites. Ecological Genomics. 2014:169-90.
589 21. Poulin R, Maure F. Host manipulation by parasites: a look back before moving
590 forward. Trends in parasitology. 2015;31(11):563-70.
591 22. Øverli Ø, Pall M, Borg B, Jobling M, Winberg S. Effects of *Schistocephalus*
592 *solidus* infection on brain monoaminergic activity in female three-spined sticklebacks
593 *Gasterosteus aculeatus*. Proceedings of the Royal Society of London B: Biological
594 Sciences. 2001;268(1474):1411-5.
595 23. Rubenstein DR, Hofmann HA. Proximate pathways underlying social behavior.
596 Current Opinion in Behavioral Sciences. 2015;6:154-9.
597 24. Macnab V, Scott A, Katsiadaki I, Barber I. Variation in the reproductive potential
598 of Schistocephalus infected male sticklebacks is associated with 11-ketotestosterone titre.
599 Hormones and Behavior. 2011;60(4):371-9.
600 25. LoBue CP, Bell MA. Phenotypic manipulation by the cestode parasite
601 *Schistocephalus solidus* of its intermediate host, *Gasterosteus aculeatus*, the threespine
602 stickleback. The American Naturalist. 1993;142(4):725-35.
603 26. Scharsack J, Kalbe M, Derner R, Kurtz J, Milinski M. Modulation of granulocyte
604 responses in three-spined sticklebacks *Gasterosteus aculeatus* infected with the tapeworm
605 *Schistocephalus solidus*. Diseases of aquatic organisms. 2004;59(2):141-50.
606 27. Scharsack JP, Koch K, Hammerschmidt K. Who is in control of the stickleback
607 immune system: interactions between *Schistocephalus solidus* and its specific vertebrate
608 host. Proceedings of the Royal Society of London B: Biological Sciences.
609 2007;274(1629):3151-8.
610 28. McDonnell CM, Alaux C, Parrinello H, Desvignes J-P, Crauser D, Durbesson E,
611 et al. Ecto-and endoparasite induce similar chemical and brain neurogenomic responses
612 in the honey bee (*Apis mellifera*). BMC ecology. 2013;13(1):25.
613 29. Aubin-Horth N, Renn SC. Genomic reaction norms: using integrative biology to
614 understand molecular mechanisms of phenotypic plasticity. Molecular Ecology.
615 2009;18(18):3763-80.
616 30. Thomas F, Poulin R, Brodeur J. Host manipulation by parasites: a
617 multidimensional phenomenon. Oikos. 2010;119(8):1217-23.
618 31. Scharsack JP, Gossens A, Franke F, Kurtz J. Excretory products of the cestode,
619 *Schistocephalus solidus*, modulate in vitro responses of leukocytes from its specific host,
620 the three-spined stickleback (*Gasterosteus aculeatus*). Fish & shellfish immunology.
621 2013;35(6):1779-87.

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622 32. Lohman BK, Steinel NC, Weber JN, Bolnick DI. Gene Expression Contributes to
623 the Recent Evolution of Host Resistance in a Model Host Parasite System. Frontiers in
624 Immunology. 2017;8(1071).
625 33. Whitfield CW, Cziko A-M, Robinson GE. Gene expression profiles in the brain
626 predict behavior in individual honey bees. Science. 2003;302(5643):296-9.
627 34. Aubin-Horth N, Letcher BH, Hofmann HA. Gene-expression signatures of
628 Atlantic salmon's plastic life cycle. General and comparative endocrinology.
629 2009;163(3):278-84.
630 35. Segal E, Friedman N, Koller D, Regev A. A module map showing conditional
631 activity of expression modules in cancer. Nature genetics. 2004;36(10):1090-8.
632 36. Barber I. Sticklebacks as model hosts in ecological and evolutionary parasitology.
