Altered Circadian Rhythms in a Mouse Model of Neurodevelopmental Disorders Based on Prenatal Maternal Immune Activation

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Abstract

Individuals with neurodevelopmental disorders, such as schizophrenia and autism spectrum disorder, exhibit various sleep and circadian rhythm disturbances that often persist and worsen throughout the lifespan. To study the interaction between circadian rhythm disruption and neurodevelopmental disorders, we utilized a mouse model based on prenatal maternal immune activation (MIA). We hypothesized that MIA exposure would lead to impaired circadian locomotor activity rhythms in adult mouse offspring. We induced MIA by injecting pregnant dams with polyinosinic:polycytidylic acid (poly IC) at embryonic day 9.5, then aged resulting offspring to adulthood. We firstly confirmed that poly IC injection in pregnant dams elevated plasma levels of pro- and anti-inflammatory cytokines and chemokines. We then placed adult offspring in running wheels and subjected them to various lighting conditions. Overall, poly IC-exposed male offspring exhibited altered locomotor activity rhythms, reminiscent of individuals with neurodevelopmental disorders. In particular, we report increased (subjective) day activity across 3 different lighting conditions: 12 h of light, 12 h of dark (12:12LD), constant darkness (DD) and constant light. Further data analysis indicated that this was driven by increased activity in the beginning of the (subjective) day in 12:12LD and DD, and at the end of the day in 12:12LD. This effect was sex-dependent, as female poly IC-exposed offspring showed overall much milder alterations in locomotor activity rhythms than saline-exposed offspring. We also confirmed that the observed behavioral impairments in adult poly IC-exposed offspring were not due to differences in maternal behavior. These data further our understanding of the link between circadian rhythm disruption and neurodevelopmental disorders and may have implications for mitigating risk to the disorder and/or informing the development of circadian-based therapies.

Keywords: circadian rhythms; neurodevelopmental risk factor; schizophrenia; autism spectrum disorder; interaction; disrupted activity rhythms; prenatal immune challenge; poly IC; wheel running; mouse model

1. Introduction

Neurodevelopmental disorders, such as schizophrenia (SCZ) and autism spectrum disorder (ASD), are chronic and debilitating disorders, each with a worldwide prevalence of around 1% (Baron-Cohen et al., 2009; McGrath et al., 2008). SCZ is typically diagnosed in late adolescence or early adulthood and is characterized by hallucinations, delusions, lack of emotional reactivity and impairments in learning and attention (Tandon et al., 2013). ASD is typically diagnosed before the age of 3 and is characterized by deficits in social interaction and communication and stereotyped repetitive behaviors (American Psychiatric Association, 2013). Interestingly, both of these psychiatric disorders have common genetic and environmental risk factors, and overlapping symptoms, such as disruptions in sleep and circadian rhythms.

Circadian rhythms are endogenous cycles that occur with a periodicity of ~24 h. They are governed by a master clock, located in the hypothalamic suprachiasmatic nucleus, and are also influenced by clocks in other brain regions and peripheral tissues (Hastings et al., 2018). Circadian clocks have to be periodically synchronized to cyclic signals in the environment, such as the light-dark cycle. The most documented behavior that follows a circadian cycle is the sleep-wake cycle. Sleep and circadian rhythms control a variety physiological and behavioral processes, thus disruptions in these systems have widespread and detrimental effects.

Strikingly, disrupted sleep and circadian rhythms are exhibited in up to 80% of individuals with SCZ (Cohrs, 2008; Delorme et al., 2020) and ASD (Couturier et al., 2005; Souders et al., 2009). Various clinical findings suggest that sleep and circadian rhythm disturbances are a characteristic feature of SCZ (Cosgrave et al., 2018; Kaskie et al., 2017) and ASD (Missig et al., 2020; Wintler et al., 2020), potentially involved in the development of the

disorders. In both disorders, these disturbances generally appear at an early age and persist throughout the lifespan (Goldman et al., 2012; Martin et al., 2005). In SCZ specifically, sleep disturbances often precede prodromal psychotic symptoms (Tan and Ang, 2001), and in both disorders, poorer sleep quality correlated with greater symptom severity (Korenic et al., 2019; Laskemoen et al., 2019). Many individuals with SCZ or ASD exhibited shifted and/or blunted 24 h melatonin profiles, a circadian hormone with a primary role in the regulation of the sleep-wake cycle (Tordjman et al., 2012; Wiggs and Stores, 2004; Wulff et al., 2012). In addition, reports suggest that individuals with SCZ also have alterations in circadian gene expression in various tissues (Johansson et al., 2016; Seney et al., 2019). Overall, the topic of sleep and circadian rhythms in individuals with SCZ and ASD is a rapidly growing field, yet the distinct biological mechanism leading to these disruptions is unknown. One avenue to study this interaction is by utilizing an animal model based on prenatal infection, which is a known neurodevelopmental risk factor for SCZ and ASD.

