Title: Sex differences and similarities in the neuroimmune response to central administration of poly I:C

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Keywords: poly I:C; sex differences; neuroimmune; cytokines; hippocampus; chemokines; male; female; interferon; mouse

ABSTRACT

The neuroimmune system is required for normal neural processes, including modulation of cognition, emotion, and adaptive behaviors. Aberrant neuroimmune activation is associated with dysregulation of memory and emotion, though the precise mechanisms at play are complex and highly context dependent. Sex differences neuroimmune activation and function further complicate our understanding of its roles in cognitive and affective regulation. Here, we characterized the physiological sickness and inflammatory response of the hippocampus following intracerebroventricular (ICV) administration of a synthetic viral mimic, polyinosinic:polycytidylic acid (poly I:C), in both male and female C57Bl/6 mice. We observed that poly I:C induced weight loss, fever, and elevations of cytokine and chemokines in the hippocampus of both sexes. Specifically, we found transient increases in gene expression and protein levels of IL-1α, IL-1β, IL-4, IL-6, TNFα, CCL2, and CXCL10, where males showed a greater magnitude of response compared with females. Only males showed increased IFNα and IFNγ in response to poly I:C, whereas both males and females exhibited elevations of IFNβ, demonstrating a specific sex difference in the anti-viral response in the hippocampus. This suggests that type I interferons are one potential node mediating sex-specific cytokine responses and neuroimmune effects on synaptic plasticity and cognition. These findings highlight the importance of using both males and females and analyzing a broad set of inflammatory markers in order to identify the precise, sex-specific roles for neuroimmune dysregulation in neurological diseases and disorders including Alzheimer's Disease and depression.

1. INTRODUCTION

The neuroimmune system is required for neural processes, including synaptic plasticity (Marin and Kipnis, 2013; Rizzo et al., 2018; Yirmiya and Goshen, 2011), and is thus well-placed to modify or disrupt learning and memory. Aberrant immune signaling has also been implicated in disorders of affect and cognition, many of which show sex-biases in prevalence and outcomes (Bekhbat and Neigh, 2018; Dantzer et al., 2008; Neigh et al., 2018). There is significant evidence for sex differences in immune responses in the periphery (Klein and Flanagan, 2016; Klein and Huber, 2010). Knowing whether these sex differences are upheld in the neuroimmune system is important for understanding exactly how neuroimmune dysregulation impacts cognition, and contributes to psychiatric and neurological disorders, in both sexes.

Illness, injury, or aseptic triggers of the innate immune system – either bacterial endotoxins (e.g., lipopolysaccharide, LPS) or viral mimics (e.g., polyinosinic:polycytidylic acid, poly I:C) – cause activation of neuroimmune cells, including microglia and astrocytes, and rapid production of cytokines in the brain (Ransohoff, 2009; Ransohoff and Brown, 2012). Cytokine production is associated with adaptive sickness behaviors and disruption of cognitive processes (Dantzer et al., 2008; Marin and Kipnis, 2013; Raison et al., 2006; Yirmiya and Goshen, 2011). Elevation of hippocampal cytokines, in particular, is associated with both disruption of memory processes (Barrientos et al., 2002; Cunningham et al., 2009; Czerniawski and Guzowski, 2014; Marin and Kipnis, 2013; McAfoose and Baune, 2009; Vasek et al., 2016; Yirmiya et al., 2002) and increased depression-like behaviors (Koo and Duman, 2009; Tang et al., 2016). Due to key roles in peripheral inflammation, the cytokines IL-1β (Barrientos et al., 2002; Goshen et al., 2007; Yirmiya et al., 2002), IL-6 (Sparkman et al., 2006; Trapero and Cauli, 2014; Weaver et al., 2002), and TNFα (Feuerstein et al., 1994; Lindbergh et al., 2020; Strieter et al., 1993) have been the focus of much of the research of neuroimmune function (Donzis and Tronson, 2014). More recently, other cytokines, including interferons (Bekhbat and Neigh, 2018; Blank et al.,

67 2016), CCL2 (Westin et al., 2012; Xu et al., 2017), and CXCL10 (Bajova et al., 2008; Blank et
68 al., 2016; Bradburn et al., 2018) have also been implicated in modulation of behavior, cognition,
69 and affective states, suggesting that many cytokines play important roles in these processes.
70 Sex differences in immune and neuroimmune activity have also been identified. In
71 studies of peripheral immune activation, females have a greater inflammatory response
72 compared with males (Klein and Flanagan, 2016). In contrast, neuroimmune cells *in vitro*,
73 including astrocytes derived from male cortical tissue, have a significantly greater reaction to
74 inflammatory insults compared with female-derived cells (Loram et al., 2012; Santos-Galindo et
75 al., 2011). Sex differences in neuroimmune activity are also observed *in vivo*. For example, we
76 have identified sex differences in the magnitude, time course, and pattern of cytokines activated
77 in the hippocampus following peripheral LPS (Speirs and Tronson, 2018), and in the long-lasting
78 impact of LPS on hippocampal function (Tchessalova and Tronson, 2019). Thus, sex
79 differences in neuroimmune responses are likely contributor to gender bias in immune-
80 associated cognitive and affective disorders.
81 Despite incredible advances in psychoneuroimmunology over the past decade, but there
82 are critical gaps in our knowledge that preclude a holistic understanding of neuroimmune
83 function and its impacts on cognition and disease. First, studies have typically focused on a few
84 inflammatory cytokines (e.g., IL-1β, IL-6, and TNFα) critical for neuroimmune activation and its
85 effects on cognition. Yet more recently, it has become clear that the massive, coordinated
86 cytokine response observed in the periphery also occurs in the CNS (Kronfol and Remick, 2000;
87 Speirs and Tronson, 2018). Which other cytokines are activated, their roles in neural function,
88 and the similarities and differences of this response in males versus females, are yet to be
89 defined (Donzis and Tronson, 2014). Second, the bulk of studies aimed at understanding
90 neuroimmune activation and its behavioral sequelae have used the gram-negative bacterial
91 shell and toll-like receptor 4 (TLR4) agonist LPS. Nevertheless, viral illnesses also trigger
92 changes in behavior, cognition, and emotional states. Given that viruses act through distinct toll-

like receptors, their impact is likely mediated by a different, albeit overlapping, pattern of cytokine activation compared with LPS or bacterial triggers. Third, due to its relevance for disease states, many *in vivo* studies of neuroimmune function use a peripheral immune challenge. Here, neuroimmune activation is primarily driven by peripheral immune signals that infiltrate the brain (Watkins et al., 1995). This complicates the interpretation of whether sex differences in cytokine levels observed in the brain are due to indirect effects based on sex differences in peripheral immune response, or to direct effect of sex differences in neuroimmune function.

In this study, we aimed to identify a broad set of inflammatory cytokines induced in the hippocampus by direct neuroimmune stimulation *via* central administration of poly I:C in both males and females. We demonstrate that poly I:C induces fever, weight loss, and changes in mRNA expression and protein levels cytokines, chemokines, and markers of glial activation across a 24-hour period in both sexes. Notably, only IFNα and IFNɣ showed male-specific patterns of activation after central poly I:C administration.

2. METHODS

2.1 Animals

A total of 99 male and female 8-9 week-old C57BL/6N mice from Envigo (Indianapolis, IN) were used in these experiments. For all experiments, mice were individually housed in standard mouse cages with *ad libitum* access to food and water in a room with maintained temperature and pressure under a 12:12-hour light:dark cycle. All mice had at least one week of acclimation to the colony room prior to any manipulations. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC).

2.2 Stereotaxic Surgeries

Bilateral guide cannulae (PlasticsOne, Roanoke, VA) targeting the lateral ventricles were

119 implanted using standard stereotaxic methods (KOPF, Tujunga, CA) at the following
120 coordinates relative to Bregma: ML: +/- 1.00mm, AP: 0.30mm, DV: -2.50mm. Animals were
121 administered a pre-surgical analgesic (5mg/kg Carprofen, subcutaneous) and anesthetized for
122 surgery using an intraperitoneal injection of 250mg/kg of Avertin (2,2,2-tribromoethanol) which
123 maintained a surgical plane of anesthesia for the duration of the craniotomy. Bilateral holes
124 were drilled into the skull at the above coordinates, and guide cannulae were implanted using
125 dental cement. Animals were given a second dose of Carprofen (5mg/kg, subcutaneous) 24
126 hours after surgery to maintain a total of 48 hours of analgesia. Mice were monitored daily for 10
127 days post-operative and were given at least 2 weeks to recover from surgery prior to use in
128 experiments.
129
130 **2.3 Poly I:C Administration**
131 Polyinosinic:polycytidylic acid (poly I:C; Cat. No. P1530; Sigma-Aldrich, St. Louis, MO)
132 was prepared according to the manufacturer's instructions and sterile-filtered using a 0.22μm
133 filter prior to administration. We infused 20μg of poly I:C (2μL of 10μg/μL poly I:C) (Zhu et al.,
134 2016) or an equal volume of 0.9% sterile saline *via* the implanted guide cannula under brief
135 isoflurane anesthesia.
136
137 **2.4 Sickness Behavior Assessment**
138 To determine whether poly I:C increased physiological measures of sickness in males
139 and females, body weights and rectal temperatures (RET-3; Physitemp, Clifton, NJ) were
140 assessed at 2, 4, 6, 12, 24, and 48 hours following ICV administration of poly I:C (n = 10 male; n
141 = 9 female) or sterile saline (n = 10 male; n = 8 female; Figure 2A). Visual and behavioral
142 measures of sickness (piloerections, squinted eyes, hunched posture, and low responsivity)

143 were assessed throughout (Hart, 1991). No changes in overt sickness behaviors were observed
144 for any experiment (data not shown).
145
146 **2.4.1. Statistical Analysis of Sickness Behaviors**
147 Analysis of body weight and temperature changes in response to poly I:C was
148 completed using a mixed repeated-measures ANOVA, using time post-infusion as the within-
149 subjects factor and treatment and sex as the between-subjects factors with Greenhouse-
150 Geisser corrections for sphericity. Significant main effects and interactions ($p < 0.05$) were
151 followed up using post-hoc tests with Bonferroni corrections for multiple comparisons, and effect
152 sizes were calculated using the partial eta squared method.
153
154 **2.5 Characterization of the Acute Neuroimmune Response**
155 We used RNA and protein endpoints to examine induction of cytokines and glial
156 activation markers. Males and females were treated with either poly I:C (*n* = 22 male; *n* = 24
157 female) or sterile saline (*n* = 8/sex) and brains collected 0.5 hours (*n* = 5 male; *n* = 6 female), 2
158 hours (*n* = 6/sex), 4 hours (*n* = 5 male; *n* = 6 female), and 24 (*n* = 6/sex) hours later. All animals
159 were transcardially perfused with 0.1M phosphate buffer to remove circulating blood from the
160 brain. Both hemispheres of dorsal hippocampus tissue were collected in separate RNase-
161 /Dnase-free, sterile microcentrifuge tubes and immediately flash frozen. All samples were stored
162 at -80°C before tissue processing.
163
164 **2.5.1 Quantitative Real-Time PCR**
165 One hemisphere of dorsal hippocampal tissue per mouse was processed for gene
166 expression analysis using quantitative real-time PCR. Frozen samples were homogenized and
167 messenger RNA (mRNA) was extracted (PureLink RNA Mini Kit; Cat. No. 12183020; Invitrogen,
168 Carlsbad, CA), under sterile, RNase-free conditions. RNA quality was assessed using gel

169 electrophoresis, and UV spectroscopy was used to assess RNA purity (A260/280 > 1.80) and
170 quantity (BioSpectrometer Basic; Eppendorf, Hamburg, Germany). Any genomic DNA in the
171 sample was removed using DNase treatment, and 800ng of cDNA was synthesized from each
172 mRNA sample (QuantiTect Reverse Transcriptase Kit; Cat. No. 205314; Qiagen, Hilden,
173 Germany). Any samples that did not have a high enough concentration of RNA to make 800ng
174 of cDNA were removed from further analyses (n = 3 male; n = 5 female). Relative gene
175 expression was measured using Power SYBR Green PCR Master Mix (Cat. No. 4368702;
176 Applied Biosystems, Foster City, CA) in 10μL reactions (ABI 7500 real-time PCR system; Cat.
177 No. 4351105; Applied Biosystems).
178 We measured expression of four commonly-used housekeeping genes: *18s*, *gapdh*,
179 *hprt1*, and *rplp0* (all QuantiTect Primer Assays: *18s* Cat. No. QT02448082, *gapdh* Cat. No.
180 QT01658692, *hprt1* Cat. No. QT00166768, *rplp0* Cat. No. QT00249375; Qiagen). We analyzed
181 the relative expression of the following genes of interest: *ccl2*, *cd11b*, *cxcl10*, *gfap*, *ifnα*, *ifnβ*,
182 *ifnγ*, *il-1α*, *il-1β*, *il-6*, *il-10*, and *tnfα*. The gene primer for *il-1α* was a QuantiTect Primer Assay
183 (Cat. No. QT00113505; Qiagen). The sequences for the remaining gene primers can be found
184 in Table 1 and were ordered through Integrated DNA Technologies and diluted to 0.13 μM to be
185 used for PCR. All Qiagen primers were diluted as per the manufacturer's instruction.
186