633 Trends in parasitology. 2013;29(11):556-66.
634 37. Adamo SA. How parasites alter the behavior of their insect hosts. Parasites and
635 Pathogens. 1997:231-45.
636 38. Helluy S, Thomas F. Effects of *Microphallus papillorobustus* (Platyhelminthes:
637 Trematoda) on serotonergic immunoreactivity and neuronal architecture in the brain of
638 *Gammarus insensibilis* (Crustacea: Amphipoda). Proceedings of the Royal Society of
639 London B: Biological Sciences. 2003;270(1515):563-8.
640 39. Tain L, Perrot-Minnot M-J, Cézilly F. Altered host behaviour and brain
641 serotonergic activity caused by acanthocephalans: evidence for specificity. Proceedings
642 of the Royal Society of London B: Biological Sciences. 2006;273(1605):3039-45.
643 40. Shaw J, Korzan W, Carpenter R, Kuris A, Lafferty K, Summers C, et al. Parasite
644 manipulation of brain monoamines in California killifish (*Fundulus parvipinnis*) by the
645 trematode *Euhaplorchis californiensis*. Proceedings of the Royal Society of London B:
646 Biological Sciences. 2009;276(1659):1137-46.
647 41. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for
648 differential expression analysis of digital gene expression data. Bioinformatics.
649 2010;26(1):139-40.
650 42. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers
651 differential expression analyses for RNA-sequencing and microarray studies. Nucleic
652 acids research. 2015:gkv007.
653 43. Tang H, Klopfenstein D, Pedersen B, Flick P, Sato K, Ramirez F, et al.
654 GOATOOLS: tools for gene ontology. Zenodo. 2015.
655 44. Berridge MJ, Irvine RF. Inositol phosphates and cell signalling. Nature.
656 1989;341(6239):197-205.
657 45. Frej AD, Otto GP, Williams RS. Tipping the scales: lessons from simple model
658 systems on inositol imbalance in neurological disorders. European Journal of Cell
659 Biology. 2017:96(2): 154-63.
660 46. Davanzo P, Thomas MA, Yue K, Oshiro T, Belin T, Strober M, et al. Decreased
661 anterior cingulate myo-inositol/creatine spectroscopy resonance with lithium treatment in
662 children with bipolar disorder. Neuropsychopharmacology. 2001;24(4):359-69.
663 47. Goodwin FK, Jamison KR. Manic-depressive illness: bipolar disorders and
664 recurrent depression. Oxford, UK.: Oxford University Press; 2007.
665 48. Ferruz N, Tresadern G, Pineda-Lucena A, De Fabritiis G. Multibody cofactor and
666 substrate molecular recognition in the myo-inositol monophosphatase enzyme. Scientific
667 Reports. 2016;6.

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668 49. Beaulieu J-M, Caron MG. Looking at lithium: molecular moods and complex
669 behaviour. Molecular interventions. 2008;8(5):230.
670 50. Schildkraut JJ, Kety SS. Biogenic Amines and Emotion. Science.
671 1967;156(3771):21-30.
672 51. Saule P, Adriaenssens E, Delacre M, Chassande O, Bossu M, Auriault C, et al.
673 Early variations of host thyroxine and interleukin-7 favor *Schistosoma mansoni*
674 development. Journal of Parasitology. 2002;88(5):849-55.
675 52. Iwata M, Yamanome T, Tagawa M, Ida H, Hirano T. Effects of thyroid hormones
676 on phototaxis of chum and coho salmon juveniles. Aquaculture. 1989;82(1-4):329-38.
677 53. Iwata M. Downstream migratory behavior of salmonids and its relationship with
678 cortisol and thyroid hormones: a review. Aquaculture. 1995;135(1-3):131-9.
679 54. Fisher SK, Heacock AM, Agranoff BW. Inositol lipids and signal transduction in
680 the nervous system: an update. Journal of neurochemistry. 1992;58(1):18-38.
681 55. Webster J, Lamberton P, Donnelly C, Torrey E. Parasites as causative agents of
682 human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-
683 parasite medication on Toxoplasma gondii's ability to alter host behaviour. Proceedings
684 of the Royal Society B: Biological Sciences. 2006;273(1589):1023-30.