Epidemiological studies have reported an association between maternal infection and an offspring's subsequent risk of developing SCZ (Brown, 2012) or ASD (Lee et al., 2015). Maternal immune activation (MIA) is a well-documented prenatal risk factor for neurodevelopmental disorders, whereby the maternal immune system is triggered by an infectious or infectious-like stimulus. Notably, maternal viral infection during the first trimester increases the offspring's risk of developing SCZ by 7-fold (Brown et al., 2004a) and ASD by 2-fold (Atladottir et al., 2010; Lee et al., 2015). The end of the first trimester in primate gestation corresponds to early gestation (E9.5) in rodents (Clancy et al., 2001). Research in humans and animal studies have shown that MIA during gestation exerts long-term changes on offspring brain development and behavior (Boulanger-Bertolus et al., 2018; Brown and Meyer, 2018;

Estes and McAllister, 2016; Guma et al., 2019; Knuesel et al., 2014). In humans, these changes have been implicated in the physical and psychiatric health of the offspring, while in rodents, MIA leads to behavioral deficits reminiscent of SCZ and ASD.

In the context of SCZ and ASD, various studies have characterized sleep and circadian rhythm disturbances in genetic animal models (Delorme et al., 2020; Wintler et al., 2020). To our knowledge, no group has explored these disturbances in a neurodevelopmental animal model. We hypothesized that prenatal infection contributes to the disrupted circadian phenotype observed in patients with neurodevelopmental disorders. In the current study, we induced MIA in mice by using the analog of viral double-stranded RNA, polyinosinic:polycytidylic acid (poly IC). Poly IC is recognized primarily by Toll-like receptor 3 (Alexopoulou et al., 2001), and upon binding, it stimulates the production and release of many pro-inflammatory cytokines and chemokines (Meyer et al., 2006). Poly IC injection leads to an inflammatory response in pregnant dams (Cunningham et al., 2007) and chronic changes in brain (frontal cortex, cingulate cortex and hippocampus) and blood cytokines in offspring at postnatal days 0, 7, 14, 30 and 60 (Garay et al., 2013). Long term neuroinflammation was also reported in offspring (Hui et al., 2018). We first validated our MIA model by assessing maternal plasma levels of several cytokines and chemokines following poly IC injection in pregnant dams. We then measured wheel running behavior in adult poly IC or saline-exposed offspring as a measure of an inherent disruption of the circadian system. Finally, we gathered evidence that the disrupted circadian phenotype was not due to differences in maternal behavior.

2. Methods

2.1. Maternal immune activation (MIA) model

Male and female C57BL/6J mice were ordered from Jackson Laboratory (product number: 000664) at 8 weeks old. Mice were placed in ventilated cages under a standard laboratory lighting condition of 12 h of light: 12 h of dark (12:12LD) for 2 weeks to acclimate to their surroundings. Three days prior to mating, soiled male bedding was placed in the female cages to increase the likelihood of copulation. Mice were mated overnight, and females were assessed between Zeitgeber Time (ZT) 0 and ZT 1 (i.e. from 0 h to 1 h after lights on) for the presence of a vaginal plug, which denoted embryonic day 0.5 (E0.5). On E9.5, to simulate viral infection, pregnant dams were intraperitoneally injected with poly IC dissolved in double-distilled water based on body weight (5 mg/kg; lot 1, 086M4045V [data in main paper], and lot 2, 096M4023V [data in Supplementary figures]; Sigma-Aldrich, St. Louis, MO, USA). A control group was injected with sterile saline solution. Dams were left to deliver their litters naturally.

For the first cohort, 16 female breeders were mated with 8 male breeders: 5 out of 10 poly IC-injected dams gave birth (average litter size: 5.8, total male offspring used=14, total female offspring used=6) and 4 out of 6 saline injected dams gave birth (average litter size: 7, total male offspring used=10, total female offspring used=5). For the second cohort, 18 female breeders were mated with 9 male breeders: 6 out of 10 poly IC-injected dams gave birth (average litter size: 6.8, total male offspring used=12, total female offspring used=6) and 6 out of 8 saline-injected dams gave birth (average litter size: 7.4, total male offspring used=12, total female offspring used=6). Litters of poly IC- and saline-exposed dams did not differ in size (cohort 1: p=.4358, cohort 2: p=.5009).