187 **Table 1.**

188 *Primer sequences used for real-time PCR.*

Gene Target	Forward Primer Sequence (5' to 3')	Reverse Primer Sequence (5' to 3')	NCBI Reference Sequence
ccl2	CCACAACCACCTCAAGCACT	AAGGCATCACAGTCCGAGT	NM_011333.3
cd11b	CGTGAATGGGGACAAACTGAC	GCACTGAGGCTGGCTATTGA	NM_008401.2
cxcl10	TCCATCACTCCCCTTTACCCA	TGGCTTGACCATCATCCTGC	NM_021274.2
gfap	AAACCGCATCACCATTCCTG	CCCGCATCTCCACAGTCTTTA	NM_010277.3
ifnα	AGAGAAGAAACACAGCCCCT	AGCACATTGGCAGAGGAAGA	NM_010502.2
ifnβ	GCTCCAAGAAAGGACGAACAT	GGATGGCAAAGGCAGTGTAA	NM_010510.1
ifnγ	GTCAACAACCCACAGGTCCA	CGACTCCTTTTCCGCTTCCT	NM_008337.4
il-1β	TGCCACCTTTTGACAGTGATG	GCTCTTGTTGATGTGCTGCT	NM_008361.4
il-6	GAGACTTCCATCCAGTTGCCT	TCATTTCCACGATTTCCCAGAG	NM_001314054.1
il-10	CTGGACAACATACTGCTAACCG	AATGCTCCTTGATTTCTGGGC	NM_010548.2
tnfα	ACCCCTTTACTCTGACCCCTT	ACTGTCCCAGCATCTTGTGT	NM_001278601.1

189

190 **2.5.1.1 Housekeeping Gene Stability Analysis**

191 To control for the transcriptional activity of the samples being analyzed, we confirmed
192 the stability of four housekeeping genes (*18s*, *gapdh*, *hprt1*, and *rplp0*) using four techniques.
193 First, we assessed the variability of the candidate genes by measuring the standard deviation of
194 the raw quantification cycle (Cq) values from all samples (Figure 1A). We found that *18s* had the
195 largest standard deviation of Cq values (1.540), followed by *gapdh* (0.527), *rplp0* (0.225), and
196 *hprt1* (0.151; Figure 1B). By this approach, *rplp0* and *hprt1* show the greatest stability compared
197 to *18s* and *gapdh*, with *hprt1* exhibiting the lowest variability.

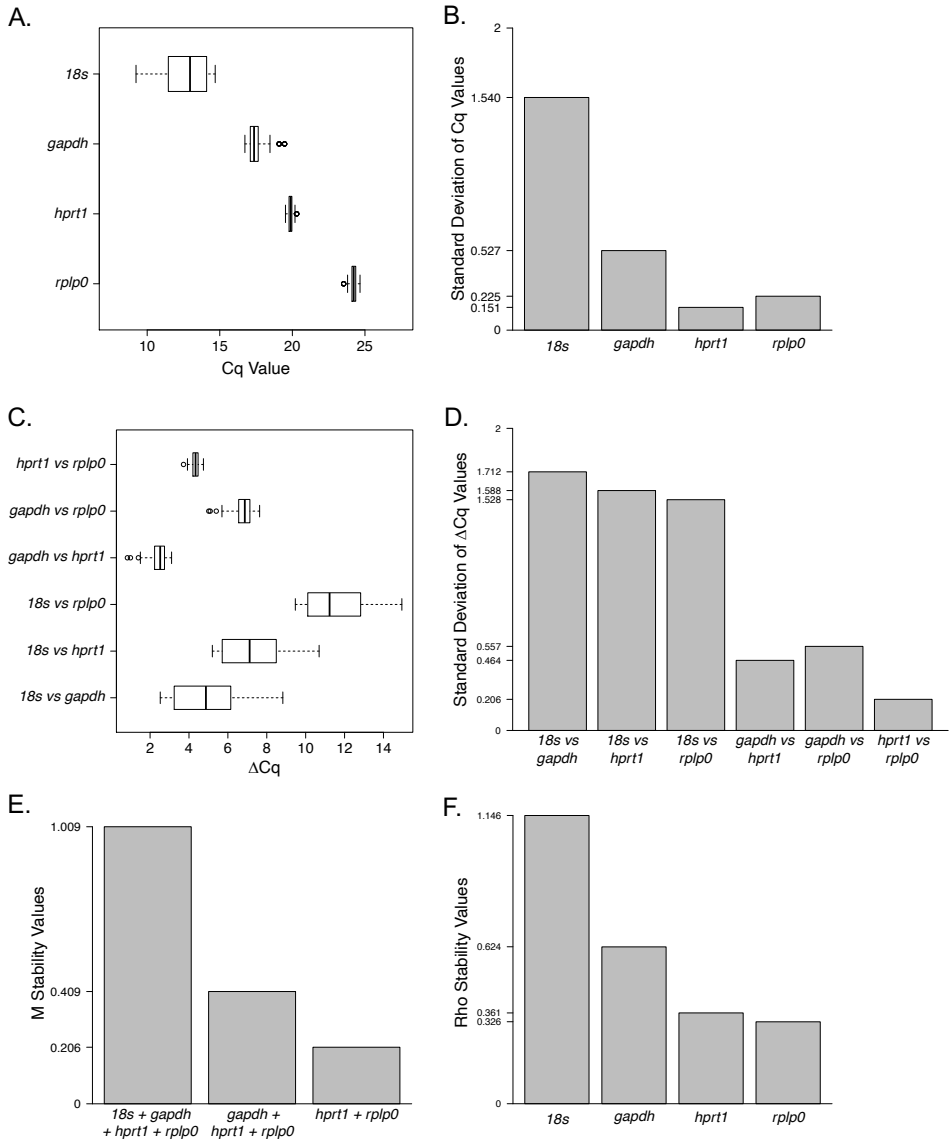
198 Second, we employed a comparative ΔCq approach in which the standard deviations of
199 the differences in Cq values (ΔCqs) between all possible pairs of candidate genes are
200 compared (Silver et al., 2006) (Figure 1C). From highest to lowest variability, the genes ranked

201 as follows: *18s* (1.609 average standard deviation), *gapdh* (0.911), *rplp0* (0.764), and *hprt1*
202 (0.753). Again, this method indicates that the most variable genes are *18s* and *gapdh* while the
203 most stable genes are *rplp0* and *hprt1*, and this is most apparent when considering the lowest
204 ΔCq standard deviation from this method is from the *rplp0* and *hprt1* comparison at 0.206
205 (Figure 1D).

206 The third method we employed was that developed by Vandesompele and colleagues,
207 which calculates the average pairwise variation of one candidate gene with all other candidate
208 genes (Vandesompele et al., 2002). We used R packages ReadqPCR and NormqPCR (Perkins
209 et al., 2012) to calculate *M* stability values, as depicted in Figure 1E. Consistent with the
210 previous methods, *hprt1* and *rplp0* are the most stable of the candidate genes, with the lowest
211 pairwise variability, *M* value, of 0.206.

212 Fourth, and last, we used a model-based stability analysis approach developed by
213 Andersen et al., an algorithm called NormFinder (v5) (Andersen et al., 2004). This method
214 protects against identifying two genes via the pairwise approach that might be misinterpreted as
215 being the most stable because they are coregulated. Using this method, again, *hprt1* and *rplp0*
216 were found to be the most stable genes with the lowest expression stability values (Figure 1F).
217 However, NormFinder resulted in *rplp0* having the lowest stability value of 0.326, indicating that
218 the model-based approach identified *rplp0* as the most stable gene.

219 Together, these methods identified the two most stable candidate housekeeping genes
220 as *hprt1* and *rplp0*. We calculated the geometric mean of the Cq values from *hprt1* and *rplp0* to
221 be used in the $2^{-\Delta\Delta Cq}$ method for calculations of relative expression for our target genes.
222



G.

	Cq Value Standard Deviations	Avg Standard Deviations of ΔCq Values	M Stability Values	Rho Stability Values
18s	1.5402440	1.609	1.609	1.1459049
gapdh	0.5266292	0.911	0.510	0.6235678
hprt1	0.1507111	0.753	0.206	0.3610396
rplp0	0.2245596	0.764	0.206	0.3256827

Figure 1. Housekeeping gene stability analysis. (A) Distribution of the quantification cycles (Cq) for housekeeping genes *18s*, *gapdh*, *hprt1*, and *rplp0***,** with **(B)** associated standard deviations. **(C)** Distribution of the difference of Cq values (ΔCq) between pairs of housekeeping genes, and **(D)** the associated standard deviations. **(E)** Stability values calculated using gene ratio method by Vandesompele et al., 2002, which uses stepwise elimination of lowest stability (highest *M* value) to rank gene stability. **(F)** Stability values calculated using a model-based approach by Andersen et al., 2004 which measures expression variation such that highest stability results in the lowest Rho value. **(G)** Summary of results from each of the four methods of housekeeping gene stability are shown.

233 **2.5.1.2. Statistical Analysis of mRNA Gene Expression**

234 For each PCR reaction, the quantification cycle (Cq) was determined, and the $2^{-\Delta\Delta Cq}$
235 method was used to calculate the relative gene expression of each gene. Any samples with
236 abnormal amplification curves, melt curves, and/or melt peaks across replicates were removed
237 from analyses (n = 1/sex).

238 Baseline sex differences in relative gene expression (qPCR) were assessed by
239 evaluating the male and female saline-treated groups. To directly compare these two groups in
240 the PCR analysis, the male saline-treated group was normalized to the female saline-treated
241 group and analyzed using independent, two-sample t-tests.

242 To analyze changes in relative gene expression (qPCR) across the 24-hour time course,
243 we normalized each group to their respective same-sex saline-treated group and used two-way
244 ANOVA tests using treatment and sex as factors. Significant main effects and interactions ($p <$
245 0.05) were followed up using post-hoc tests with Bonferroni corrections for multiple
246 comparisons, and effect sizes were calculated using the partial eta squared method.

247

248 **2.5.2 Multiplex Assays**

249 The second hemispheres of dorsal hippocampal tissue were processed as previously
250 described using low-detergent RIPA buffer sonication (Speirs and Tronson, 2018). Milliplex
251 magnetic bead panel assays (CCL2, CXCL10, IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, and IL-10;
252 Millipore Sigma, Burlington, MA) were used as per manufacturer's instructions. Cytokine
253 concentrations were calculated as pg/mg of hippocampal tissue via Luminex software. Only
254 samples that showed readable bead counts according to the Luminex software were included in
255 the analyses.

256

257 **2.5.2.1. Statistical Analysis of Protein Levels**

258 Baseline sex differences in protein levels from multiplex assays were analyzed with

independent, two-sample t-tests comparing the saline-treated groups and two-way ANOVA tests using treatment and sex as factors were used. Significant main effects and interactions ($p < 0.05$) were followed up using post-hoc tests with Bonferroni corrections for multiple comparisons, and effect sizes were calculated using the partial eta squared method.

2.6 Data Visualization and Statistical Software

Data visualization and statistical analyses were completed using R 3.6.2 (R Core Team, 2019) with the following packages: dplyr (v0.8.5; Wickham et al., 2020), tidyr (v1.0.2; Wickham and Henry, 2020), rstatix (v0.5.0; Kassambara, 2020), DescTools (v0.99.34; Signorell et al., 2020), sjstats (v0.17.9; Lüdecke, 2020), ReadqPCR and NormqPCR (Perkins et al., 2012), ggplot2 (Wickham, 2016), gridExtra (v2.3; Auguie, 2017), pheatmap (v1.0.12; Kolde, 2019), and viridis (v0.5.1; Garnier, 2018).

3. RESULTS

3.1 Central administration of poly I:C induces physiological sickness responses

Both females and males showed physiological responses to poly I:C. Whereas both saline- and poly I:C-treated animals showed changes in weight across the 48-hour period (Figure 2B, main effect of Time: $F(3.13, 96.92) = 28.899$, $p < 0.001$, $\eta^2_p = 0.482$), poly I:C caused weight loss in both sexes (main effect of Treatment: $F(1, 31) = 8.781$, $p = 0.006$, $\eta^2_p = 0.221$; trend towards a Time x Treatment interaction: $F(3.13, 96.92) = 2.476$, $p = 0.064$, $\eta^2_p = 0.074$). Specifically, males and females treated with poly I:C lost significantly more weight than the saline-treated animals at the 12- ($p = 0.004$) and 24-hour ($p = 0.022$) time points. By 48 hours post-treatment, the weights of poly I:C-treated animals had recovered and were no longer different from those of saline-treated animals ($p = 1.00$).