685 56. Berger CS, Aubin-Horth N. An eDNA-qPCR assay to detect the presence of the
686 parasite Schistocephalus solidus inside its threespine stickleback host. Journal of
687 Experimental Biology. 2018;221(9):jeb178137.
688 57. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among
689 catalases. Cellular and molecular life sciences. 2004;61(2):192-208.
690 58. Schirmer R, Schöllhammer T, Eisenbrand G, Krauth-Siegel R. Oxidative stress as
691 a defense mechanism against parasitic infections. Free Radical Research
692 Communications. 1987;3(1-5):3-12.
693 59. Rademacher T, Parekh R, Dwek R. Glycobiology. Annual review of
694 biochemistry. 1988;57(1):785-838.
695 60. Collins CM, Olstad K, Sterud E, Jones CS, Noble LR, Mo TA, et al. Isolation of a
696 novel fish thymidylate kinase gene, upregulated in Atlantic salmon (*Salmo salar* L.)
697 following infection with the monogenean parasite *Gyrodactylus salaris*. Fish & shellfish
698 immunology. 2007;23(4):793-807.
699 61. Ponton F, Lefèvre T, Lebarbenchon C, Thomas F, Loxdale H, Marché L, et al. Do
700 distantly related parasites rely on the same proximate factors to alter the behaviour of
701 their hosts? Proceedings of the Royal Society of London B: Biological Sciences.
702 2006;273(1603):2869-77.
703 62. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, et al.
704 HMDB 4.0: the human metabolome database for 2018. Nucleic acids research.
705 2018;46(D1):D608-D17.
706 63. Consortium U. UniProt: a worldwide hub of protein knowledge. Nucleic acids
707 research. 2019;47(D1):D506-D15.
708 64. Hébert FO, Phelps L, Samonte I, Panchal M, Grambauer S, Barber I, et al.
709 Identification of candidate mimicry proteins involved in parasite-driven phenotypic
710 changes. Parasites & vectors. 2015;8(1):1.
711 65. Biron DG, Marché L, Ponton F, Loxdale HD, Galéotti N, Renault L, et al.
712 Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach.
713 Proceedings of the Royal Society B: Biological Sciences. 2005;272(1577):2117-26.

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714 66. Adamo SA. Parasites: evolution's neurobiologists. Journal of Experimental
715 Biology. 2013;216(1):3-10.
716 67. Barribeau SM, Sadd BM, du Plessis L, Schmid-Hempel P. Gene expression
717 differences underlying genotype-by-genotype specificity in a host–parasite system.
718 Proceedings of the National Academy of Sciences. 2014;111(9):3496-501.
719 68. Kalbe M, Kurtz J. Local differences in immunocompetence reflect resistance of
720 sticklebacks against the eye fluke *Diplostomum pseudospathaceum*. Parasitology.
721 2006;132(01):105-16.
722 69. Piazzon MC, Estensoro I, Calduch-Giner JA, del Pozo R, Picard-Sánchez A,
723 Pérez-Sánchez J, et al. Hints on T cell responses in a fish-parasite model: *Enteromyxum*
724 *leei* induces differential expression of T cell signature molecules depending on the organ
725 and the infection status. Parasites & vectors. 2018;11(1):443.
726 70. McTaggart SJ, Cézard T, Garbutt JS, Wilson PJ, Little TJ. Transcriptome
727 profiling during a natural host-parasite interaction. BMC genomics. 2015;16(1):643.