At postnatal day 21, pups were weaned, weighed, and tagged. Two to three sex-matched littermates were group housed and offspring were aged to adulthood (8 weeks old) before testing. To minimize the effect of litter, we mated enough mice to ensure that groups contained a minimum of 4 litters for each experimental condition. We also analyzed data using a mixed effects model, which is well suited to deal with litter effects, and we visually confirmed that the significant effects that we reported were not driven by a cluster of mice from the same litter. All excess offspring were euthanized, and after the conclusion of testing, all mice were euthanized. Animal use was in accordance with the guidelines of the Canadian Council of Animal Care and was approved by the McGill University Animal Care Committee.

2.2. Verification of inflammatory response in pregnant dams after poly IC injection

The following protocol is depicted in **Fig. 1A**. Pregnant dams were anesthetized 3 h after poly IC injection and trunk blood was collected, using heparin (10 μL) as an anticoagulant. Pregnancy was confirmed via dissection. Blood samples were centrifuged at 4°C, and the supernatant was stored at -80°C, before being processed for levels of various pro- and anti-inflammatory cytokines and chemokines by Eve Technologies (Calgary, AB, Canada). The Mouse Cytokine Array Proinflammatory Focused 10-plex (MDF10) was used, for the following biomarkers: IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, MCP-1 and TNFα. Concentration values were provided by the company, and as per their instructions, samples that were below the detectable range were designated as 0 pg/mL. GM-CSF and IFNγ were also part of the Array but the data are not reported because most samples were close to or below the minimum detectable concentration.

2.3. Wheel-running activity under different lighting conditions

The following protocol is depicted in **Fig. 1B**. Mice were individually housed in running wheel cages, which were placed in light-proof ventilated cabinets. Light was controlled via an external timer, with "lights on" between 150 and 200 lux. After a 2-week acclimation and entrainment period, mice were exposed to 3 lighting conditions, each for 3 weeks: 12:12LD, constant darkness (DD) and constant light (LL) (n=10–12 per group for male offspring and n=5-6 per group for female offspring). Wheel running data was collected to evaluate the effect of MIA on circadian behaviors. 12:12LD is a condition where external timing cues (the light:dark cycle) guide entrainment. Exposure to DD allows for the assessment of free-running rhythms without the influence of light as a timing cue. Exposure to LL has been shown to weaken the suprachiasmatic nucleus neuronal network (Ohta et al., 2005), thus, may uncover pre-existing network connectivity impairments in the poly IC-exposed mice. Running wheel activity data was collected and analyzed using ClockLab software, version 6 (Actimetrics, Wilmette, IL, USA). The analysis was carried out on the last 10 days of wheel-running recordings for each condition. We calculated various circadian locomotor activity variables, including circadian period (tau; calculated using a chi-square periodogram), duration of the active period (*alpha*; denoted by the numbers of hours between activity onset and offset), total amount of daily activity and percent day activity relative to total daily activity. In constant conditions (DD and LL), the subjective night was defined to be from the beginning of activity onset until half a period later, and the rest of the circadian cycle was defined as the subjective day. Analyses for non-parametric variables included interdaily stability, which quantifies the synchronization to the 24h light-dark cycle,

intradaily variability, which quantifies rhythm fragmentation, and relative amplitude, which quantifies the robustness of the rhythm. A detailed analysis of activity bouts was also done, where a bout is defined as a sustained period of activity. Namely, we calculated the number of counts per bout of activity, the duration of activity bouts, the number of activity bouts per day and the peak rate of activity (defined as the peak number of counts per min). Onset variability was defined as the temporal relation between the internal circadian clock and the light-dark cycle. It was calculated as the difference between the onset of activity and the time of lights off. In constant conditions (DD and LL), the onset variability was computed by calculating the deviation of the observed activity onset from the predicted activity onset. The predicted activity onset was calculated using the free-running period, which assumes no day-to-day variability. Lastly, the 24 h profiles were created by averaging the total counts from a 10-day period over the 24 h day for each mouse, then calculating group averages. In the constant conditions, the 24 h profiles were created similarly to those in the 12:12LD condition except that the group averages were created by aligning profiles at the onset of activity for each mouse.

2.4. Maternal behavior

The following protocol is depicted in **Supplementary Fig. 1**. Postnatal maternal behavior was video-recorded in the home cage for 24 hours between postnatal day 4 and 5. Each dam (n=4-7) was observed in the home cage for four 72-min observation periods, at the following times: the beginning of the light phase (starting at ZT 0.5) and the dark phase (starting at ZT 12), and in the middle of the light phase (starting at ZT 4) and the dark phase (starting at ZT 16). Within the observation period, the behavior of each dam was scored every minute (72

observations per session, total of 288 observations (epochs) per dam in a day). Three variables were recorded during both the day and night: out of nest behavior, in nest behavior and nest building. A more detailed analysis of maternal behavior is presented in the Supplementary figures.