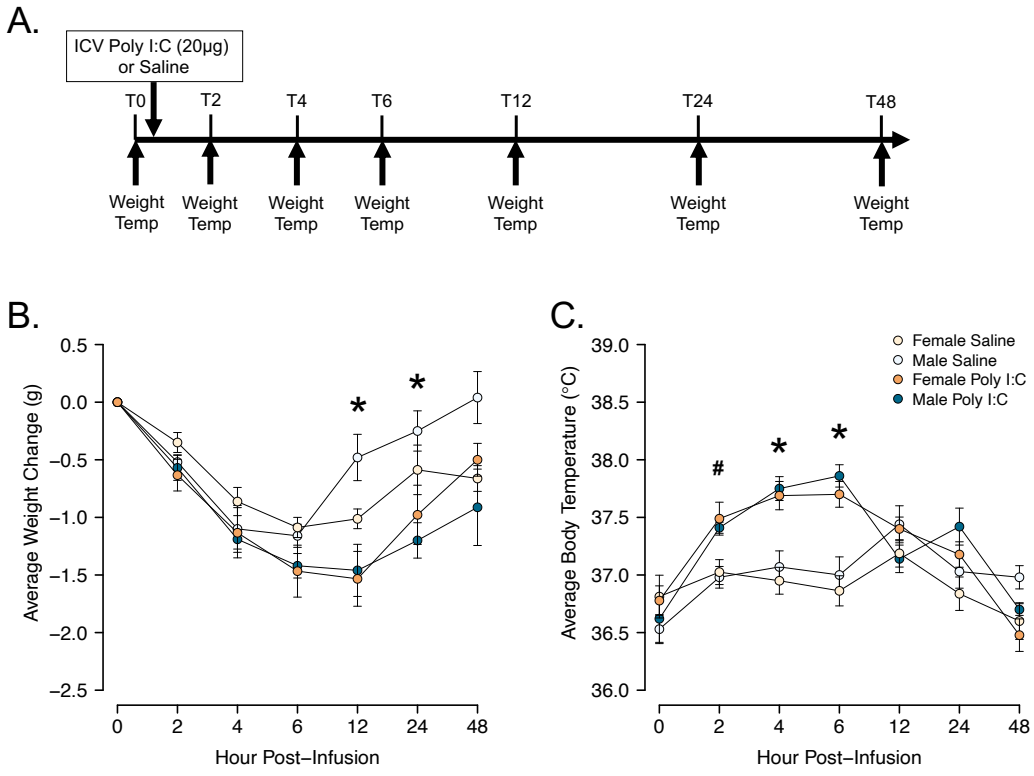


Figure 2. Analysis of sickness behaviors following poly I:C administration. (A) Timeline of body weight and temperature measurements following poly I:C or sterile saline administration. **(B)** Average weight change from baseline (Time = 0 hour) prior to treatment. **(C)** Average body temperature as measured via rectal thermometer. Analyzed using mixed repeated-measures ANOVA. **#**: trend towards significance with $p < 0.08$ poly I:C- vs saline-treated groups; *****: $p < 0.05$ poly I:C- vs saline-treated groups.

In both males and females, poly I:C caused significant increases in body temperature relative to the saline-treated group (Figure 2C; main effect of Treatment: $F(1, 31) = 23.759$, $p < 0.001$, $\eta^2_p = 0.434$; Time x Treatment interaction: $F(4.6, 142.62) = 11.635$, $p < 0.001$, $\eta^2_p = 0.273$). Post-hoc tests revealed that body temperature began to increase 2 hours following poly I:C ($p = 0.068$), remained elevated at the 4- ($p < 0.001$) and 6-hour ($p < 0.001$) time points, and recovered to saline-treated body temperatures by 12 hours post treatment (all $p = 1.00$).

299 **3.2 Baseline sex differences in mRNA expression and protein levels of select**
300 **hippocampal immune molecules**

301 **3.2.1. Interleukins**

302 Select immune signaling markers showed baseline sex differences. mRNA expression of
303 interleukin *(il)-1α* exhibited a trend towards a sex difference in baseline expression (Figure 3C1;
304 $t(12) = 2.006$, $p = 0.068$), and *il-6* showed a significant sex difference (Figure 3E1; $t(11) = 3.079$,
305 $p = 0.01$, 95% CI [0.182, 1.062]). These results did not hold true for protein levels of IL-1α or IL-
306 6. Rather, multiplex data showed that protein levels of IL-1β and IL-10 were significantly
307 different between saline-treated males and females (Figures 4D and 4F, respectively; IL-1β:
308 $t(13) = 4.275$, $p = 0.001$, 95% CI [5.682, 17.291]; IL-10: $t(13) = 2.236$, $p = 0.044$, 95% CI [0.672,
309 39.314]).

310 **3.2.2. Interferons**

311 Two interferons (IFN), *ifnα* and *ifnγ*, also showed baseline sex differences in relative
312 mRNA expression (Figures 3G1 and 3I1: IFNα: $t(12) = 5.546$, $p = 0.0001$, 95% CI [0.441, 1.01];
313 IFNγ: $t(11) = 2.995$, $p = 0.012$, 95% CI [0.259, 1.694]). Likewise, protein levels of IFNγ exhibited
314 a sex difference in saline-treated groups (Figure 4G; $t(14) = 6.475$, $p < 0.001$, 95% CI [6.534,
315 13.006]).

316 **3.2.3. Chemokines**

317 Expression of CC chemokine *ccl2* similarly followed this pattern at baseline (Figure 3K1;
318 $t(12) = 3.287$, $p = 0.006$, 95% CI [0.259, 1.279]), and this held true for protein levels of CCL2 as
319 well (Figure 4H; $t(12) = 2.751$, $p = 0.018$, 95% CI [12.798, 110.318]). Interestingly, all genes with
320 sex differences in saline-treated animals showed that both female baseline mRNA expression
321 and protein levels were at least twice as high as that of males.

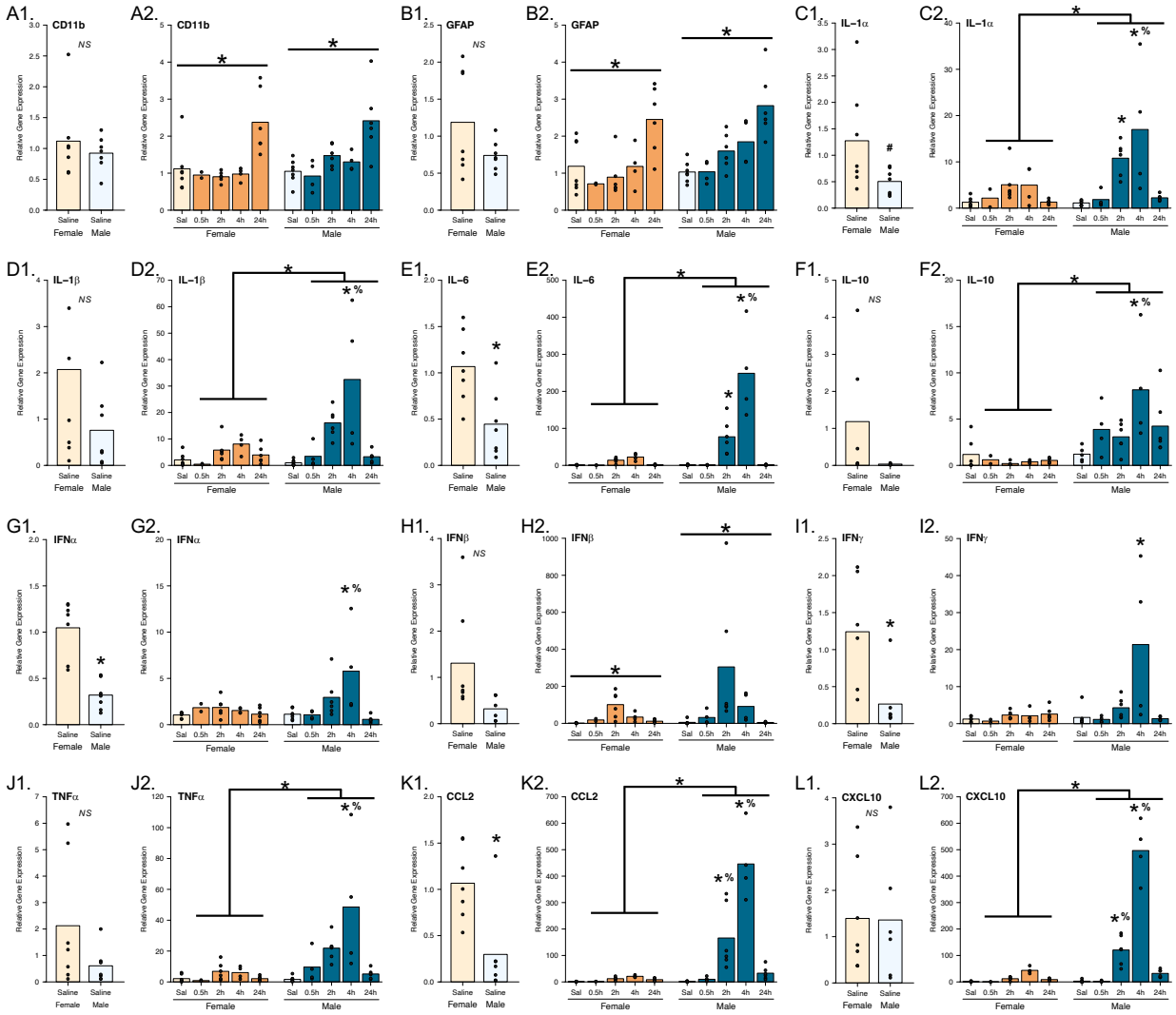


Figure 3. mRNA gene expression of cytokines, chemokines, and markers of glial activation in the hippocampus. Baseline gene expression was measured by normalizing the male saline-treated group to the female saline-treated group and analyzed using independent, two-sample t-tests. Baseline expression of **(A1)** CD11b, **(B1)** GFAP, **(C1)** IL-1α, **(D1)** IL-1β, **(E1)** IL-6, **(F1)** IL-10, **(G1)** IFNα, **(H1)** IFNβ, **(I1)** IFNγ, **(J1)** TNFα, **(K1)** CCL2, and **(L1)** CXCL10 are shown. Gene expression changes following poly I:C treatment were calculated by normalizing time points after poly I:C treatment to the saline-treated groups within sex to eliminate confounding variables of baseline sex differences. Gene expression was analyzed using two-way ANOVA tests for **(A2)** CD11b, **(B2)** GFAP, **(C2)** IL-1α, **(D2)** IL-1β, **(E2)** IL-6, **(F2)** IL-10, **(G2)** IFNα, **(H2)** IFNβ, **(I2)** IFNγ, **(J2)** TNFα, **(K2)** CCL2, and **(L2)** CXCL10. **#**: trend towards significance with $p < 0.08$ vs females; ***** above a bracket indicates a significant main effect ($p < 0.05$); ***** above a single bar indicates a significant post-hoc test ($p < 0.05$) vs saline-treated group within sex; **%** above a single bar indicates a significant post-hoc test ($p < 0.05$) vs females at the same time point.