728 71. Perrot-Minnot M-J, Cézilly F. Investigating candidate neuromodulatory systems
729 underlying parasitic manipulation: concepts, limitations and prospects. Journal of
730 Experimental Biology. 2013;216(1):134-41.

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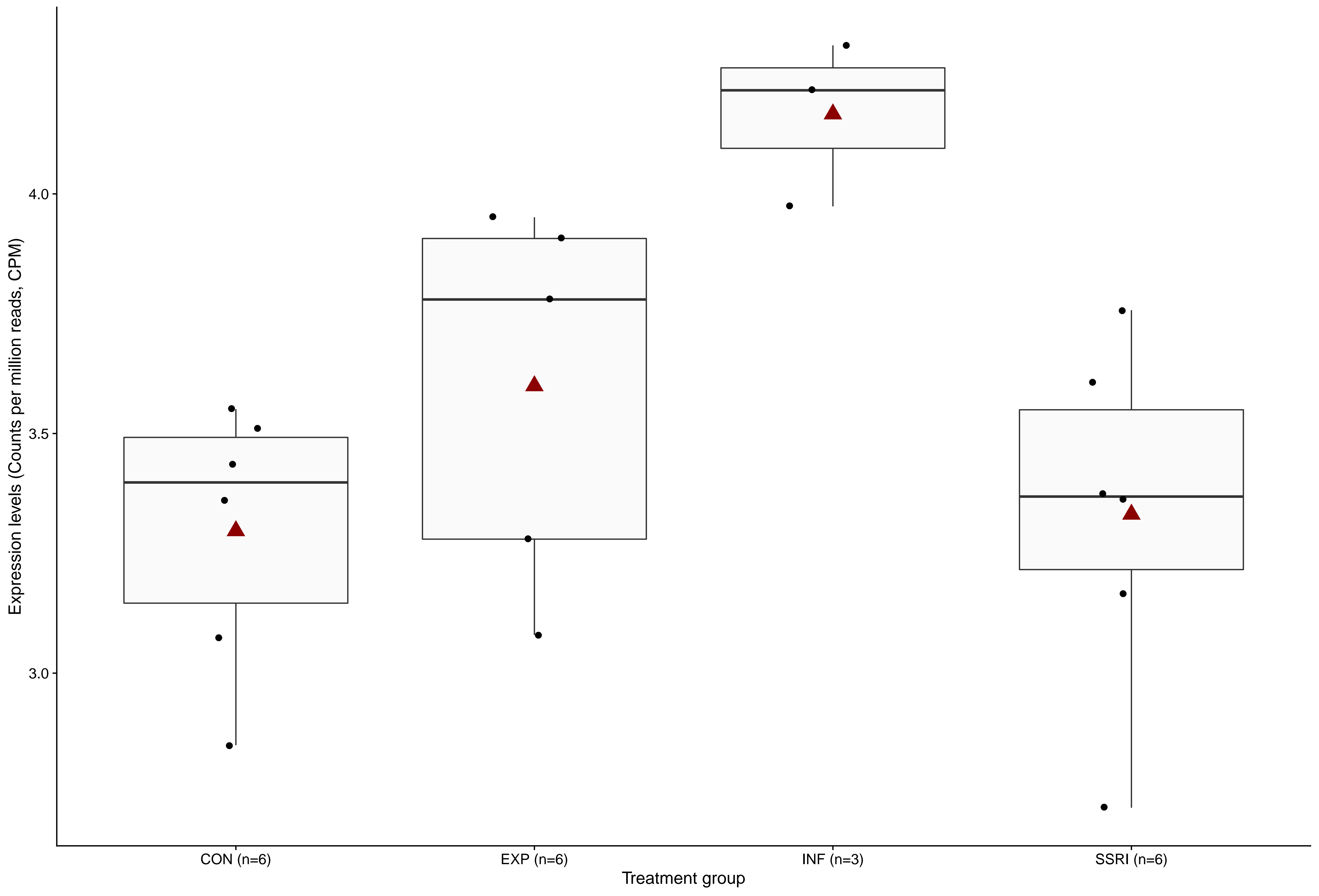

Expression levels (Counts per million reads, CPM)
4.0
3.5
3.0
CON (n=6)
EXP (n=6)
INF (n=3)
SSRI (n=6)
Treatment group

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