2.5 Statistics

Data were analyzed and graphed using Prism (GraphPad, version 8). Maternal cytokine and chemokine expression levels, and postnatal maternal behavior data were analyzed using an independent samples t-test between poly IC and saline-injected pregnant dams. Data that did not pass the Shapiro-Wilk normality test were analyzed by Mann-Whitney U test, and data with unequal variances (assessed by an *F*-test) were analyzed by an independent samples t-test with Welsh's correction. Wheel-running data were analyzed using a mixed-effects analysis with Geisser-Greenhouse correction, and Sidak's *post-hoc* tests, to determine interactions between lighting condition (12:12LD, DD and LL) and group (poly IC or saline exposure). If no interaction was found, then main effects were explored. To further analyze our data, we performed an independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) between poly IC or saline-exposed adult offspring at the beginning and end of the (subjective) day. Males and females were assessed separately. Individual data points represent data from individual mice, bars show mean ± SEM. $p<0.05$ was considered statistically significant.

3. Results

3.1. Verification of inflammatory response in pregnant dams after poly IC injection

Various cytokines and chemokines are known to be acutely elevated in blood plasma of pregnant dams following poly IC injection (Arrode-Bruses and Bruses, 2012; Meyer et al., 2006). We confirmed that the lot of poly IC used induced an inflammatory response in pregnant dams (n=4–5 per group). We sacrificed pregnant dams 3 h post poly IC or saline injection on E9.5 and quantified blood levels of pro- and anti-inflammatory cytokines and chemokines (see experimental timeline in **Fig. 1A**). Poly IC-injected dams had a significant elevation in blood plasma expression of IL-6 (p =.0137), IL-1β (p <.0001), IL-10 (p =.0001), TNFα (p =.0159), IL-12(p70) (p =.0159) and MCP-1 (p =.0190) compared to controls, and a trending difference in IL-2 (p =.0642) and IL-4 (p =.0714) (**Fig. 2A-H**). When we replicated this experiment, we used a second lot of poly IC. In our second lot, similar differences were observed compared to our control group, although we generally observed about a 2-fold reduction in cytokine and chemokine levels compared to our first lot (**Supplementary Fig. 2A-H**). Overall, we confirmed that both lots of poly IC induced an inflammatory immune response in pregnant dams compared to a saline injection.

3.2. Wheel-running activity under different lighting conditions

We explored if circadian locomotor activity rhythms were altered in the MIA mouse model using *in utero* poly IC exposure at E9.5 (see experimental timeline in **Fig. 1B**). In this

experiment, poly IC and saline–exposed mice (n=10-12 male offspring per group, n=5-6 female offspring per group) were subjected to different lighting conditions (12:12LD, DD and LL). Representative actograms for each lighting condition are shown for saline-exposed animals (**Fig. 3A-C,** males; **Fig. 4A-C**, females) and for poly IC-exposed animals (**Fig. 3D-F**, males; **Fig. 4D-F**, females). Activity profiles, with the average daily activity for all mice in each group, are also shown for each lighting condition (**Fig. 5A-C**, males; **Fig. 6A-C**, females). **The Tables** list all analyzed parameters. In the next sections, we describe the analyses for the first cohort of mice, then compare it to the results obtained in the second cohort to confirm our findings. Males are presented first, followed by females.

3.2.1. Circadian Locomotor Activity Variables

We report a group x lighting condition interaction trending significance for circadian period [$F_{(2, 62)} = 2.878$, $p = .0638$], and *post-hoc* analysis revealed a longer period in poly IC-exposed males under DD ($p = .0190$) (**Fig. 3G**). Overall, poly IC-exposed males had a significantly longer *alpha*, which is defined by the duration between the onset and offset of activity, than controls [Group, $F_{(1, 62)}=7.126$, $p = .0097$], without a group x lighting condition interaction. *Post-hoc* analysis revealed a longer *alpha* in poly IC-exposed males under 12:12LD ($p = .0001$) and DD ($p = .0422$) (**Fig. 3H**). Interestingly, poly IC-exposed males had more (subjective) day activity counts across lighting conditions than controls [Group, $F_{(1, 62)} = 35.90$, $p < .0001$]. *Post-hoc* analysis revealed more activity in poly IC-exposed males during 12:12LD ($p = .0085$), DD ($p = .0042$) and LL ($p = .0461$) (**Fig. 3I**). Similarly, poly IC-exposed males had significantly more percent (subjective) day activity than controls [$F_{(1, 62)} = 25.30$, $p < .0001$],

without a group x lighting condition interaction. *Post-hoc* analysis revealed increased activity in poly IC-exposed males during 12:12LD (p =.0041), DD (p =.0340) and LL (p =.0153) (**Fig. 3J**). Males showed no group x lighting condition interactions or group differences in onset variability, (subjective) night activity counts and total activity (**Table 1**).