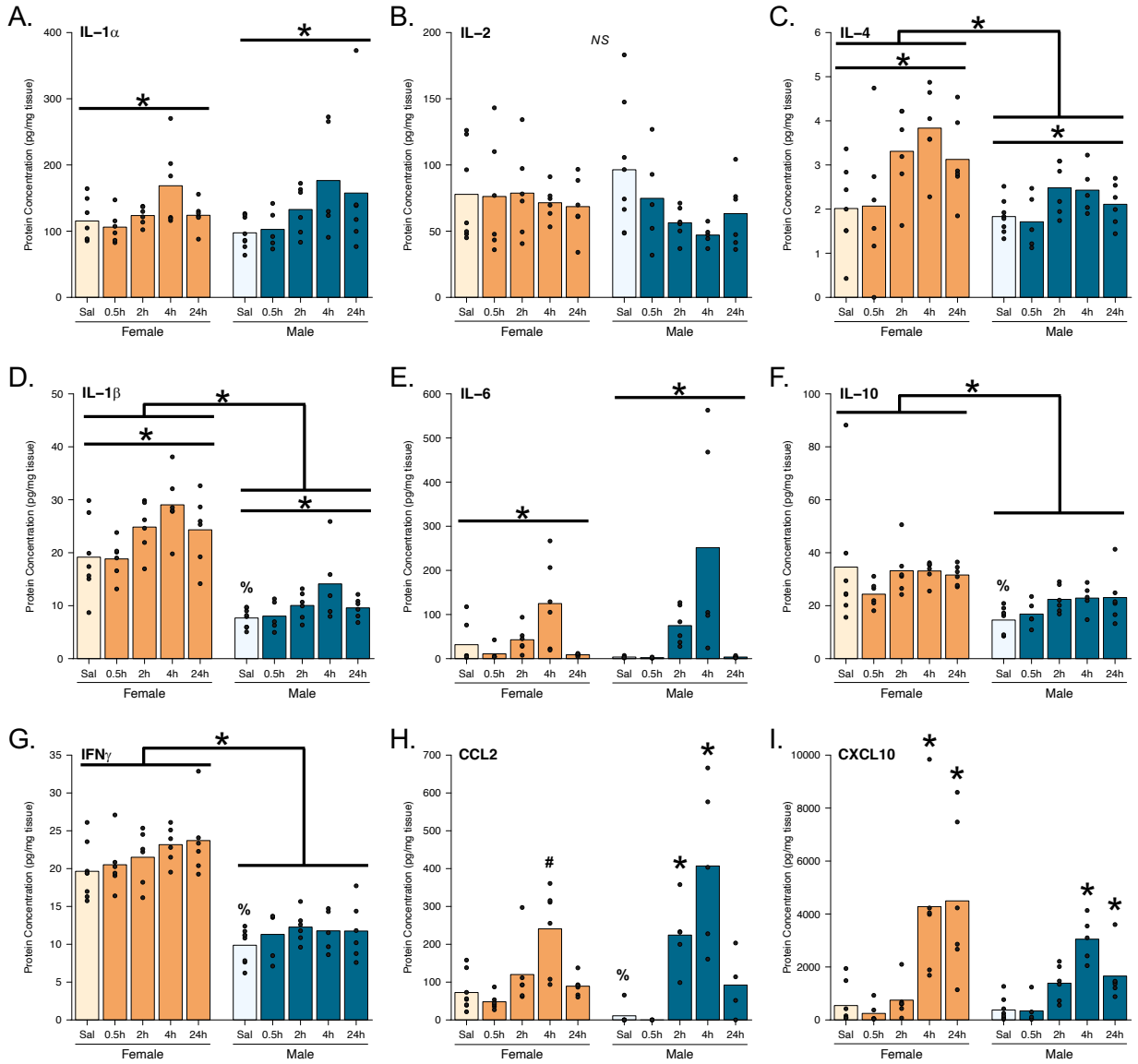


Figure 4. Protein levels of cytokines and chemokines in the hippocampus. Protein concentration (pg/mg) of **(A)** IL-1α, **(B)** IL-2, **(C)** IL-4, **(D)** IL-1β, **(E)** IL-6, **(F)** IL-10, **(G)** IFNγ, **(H)** CCL2, and **(I)** CXCL10 are shown. Two-way ANOVA tests were used to analyze these data. **#** above a single bar indicates a trend towards significance, with a post-hoc test result of $p < 0.08$ vs saline-treated group within sex; ***** above a bracket indicates a significant main effect ($p < 0.05$); ***** above a single bar indicates a significant post-hoc test ($p < 0.05$) vs saline-treated group within sex; **%** above a single bar indicates a significant post-hoc test ($p < 0.05$) vs females at the same timepoint.

3.2.4. Glial activation markers and other cytokines did not show baseline sex differences

It is equally important to note the markers that did not show sex differences at baseline.

350 Both the microglial activation marker *cd11b* and the astrocyte activation marker *gfap* showed
351 equivalent levels of gene expression in the saline-treated groups in both sexes (Figures 3A1
352 and 3B1, respectively; *cd11b*: $t(12) = 0.723$, $p = 0.483$; *gfap*: $t(12) = 1.603$, $p = 0.135$). Other
353 commonly examined inflammatory cytokines, including IL-2, IL-4, and tumor necrosis factor
354 (TNF)α similarly showed no sex difference at baseline (IL-2 p = 0.420; IL-4 p = 0.633; TNFα =
355 0.139). No other sex differences in mRNA gene expression or protein levels were observed
356 under baseline conditions.
357
358 **3.3 Central poly I:C causes greater increases in male mRNA expression of hippocampal**
359 **cytokines compared with females**
360 **3.3.1. Glial Activation Markers**
361 Poly I:C treatment significantly increased expression of both *cd11b* and *gfap*, although
362 this appeared to be true only at the 24-hour time point (Figures 3A2 and 3B2; *cd11b* main effect
363 of Treatment: $F(4, 42) = 12.96$, $p < 0.001$, $\eta^2_p = 0.552$; *gfap* main effect of Treatment: $F(4, 42) =$
364 12.992, $p < 0.001$, $\eta^2_p = 0.553$). Sex did not affect the response of either *cd11b* or *gfap* to poly
365 I:C (insignificant Sex x Treatment interactions: *cd11b*: $F(4, 42) = 0.684$, $p = 0.607$; *gfap*: $F(4, 42)$
366 $= 0.923$, $p = 0.460$).
367 **3.3.2. Interleukins**
368 Poly I:C caused increased expression of *il-1*α, *il-1*β, and *il-6* in both males and females
369 (Figures 3C2, 3D2, and 3E2; main effects of Treatment: *il-1*α: $F(4, 42) = 9.784$, $p < 0.001$, $\eta^2_p =$
370 0.482; *il-1*β: $F(4, 42) = 9.512$, $p < 0.001$, $\eta^2_p = 0.475$; *il-6*: $F(4, 42) = 22.28$, $p < 0.001$, $\eta^2_p =$
371 0.680). In males, expression began to increase at the 2-hour time point following poly I:C
372 treatment for *il-1*α ($p = 0.015$), *il-1*β ($p = 0.057$), and *il-6* (p = 0.029), showed peaks at the 4-
373 hour time point ($p < 0.001$ for all), and decreased to saline-treated levels by 24 hours ($p = 1.00$
374 for all). Each of these genes also showed an overall greater expression in males than females

375 (main effects of Sex: *il-1α*: $F(1, 42) = 6.398$, $p = 0.015$, $\eta^2_p = 0.132$; *il-1β*: $F(1,42) = 6.695$, $p =$
376 0.013, $\eta^2_p = 0.137$; *il-6*: $F(1, 42) = 21.1$, $p < 0.001$, $\eta^2_p = 0.334$), and a significantly greater
377 magnitude of response in males compared with females (Sex x Treatment interactions: *il-1α*:
378 $F(4, 42) = 3.103$, $p = 0.025$, $\eta^2_p = 0.228$; *il-1β*: $F(4, 42) = 4.288$, $p = 0.005$, $\eta^2_p = 0.290$; *il-6*: $F(4,$
379 $42) = 15$, $p < 0.001$, $\eta^2_p = 0.588$). Post-hoc tests revealed for all three genes, males exhibited an
380 even greater response at only the 4-hour time point compared with females ($p < 0.05$ for all).
381 Notably, the peak *il-1α* and *il-1β* expression in males was roughly 3-fold higher than that of the
382 peak female expression for these cytokines, and the *il-6* peak expression in males was more
383 than 10-fold higher than that of females (Figures 3C2, 3D2, and 3E2).
384 Males showed greater *il-10* gene expression across all time points compared with
385 females (Figure 3F2; main effect of Sex: $F(1, 39) = 25.642$, $p < 0.001$, $\eta^2_p = 0.397$). Additionally,
386 poly I:C significantly increased gene expression of *il-10* in males, but not females (Sex x
387 Treatment interaction: $F(4, 39) = 3.304$, $p = 0.02$, $\eta^2_p = 0.253$). Specifically, male expression of
388 *il-10* at the 4-hour time point following poly I:C was significantly greater than that of saline-
389 treated controls ($p = 0.001$), and this was also greater than the 4-hour expression in females (p
390 $= 0.001$).
391 **3.3.3. Tumor Necrosis Factor Alpha**
392 Gene expression of *tnfα* increased in response to poly I:C overall, males had
393 significantly higher expression than females overall, and males showed a greater magnitude of
394 response compared with females (Figure 3J2; main effect of Treatment: $F(4, 42) = 6.407$, $p =$
395 0.0004, $\eta^2_p = 0.379$; main effect of Sex: $F(1, 42) = 10.1$, $p = 0.003$, $\eta^2_p = 0.194$; Sex x Treatment
396 interaction: $F(4, 42) = 4.117$, $p = 0.007$, $\eta^2_p = 0.282$). Post-hoc tests showed that males 4 hours
397 post-treatment had significantly greater expression than those treated with saline ($p < 0.001$),
398 and this was again greater than the 4-hour peak expression in females ($p = 0.001$).
399 **3.3.4. Interferons**

400 Both *ifnα* and *ifnγ* showed a similar response pattern to poly I:C whereby males treated
401 with poly I:C exhibited a significant acute increase in gene expression of both cytokines, but
402 females did not show the same response (Figures 3G2 and 3I2; *ifnα*: main effect of Treatment:
403 $F(4, 42) = 5.007$, $p = 0.002$, $\eta^2_p = 0.323$; Sex x Treatment interaction: $F(4, 42) = 3.35$, $p = 0.018$,
404 $\eta^2_p = 0.242$; *ifnγ*: main effect of Treatment: $F(4, 40) = 4.698$, $p = 0.003$, $\eta^2_p = 0.32$; Sex x
405 Treatment interaction: $F(4, 40) = 4.178$, $p = 0.006$, $\eta^2_p = 0.295$). Specifically, 4 hours after poly
406 I:C treatment, males showed significantly elevated expression compared to the saline-treated
407 controls ($p = 0.0001$), and this was greater in magnitude than the 4-hour time point in females (p
408 $= 0.001$). Female *ifnα* and *ifnγ* did not seem to respond to poly I:C treatment at any time point.
409 In contrast, poly I:C transiently increased expression of *ifnβ* in both males and females,
410 and there were no sex differences seen in magnitude of expression increase (Figure 3H2; main
411 effect of Treatment: $F(4, 42) = 4.855$, $p = 0.003$, $\eta^2_p = 0.316$; insignificant Sex x Treatment
412 interaction: $F(4, 42) = 1.297$, $p = 0.287$). Unlike all other cytokines examined in this study, peak
413 expression appeared to be at the 2-hour time point, and expression began decreasing again by
414 4 hours post-treatment. The magnitude increase was also notable, with a 100-fold increase in
415 females and a 300-fold increase in males.

416 **3.3.5. Chemokines**

417 Poly I:C significantly increased the expression of both *ccl2* and *cxcl10* in males and
418 females, with a peak increase in expression at 4-hours post-infusion (Figures 3K2 and 3L2;
419 main effects of Treatment: *ccl2*: $F(4, 41) = 25.47$, $p < 0.001$, $\eta^2_p = 0.713$; *cxcl10*: $F(4, 42) =$
420 87.37, $p < 0.001$, $\eta^2_p = 0.893$). Also consistent with many other cytokines, expression of both
421 *ccl2* and *cxcl10* was greater overall in males compared with females (main effects of Sex: *ccl2*:
422 $F(1, 41) = 44.55$, $p < 0.001$, $\eta^2_p = 0.521$; *cxcl10*: $F(1, 42) = 92.79$, $p < 0.001$, $\eta^2_p = 0.688$). Finally,
423 males showed a markedly greater magnitude of response than did females for both chemokines
424 (Sex x Treatment interactions: *ccl2*: $F(4, 41) = 20.96$, $p < 0.001$, $\eta^2_p = 0.672$; *cxcl10*: $F(4, 42) =$

425 60.51, $p < 0.001$, $\eta^2_p = 0.852$). Remarkably, male *ccl2* expression peaked at nearly 450-fold
426 greater than the expression of saline-treated males compared to a roughly 20-fold increased
427 peak in females (Figure 3K2). Similarly, *cxcl10* expression in males peaked at nearly 500-times
428 that of saline-treated males while female *cxcl10* expression peaked at just over 40-times greater
429 than saline-treated females (Figure 3L2). These massive increases in gene expression are
430 reflected in the strong effect sizes noted for the interaction effect above. Post-hoc tests
431 confirmed that the male 2- and 4-hour time points post-treatment showed significantly greater
432 gene expression of both *ccl2* and *cxcl10* than saline-treated males ($p < 0.001$). Additionally,
433 both the male 2- and 4-hour time points of both genes proved to be significantly greater than the
434 2- and 4-hour time points in females, respectively ($p < 0.01$).

435 **3.4 Central poly I:C increases select hippocampal cytokine protein levels in males and**
436 **females**

437 **3.4.1. Interleukins**

438 We analyzed several members of the interleukin family of cytokines, including classically
439 inflammatory IL-1α, IL-1β, IL-2, IL-4, and IL-6, and classically anti-inflammatory IL-10. Of these,
440 IL-1α, IL-1β, IL-4, and IL-6 significantly increased following ICV poly I:C administration in both
441 males and females (Figures 4A, 4C, 4D, 4E; main effects of Treatment: IL-1α: $F(4, 51) = 3.523$,
442 $p = 0.013$, $\eta^2_p = 0.216$; IL-1β: $F(4, 51) = 5.721$, $p = 0.001$, $\eta^2_p = 0.31$; IL-4: $F(4, 51) = 5.146$, $p =$
443 0.001, $\eta^2_p = 0.288$; IL-6: $F(4,51) = 10.298$, $p < 0.001$, $\eta^2_p = 0.447$). In all cases, protein levels
444 appear to increase to a peak 4 hours following poly I:C, similar to the effects seen in mRNA
445 expression. In addition, both IL-1β and IL-4 also exhibited a main effect of Sex such that protein
446 levels of these cytokines, regardless of time point, were significantly higher in females
447 compared with males (Figures 4C and 4D; IL-1β: $F(1, 51) = 114.226$, $p < 0.001$, $\eta^2_p = 0.691$; IL-
448 4: $F(1, 51) = 11.03$, $p = 0.002$, $\eta^2_p = 0.178$). No interactions of sex and treatment were found for
449 any of the interleukin cytokines examined here (IL-1α: $F(4, 51) = 0.446$, $p = 0.775$; IL-1β: $F(4,$

450 51) = 0.513, p = 0.726; IL-4: $F(4, 51) = 0.982$, p = 0.426; IL-6: $F(4, 51) = 1.779$, p = 0.148).
451 Neither IL-2 nor IL-10 showed any effects of poly I:C treatment in either sex (Figures 4B and 4F;
452 insignificant main effects of Treatment: IL-2: $F(4, 51) = 1.498$, p = 0.217; IL-10: $F(4, 51) = 1.122$,
453 p = 0.357). Similar to IL-1β and IL-4, though, we found that females had overall higher levels of
454 IL-10 than did males (main effect of Sex; $F(1, 51) = 20.27$, $p < 0.001$, $\eta^2_p = 0.284$).

