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

6 Lucie Grecias¹, Francois Olivier Hebert¹, Verônica Angelica Alves¹, Iain Barber²,
7 Nadia Aubin-Horth¹

8

9 ¹*Département de Biologie et Institut de Biologie Intégrative et des Systèmes*
10 *(IBIS), Université Laval, Québec, Canada*

11

12 ² *School of Animal, Rural and Environmental Sciences, Nottingham Trent*
13 *University, Nottingham, United Kingdom*

14

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.

37

38

Introduction

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

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

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

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

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

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

Materials and Methods

Exposure of fish host to its parasite or SSRI

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

Gene expression quantification by RNA-seq

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

Analysis of differential gene expression

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

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

Functional analysis in infected sticklebacks: pharmacological rescue of behaviour

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

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

Results and discussion

Altered molecular pathways in the brain following an infection by *S. solidus*

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

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

i) Molecular changes associated with behavioural alteration

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

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

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

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

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

ii) Molecular changes associated with the host response

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

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

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

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

ii) Exposed fish also have a distinct gene expression profile

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

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

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

Overlap of SSRI-treated and infected fish transcriptomes

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

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

Conclusion

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

Acknowledgments

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

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.

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

FIGURES

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

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

REFERENCES

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

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

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

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

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

a)

b)