455 **3.4.2. Interferon**

456 We also measured IFNγ levels in response to poly I:C. Unlike mRNA expression, IFNγ
457 protein levels did not change following poly I:C administration in either sex (Figure 4G;
458 insignificant main effect of Treatment: $F(4, 52) = 1.93$, p = 0.119). However, IFNγ levels were
459 higher in females relative to males (main effect of Sex: $F(1, 52) = 150.64$, $p < 0.001$; $\eta^2_p =$
460 0.743). This was consistent with mRNA expression data where saline-treated females also
461 showed significantly higher expression of *ifnγ* at baseline than did males (see Figure 3Q).

462 **3.4.3. Chemokines**

463 Finally, we measured protein levels of the chemokines CCL2 and CXCL10 in both
464 sexes. Both chemokines were significantly affected by central administration of poly I:C and in
465 different ways in males and females (Figures 4H and 4I; CCL2: main effect of Treatment: $F(4,$
466 $46) = 18.517$, $p < 0.001$, $\eta^2_p = 0.617$; Sex x Treatment interaction: $F(4, 46) = 3.381$, p = 0.017,
467 $\eta^2_p = 0.227$; CXCL10: main effect of Treatment $F(4, 52) = 14.54$, $p < 0.001$, $\eta^2_p = 0.528$; Sex x
468 Treatment interaction: $F(4, 52) = 2.796$, p = 0.035, $\eta^2_p = 0.177$). In males, CCL2 levels increased
469 earlier (at 2 hours) post-infusion relative to females. For CXCL10, females took longer for
470 protein levels to begin to decrease as compared to the time course in males, with females still
471 showing the massive elevation at 24 hours post-infusion as they did at 4 hours.

472 Notably, both CCL2 and CXCL10 levels showed the most substantial increases out of all
473 cytokines measured in protein analysis in the hippocampus. Specifically, female CCL2 levels
474 induced by poly I:C peaked at approximately 4 times that of the saline-treated animals, and

475 male CCL2 levels peaked nearly 8 times that of saline-treated males (Figure 4H). More
476 remarkably, female CXCL10 levels rose roughly 16-fold by 4 hours after poly I:C administration,
477 and males showed a 12-fold increase by 4-hours (Figure 4I).
478
479 **3.5. Summary**
480 Ultimately, we observed that most, but not all, protein levels of cytokines and
481 chemokines examined here responded to central administration of poly I:C in males and
482 females. Of those that did respond (IL-1α, IL-1β, IL-4, IL-6, CCL2, and CXCL10), peak protein
483 levels were seen at 4 hours following poly I:C treatment. Overall, the pattern of immune
484 response as measured through protein was similar between males and females, with only 2
485 chemokines, CCL2 and CXCL10, showing sex differences in the time course of response.
486 Importantly, these data largely overlap with the timing and patterns of response seen in the
487 same genes measured using relative mRNA expression (see Figures 5A and 5B).

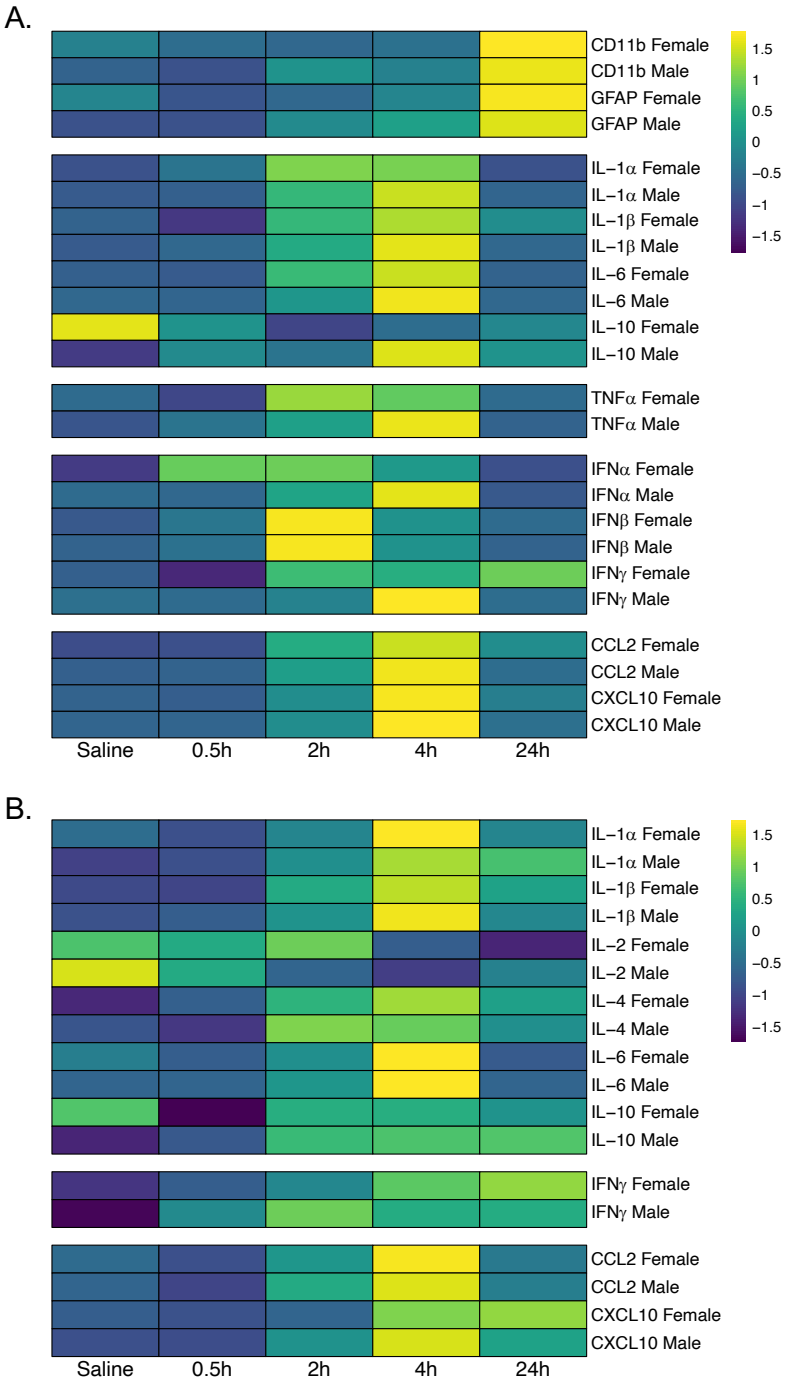


Figure 5. Heatmaps of gene expression and protein levels in the hippocampus. Changes in **(A)** mRNA gene expression and **(B)** protein levels for cytokines, chemokines, and markers of glial activation are shown. Values are centered and scaled across rows to highlight changes across the time course for each gene of interest; thus, differences in magnitude between genes are not depicted.

496 **4. DISCUSSION**

497 Here we demonstrated that central administration of poly I:C induced both physiological
498 measures of sickness and concomitant neuroinflammatory activation in both male and female
499 mice. Both males and females treated with poly I:C mounted an acute fever response and
500 showed weight loss that recovered to saline-treated levels 48 hours later. Additionally, both
501 sexes showed acute cytokine and chemokine responses in the hippocampus, as measured by
502 both mRNA expression and protein levels, that followed the time course of fever. Whereas most
503 neuroimmune activation markers were induced in both sexes, *il-10*, *ifn*α, and *ifn*γ increased in
504 males only. Given the specific role of type I interferons in viral response (Seth et al., 2006;
505 Stetson and Medzhitov, 2006; Teijaro, 2016), this sex difference may have important
506 implications for how neuroimmune activation differentially disrupts cognition in males and
507 females.

508 Poly I:C treatment in both sexes resulted in a significant and transient increase in gene
509 expression and protein levels of most, but not all, cytokines and chemokines in the
510 hippocampus, including IFNβ, IL-1α, IL-1β, IL-6, TNFα, CCL2, and CXCL10. That administration
511 of an immune stimulant, including viral mimics such as poly I:C, induces a neuroimmune
512 response is not new; however, most of the previous studies on poly I:C used peripheral
513 administration (Cunningham, Campion, Teeling, Felton, & Perry, 2007a; Fortier et al., 2004;
514 Kamerman, Skosana, Loram, Mitchell, & Weber, 2011; Murray et al., 2015). As such, multiple,
515 indirect mechanisms are likely involved in causing inflammation in the brain (Watkins et al.,
516 1995). Peripheral administration of poly I:C, specifically, was found to induce neuroinflammation
517 through a separate and distinct pathway than central administration (Zhu et al., 2016).
518 Additionally, these studies examined a subset of cytokines or chemokines, including the most
519 commonly examined IL-1β, IL-6, and TNFα, and type I interferons that typically respond to
520 viruses. Given evidence of mechanistic complexities governing neuroinflammation, particularly

521 from stimulants such as poly I:C, and given that there are over 300 cytokines with important
522 roles in the immune system and neural function, it is critical to begin looking beyond IL-1β, IL-6,
523 and TNFα and more strongly consider implications of such limits in experimental design for the
524 field of psychoneuroimmunology.

525 In addition to the intricacies of how immune stimulants such as poly I:C activate the
526 neuroimmune system, males and females differ in immune and neuroimmune responses. The
527 direction of these differences depends on whether you are looking in the periphery (Klein and
528 Flanagan, 2016) or the brain (Loram et al., 2012; Santos-Galindo et al., 2011). However, sex
529 differences in peripheral immune function are not just about size of the response, but also the
530 type of response. For example, women show stronger inflammatory responses to vaccines
531 (Klein and Flanagan, 2016) and greater rates of antibody-mediated autoimmune disorders (e.g.,
532 multiple sclerosis and systemic lupus erythematosus). In contrast, men are at greater risk of an
533 exaggerated and pathological acute inflammatory response and "cytokine storm" and show
534 greater risk of cell-mediated autoimmune disorders (e.g., myocarditis and ankylosing
535 spondylitis) (Fairweather et al., 2008). Additional considerations include accounting for levels of
536 inflammatory signals at baseline compared with the responsiveness to immune triggers. Here
537 we observed that males showed greater responsiveness to poly I:C, and females had higher
538 basal levels of cytokines prior to poly I:C treatment, emphasizing the complexity of
539 neuroimmune processes.

540 Viral infections are not immune to observations of sex differences. Specifically, males
541 and females show sex differences in prevalence, intensity, and survival outcomes of viruses
542 (Barna et al., 1996; Klein and Flanagan, 2016; Klein and Huber, 2010). Many report that when
543 females show better survival outcomes or seem more protected against certain viruses, it is due
544 to the female immune system having a greater anti-viral response that allows the virus to be
545 cleared faster (Barna et al., 1996; Channappanavar et al., 2017; Klein, 2012; vom Steeg and

546 Klein, 2016). Data on SARS-CoV-2, the virus that causes the coronavirus disease 2019
547 (COVID-19), has showed a similar sex difference in intensity of infection, with more males
548 having worse symptoms, including neurological complications, and survival outcomes relative to
549 females (Chen et al., 2020; Gebhard et al., 2020; Guan et al., 2020; Klein et al., 2020; Onder et
550 al., 2020). However, in contrast to the data that suggests a greater immune response results in
551 faster recovery, recent studies indicate that higher levels of inflammatory cytokines including IL-
552 6, IL-1β, CXCL10, and CCL2 are correlated with worse COVID-19 outcomes (Liu et al., 2020;
553 Takahashi et al., 2020). Unequivocally, sex matters in the context of immune and neuroimmune
554 responses as well as in the acute and chronic implications of neuroimmune modulation of neural
555 function, cognition, and emotion in males and females.

556 Similar to extant sex differences in immune and neuroimmune responses, we observed
557 that most cytokines examined, including IL-1α, IL-1β, IL-6, IL-10, IFNα, TNFα, CCL2, and
558 CXCL10 showed a stronger response in males compared with females. Whereas others have
559 reported increases in select inflammatory markers following poly I:C treatment, these studies
560 used either only used male (Fortier et al., 2004; Kamerman et al., 2011) or female rodents
561 (Cunningham et al., 2007b; Murray et al., 2015). To the best of our knowledge, this is the first
562 direct comparison of hippocampal cytokines in males and females as a consequence of poly
563 I:C. Whether the greater magnitude in male response to poly I:C indicates greater
564 neuroprotection or vulnerability remains to be determined. Additionally, whether sex differences
565 in the cytokine and chemokine responses observed here result in functional differences in
566 modulation of neural function, including cognition and emotion, is a critical knowledge gap yet to
567 be addressed.

568 We observed that most cytokines examined showed a greater magnitude of response in
569 males compared with females. This is consistent with previous findings that male-derived
570 astrocytes have a greater reaction to inflammatory insults compared with females (Astiz et al.,

571 2014; Chistyakov et al., 2018; Loram et al., 2012; Santos-Galindo et al., 2011). Poly I:C is
572 recognized by microglia, astrocytes, and neurons via toll-like receptor 3 (TLR3) (Alexopoulou et
573 al., 2001; Kielian, 2006; Matsumoto and Seya, 2008). The interaction of these 3 cell types is
574 crucial in mediating inflammatory responses (Cerbai et al., 2012; Scholz and Woolf, 2007).
575 Given that TLR3 shows much greater expression in astrocytes relative to microglia (Bsibsi et al.,
576 2002), the reaction of astrocytes in males may be driving the sex differences in magnitude gene
577 expression response of cytokines following poly I:C. The astrocyte activation marker, GFAP,
578 and the microglial activation marker, CD11b, did not increase until 24 hours after poly I:C
579 treatment and did not show sex differences. However, this does not absolve astrocytes or
580 microglia from the acute response to poly I:C. Specifically, Norden and colleagues found that
581 cytokine gene expression from both astrocytes and microglia preceded increases in astrocyte
582 and microglial activation markers, (GFAP and Iba1, respectively), and that these activation
583 markers similarly did not show reliable increases until the 24-hour time point (Norden et al.,
584 2016). It is likely, therefore, that the greater astrocytic reaction in males is driving the greater
585 magnitude of response to poly I:C by males.

586 The interferon family of cytokines showed a sex-specific pattern of expression in the
587 hippocampus. Specifically, males showed increases in IFNα, IFNβ, and IFNγ, but females only
588 showed a significant response in IFNβ. This is consistent with previous findings that showed
589 females increased gene expression of IFNβ, but not IFNα, in response to peripheral poly I:C,
590 though this study did not measure these effects in males for comparison (Murray et al., 2015).
591 Type I interferons, IFNα and IFNβ, are key to the anti-viral response of the immune system and,
592 as such, are known to respond to viral stimulants including poly I:C (Costello and Lynch, 2013;
593 Scumpia et al., 2005; Seth et al., 2006; Stetson and Medzhitov, 2006; Teijaro, 2016). Consistent
594 with our data, in which IFNβ showed an early peak expression levels, type I interferon activity is
595 responsible for inducing inflammatory cytokines such as IL-6 and TNFα (Fortier et al., 2004;

596 Murray et al., 2015). Additionally, interferon signaling from poly I:C treatment also results in
597 altered glutamatergic signaling (Costello and Lynch, 2013; Scumpia et al., 2005), which is
598 known to be critical for hippocampal memory formation (Katagiri et al., 2001). Given that males
599 induce both IFNα and IFNβ following poly I:C whereas females only induce IFNβ, interferons
600 have important implications for sex differences in neuroimmune disruption of cognition from
601 virus or viral-like stimulants.

602 Taken together, this study characterizes the sickness and neuroimmune responses to
603 central administration of poly I:C, and we observed sex-specific patterns of hippocampal
604 cytokine responses. Specifically, we identified type I interferons as one potential node mediating
605 sex-specific cytokine responses and neuroimmune effects on synaptic plasticity and cognition.
606 Additionally, the magnitude of response of cytokines such as CCL2 and CXCL10 highlight the
607 importance of examining a comprehensive set of inflammatory markers using multiple
608 endpoints. Neuroimmune activation is known to play a role in cognitive deficits and affective
609 dysregulation in diseases such as Alzheimer's Disease and other dementias (Krstic and
610 Knuesel, 2013), Post-Traumatic Stress Disorder (Pace and Heim, 2011; Wang and Young,
611 2016), depression (Bekhbat and Neigh, 2018; Hodes et al., 2015), and now even in COVID-19
612 (Zhou et al., 2020). Given the sex/gender biases in prevalence, severity, and/or survival
613 outcomes, identifying sex-specific neuroimmune responses will provide novel targets for
614 personalized prevention and treatment of these diseases.
615

ACKNOWLEDGEMENTS

We would like to thank Ashley Keiser, Daria Tchessalova, Brynne Raines, and Sarah Jacob for assistance with the techniques, reagents, and data collection for these experiments.

FINANCIAL SUPPORT

These experiments were supported by a University of Michigan Office of Research Award to NCT.

COMPETING INTERESTS

The authors have no competing interests to declare.

626

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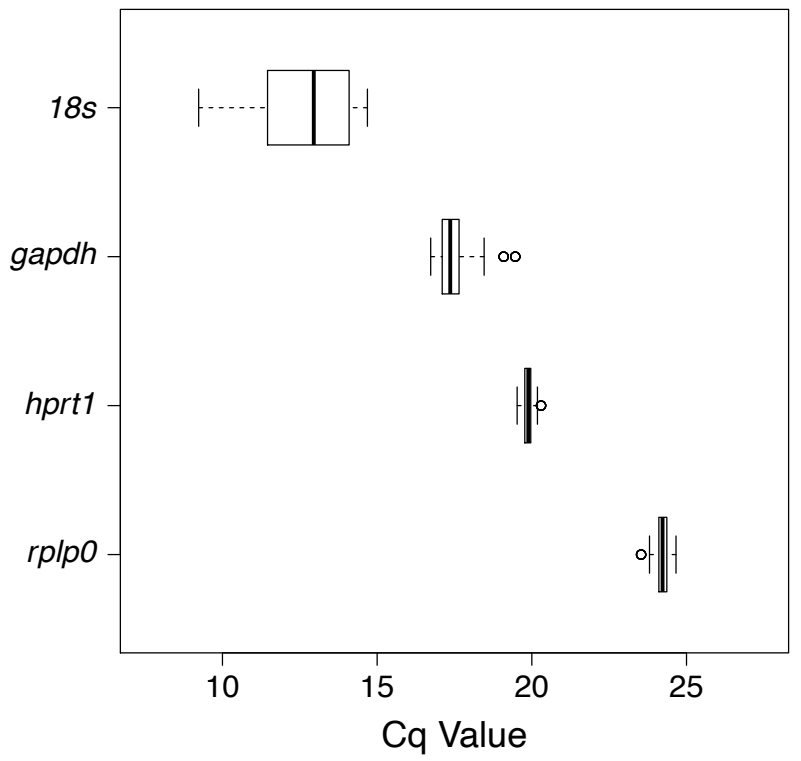
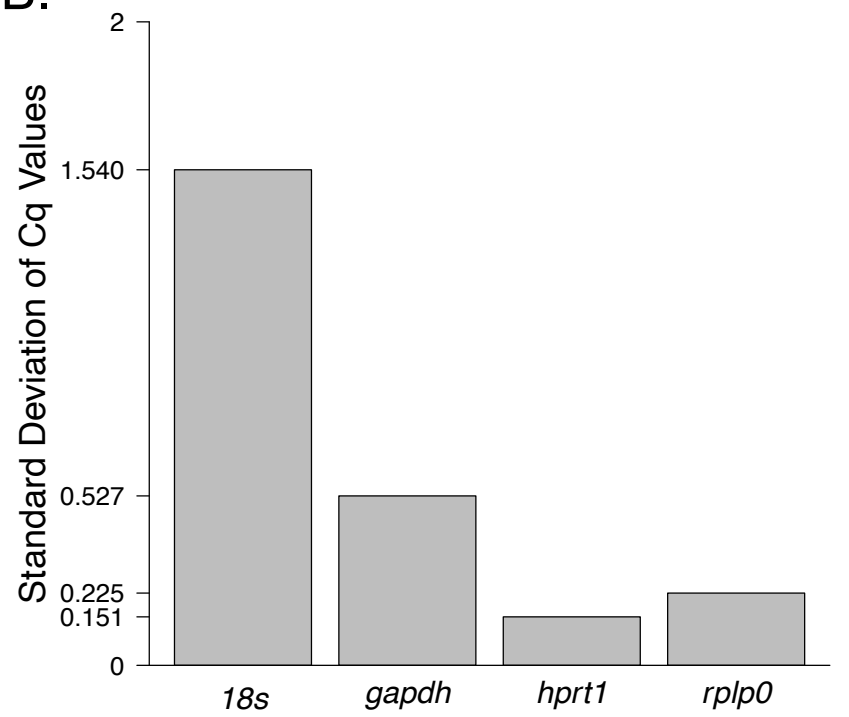
A.



B.



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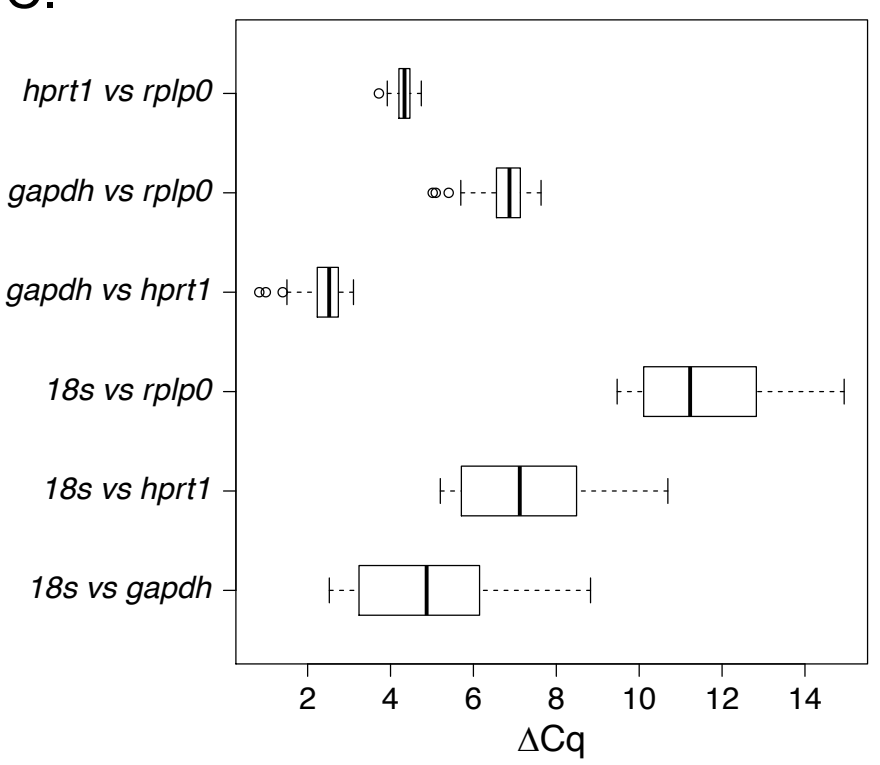

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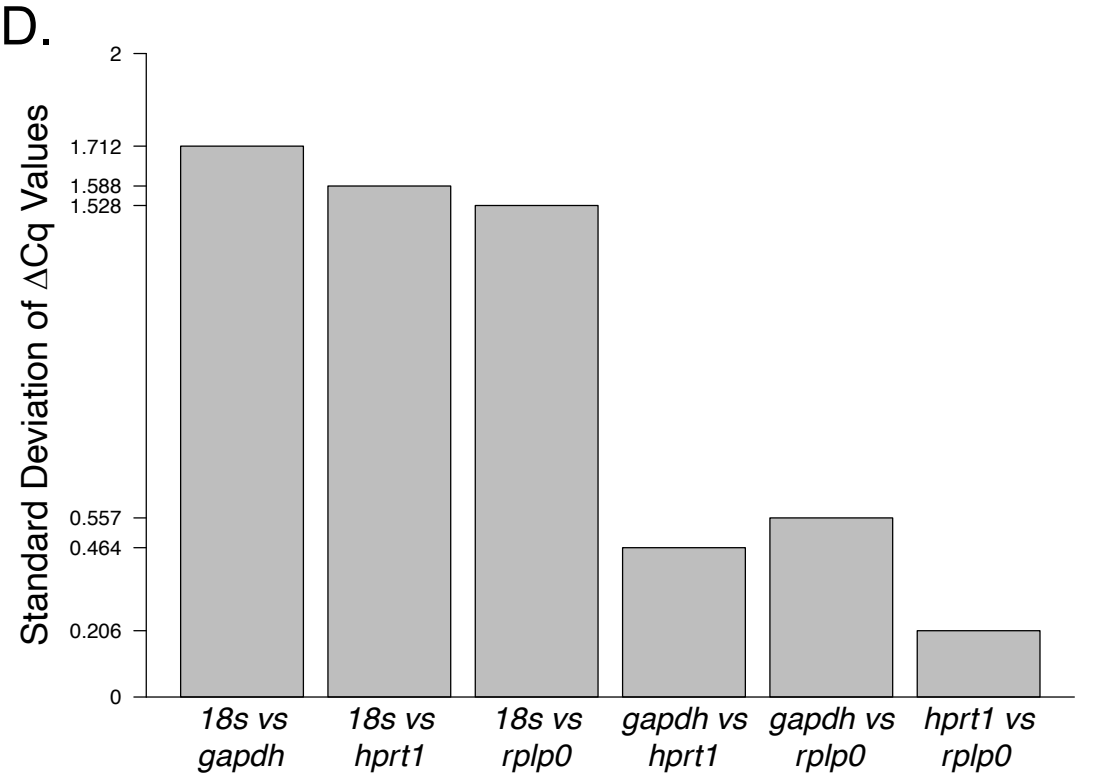
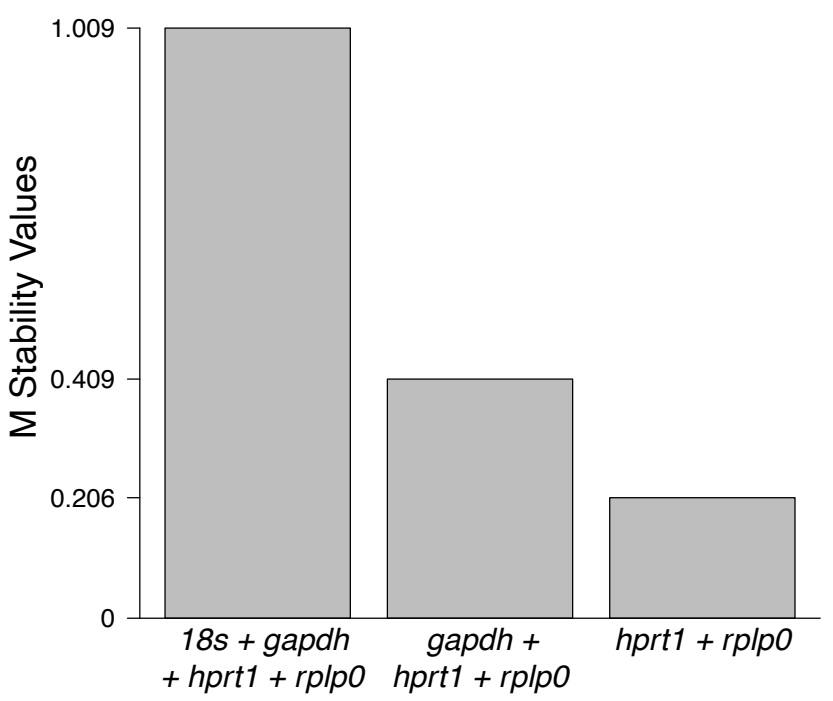
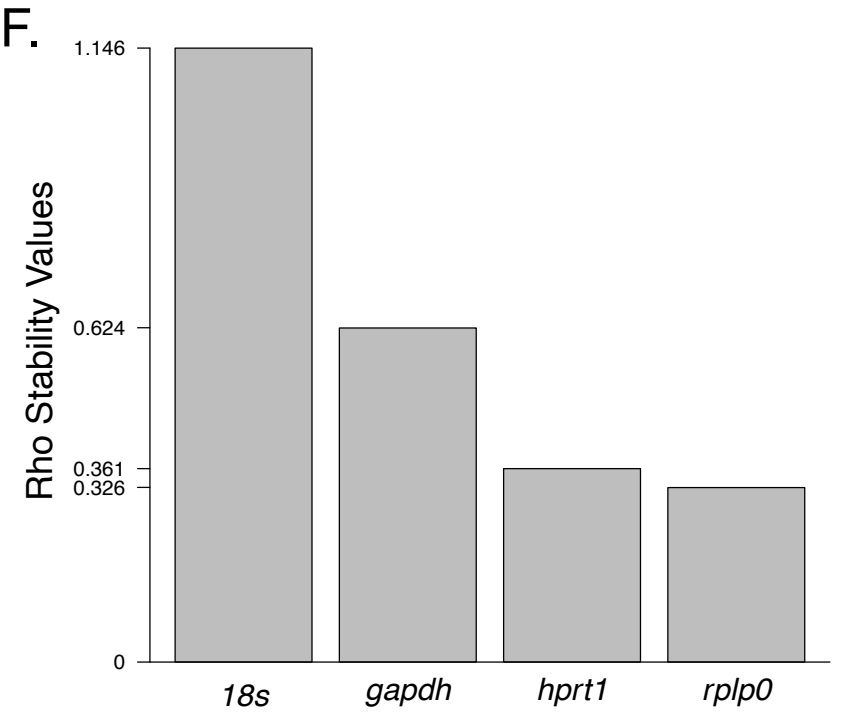


E.

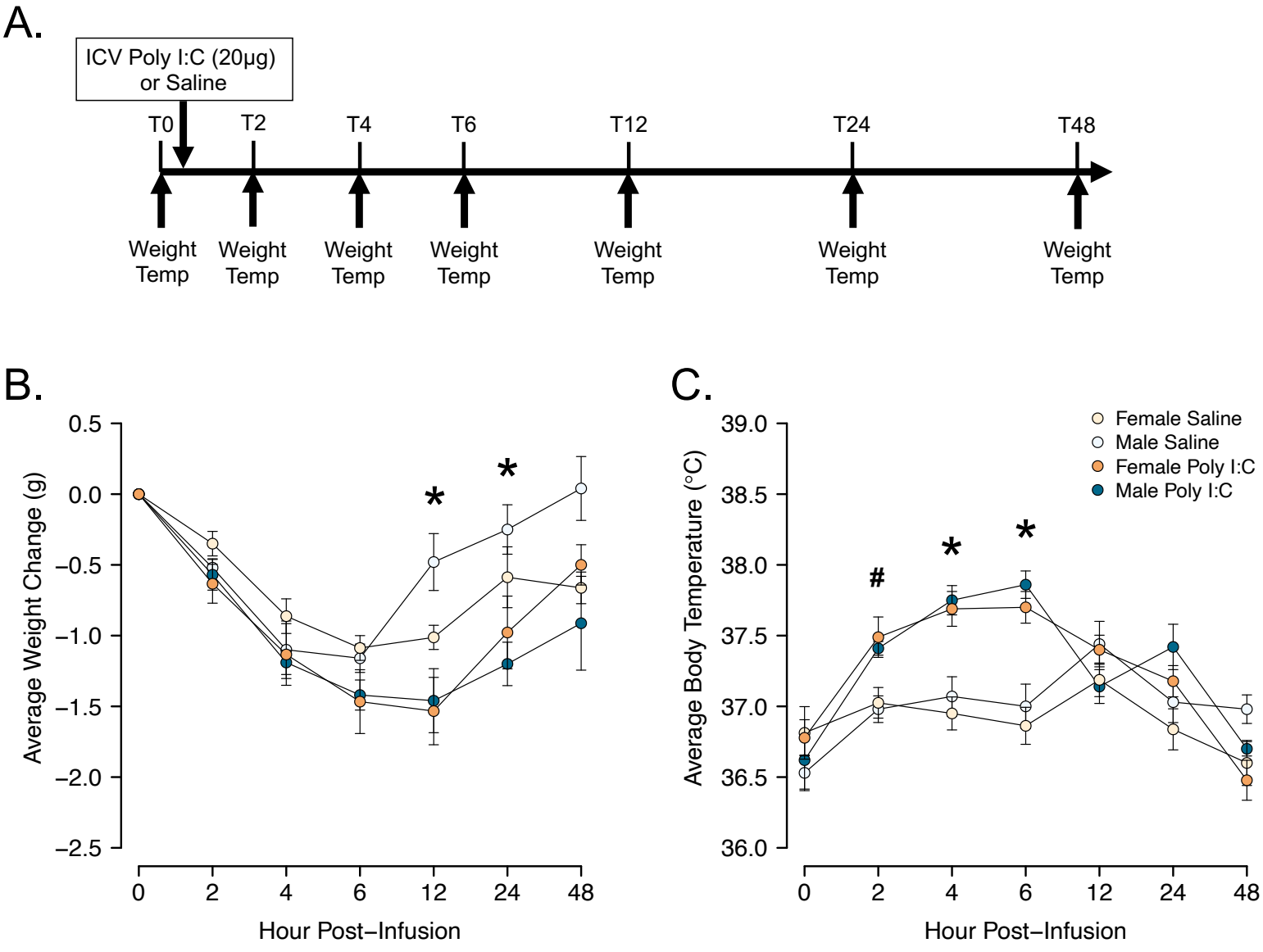


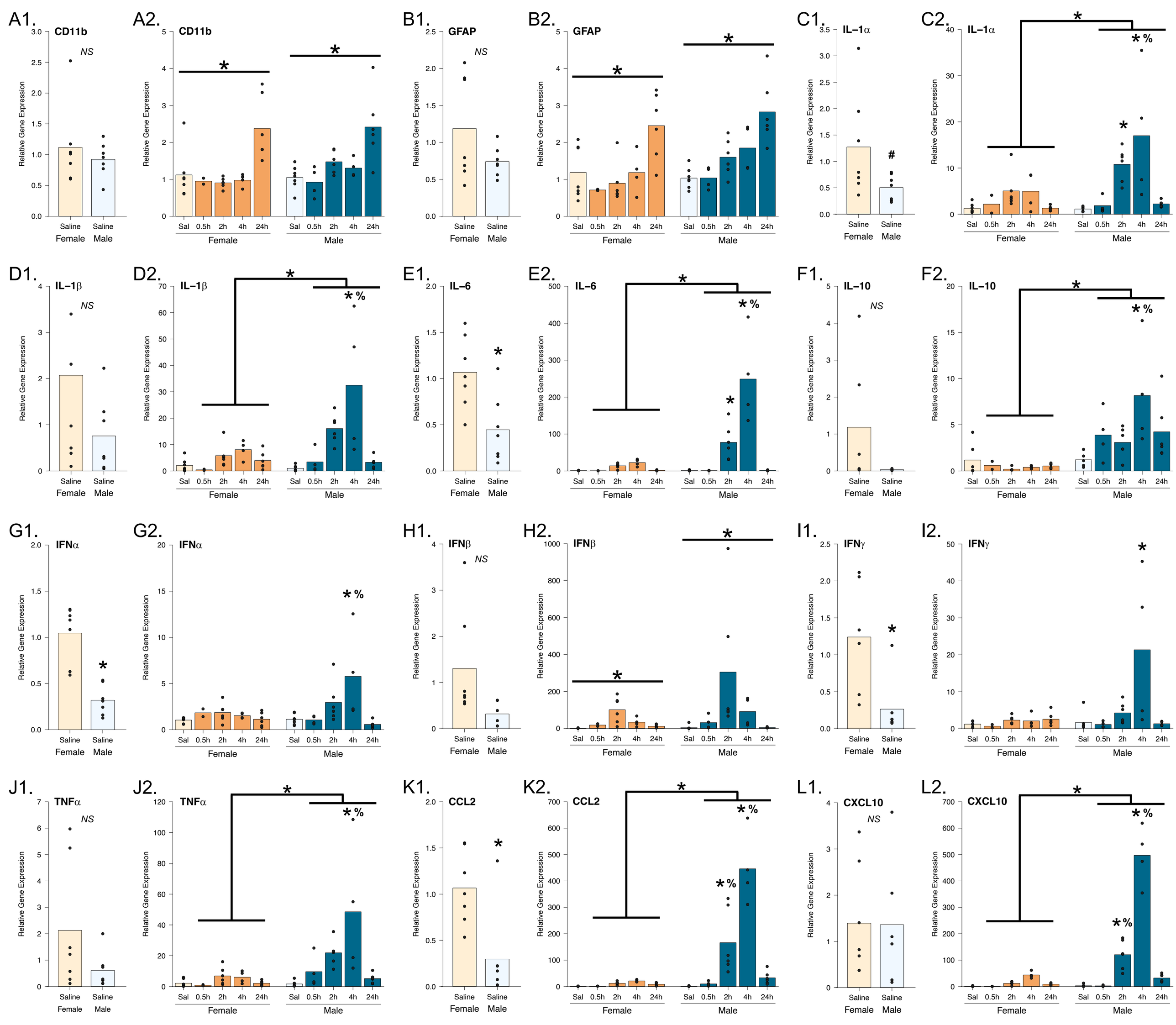
F.

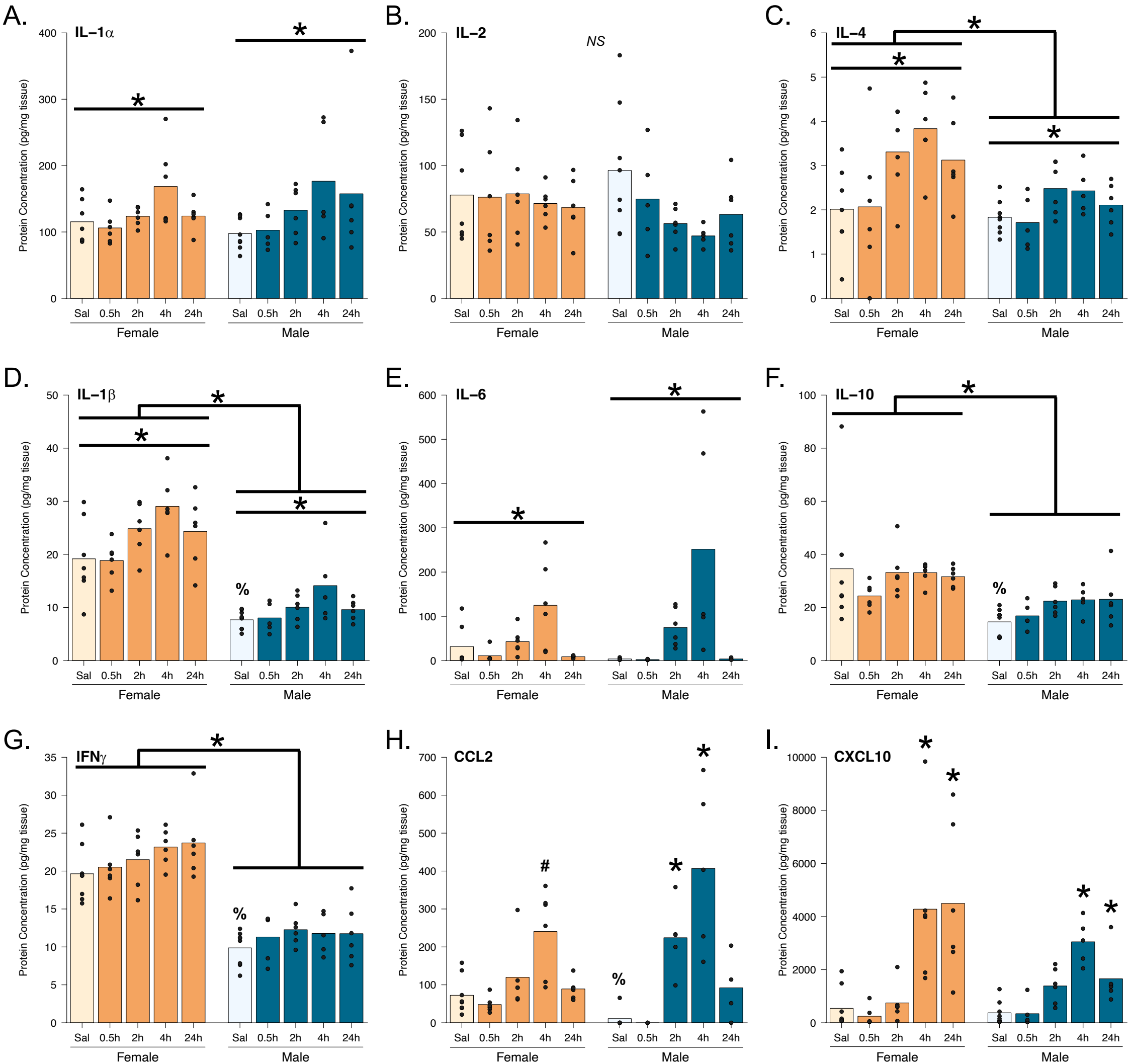


G.

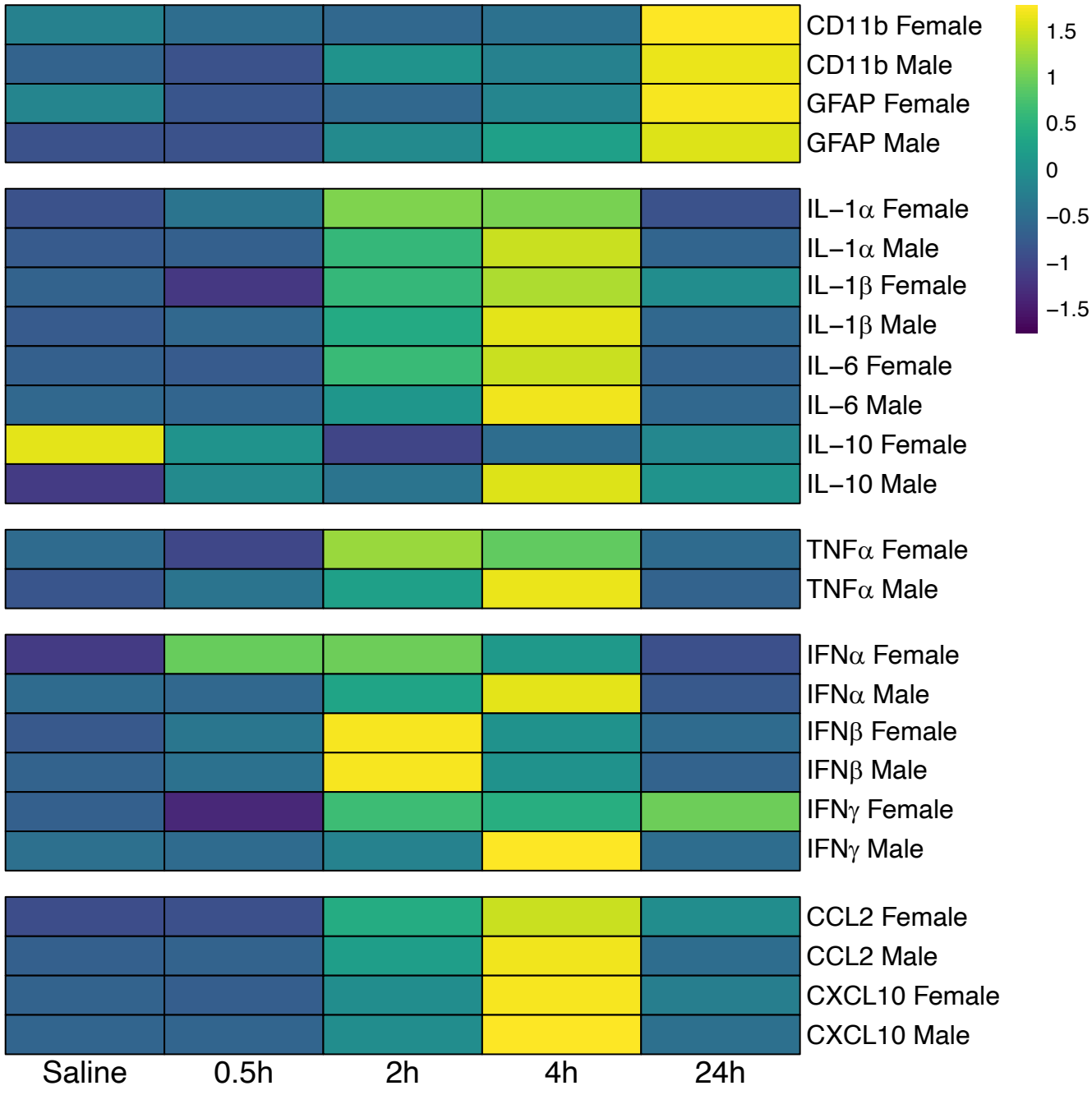
	Cq Value Standard Deviations	Avg Standard Deviations of ΔCq Values	M Stability Values	Rho Stability Values
18s	1.5402440	1.609	1.609	1.1459049
gapdh	0.5266292	0.911	0.510	0.6235678
hprt1	0.1507111	0.753	0.206	0.3610396
rplp0	0.2245596	0.764	0.206	0.3256827


A.
ICV Poly I:C (20μg) or Saline
T0
T2
T4
T6
T12
T24
T48
Weight Temp
B.
Average Weight Change (g)
0.5
0.0
−0.5
−1.0
−1.5
−2.0
−2.5
*
*
0
2
4
6
12
24
48
Hour Post−Infusion
C.
Average Body Temperature (°C)
39.0
38.5
38.0
37.5
37.0
36.5
36.0
Female Saline
Male Saline
Female Poly I:C
Male Poly I:C
#
*
*
0
2
4
6
12
24
48
Hour Post−Infusion

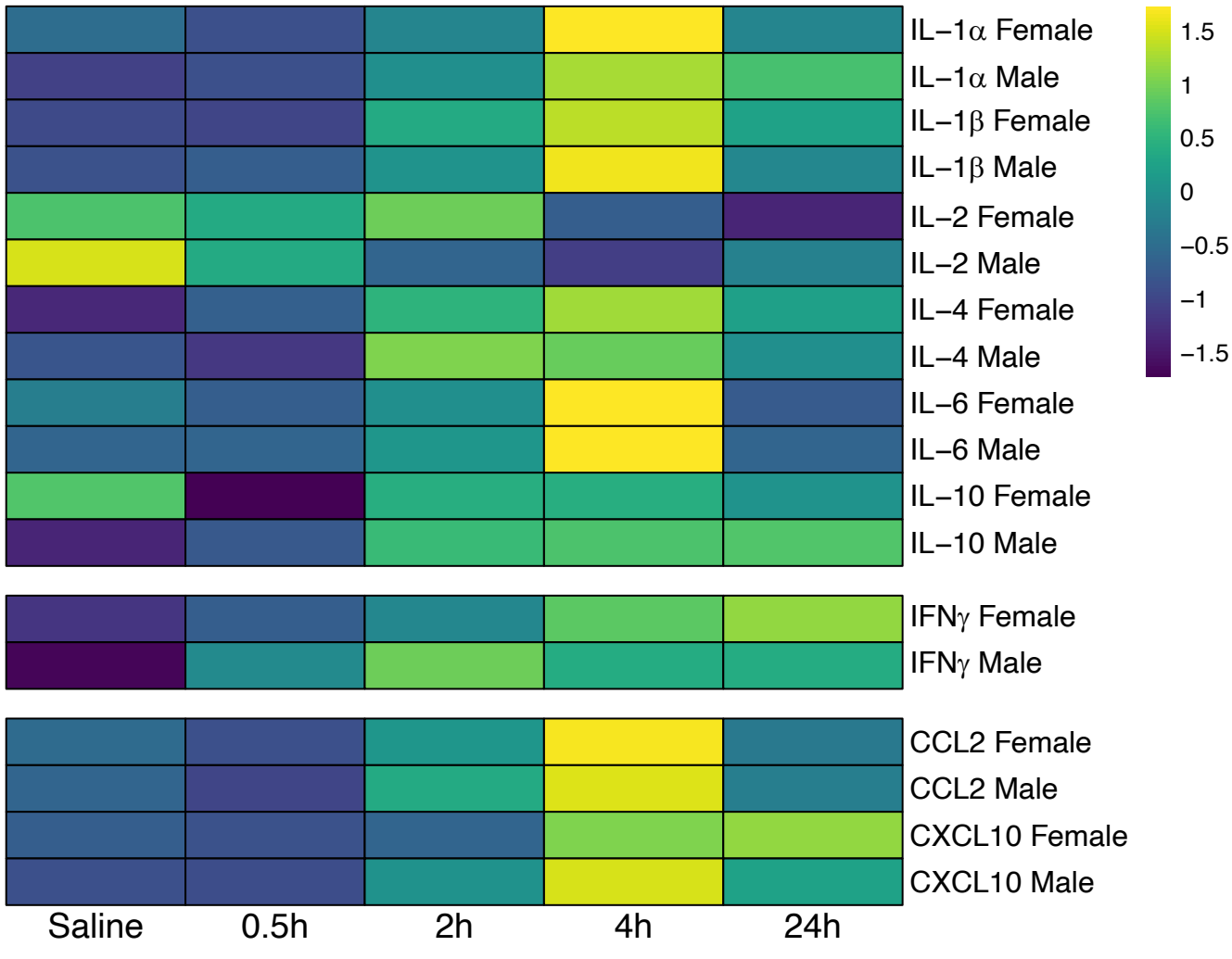

A1.
CD11b
NS
A2.
CD11b
B1.
GFAP
NS
B2.
GFAP
C1.
IL−1α
#
C2.
IL−1α
D1.
IL−1β
NS
D2.
IL−1β
E1.
IL−6
E2.
IL−6
F1.
IL−10
NS
F2.
IL−10
G1.
IFNα
G2.
IFNα
H1.
IFNβ
NS
H2.
IFNβ
I1.
IFNγ
I2.
IFNγ
J1.
TNFα
NS
J2.
TNFα
K1.
CCL2
K2.
CCL2
L1.
CXCL10
NS
L2.
CXCL10
Relative Gene Expression
Saline
Female
Male
Sal
0.5h
2h
4h
24h


A.
IL–1α
B.
IL–2
NS
C.
IL–4
D.
IL–1β
E.
IL–6
F.
IL–10
G.
IFNγ
H.
CCL2
I.
CXCL10
Protein Concentration (pg/mg tissue)
Sal
0.5h
2h
4h
24h
Female
Male
*
%
#

A.


CD11b Female
CD11b Male
GFAP Female
GFAP Male
IL−1α Female
IL−1α Male
IL−1β Female
IL−1β Male
IL−6 Female
IL−6 Male
IL−10 Female
IL−10 Male
TNFα Female
TNFα Male
IFNα Female
IFNα Male
IFNβ Female
IFNβ Male
IFNγ Female
IFNγ Male
CCL2 Female
CCL2 Male
CXCL10 Female
CXCL10 Male
Saline
0.5h
2h
4h
24h
1.5
1
0.5
0
−0.5
−1
−1.5

B.


IL−1α Female
IL−1α Male
IL−1β Female
IL−1β Male
IL−2 Female
IL−2 Male
IL−4 Female
IL−4 Male
IL−6 Female
IL−6 Male
IL−10 Female
IL−10 Male
IFNγ Female
IFNγ Male
CCL2 Female
CCL2 Male
CXCL10 Female
CXCL10 Male
Saline
0.5h
2h
4h
24h
1.5
1
0.5
0
−0.5
−1
−1.5