Interestingly, when we repeated this experiment with a different lot of poly IC, we reproduced the difference between groups on (subjective) day activity counts [Group, $F_{(1, 64)}$ = 12.34, p =.0008], without a group x lighting condition interaction. *Post-hoc* analysis revealed increased activity in poly IC-exposed males during 12:12LD (p =.0041), but not DD (p =.1395) or LL (p =.1402) (**Supplementary Fig. 3I**). We surprisingly did not see a difference between groups on percent (subjective) day activity [Group, $F_{(1, 22)}$ = 1.245, p =.2765] (**Supplementary Fig. 3J**), but this is likely due to the trending difference we found in (subjective) night activity counts [Group, $F_{(1, 22)}$ = 3.745, p =.0659] and total activity [Group, $F_{(1, 22)}$ = 4.646, p =.0423] (**Supplementary Table 1**).

In females, we did not find a group x lighting condition interaction nor any group differences for circadian locomotor activity variables **(Fig. 4G-J and Table 2)**. When we repeated this experiment with a different lot of poly IC, we found a trend for poly IC-exposed females to have more percent (subjective) day activity [$F_{(1, 30)}$ = 3.602, p =.0674] and (subjective) day activity counts [$F_{(1, 10)}$ = 4.052, p =.0718], but no differences in other circadian locomotor activity variables (**Supplementary Fig. 4G-J and Supplementary Table 2).**

Overall, females exposed to *in utero* poly IC did not show as severe alterations as their male counterparts. Poly IC-exposed males primarily showed increased (subjective) day activity counts across the 3 different lighting conditions, which was consistent in both cohorts. In the first

cohort, we also report a longer period in DD, an extended *alpha*, and increased percent (subjective) day activity in poly IC-exposed mice compared to controls.

3.2.2 Daily Activity Profiles

To further analyze our data, daily activity profiles were created by plotting average running activity per group from ZT 0-24 in 12:12LD, and CT 0-24 in DD and LL. When observing the first 3 h of the (subjective) day, male poly IC exposed offspring exhibited increased activity in 12:12LD (p =.0031) and DD (p =.0067), but not LL (**Fig. 5D-F**). Additionally, poly IC-exposed males also showed increased activity in the last 1.5 h of the day in 12:12LD (p <.0001) but not in DD or LL (**Fig. 5G-I**). In the second cohort, we similarly found an increase in activity in the first h of the (subjective) day under 12:12LD (p =.0090) and DD (p =.0068), but not LL (**Supplementary Fig. 5D-F**). These differences were not observed in females, due to the lack of day activity counts in the poly IC-exposed females (**Fig. 6D-I**). Overall, these analyses complemented the ANOVAs we conducted, by suggesting that the differences between groups in 12:12LD and DD are mainly driven by increased activity in the beginning of the (subjective) day.

3.2.3. Non-parametric parameters and Analysis of Activity Bouts

When exploring non-parametric rest-activity parameters in males, poly IC-exposed male mice had overall greater intradaily variability scores than controls [$F_{(1, 22)} = 6.621$, p =.017], without a group x lighting condition interaction (**Fig. 7A**). No group differences were found in

scores of relative amplitude (**Fig. 7B**). In the second cohort, no differences were found in non-parametric variables (**Supplementary Fig. 7A-B**). As well, no differences were found in onset variability scores in either cohort (**Fig. 7C, Supplementary Fig. 7C).**

In the first cohort of male mice, we did not find any differences in the bout analysis **(Fig. 7D, Table 1)**. However, in the second cohort, there was a trend for poly IC-exposed male mice to have a greater average bout length [$F_{(1, 22)} = 3.286$, $p = .0836$], and a significantly greater average peak rate [$F_{(1, 22)} = 4.568$, $p = .0439$] than controls (**Supplementary Fig. 7D, Supplementary Table 1**).

In females, poly IC-exposed mice had a trend for overall higher intradaily variability scores [$F_{(1, 9)} = 4.27$, $p = .0687$], without a poly IC x lighting condition interaction (**Fig. 7E**). No differences were found in scores of relative amplitude (**Fig. 7F**). In the second cohort, no differences were found on non-parametric measures in females, nor in onset variability scores in either cohort (**Fig. 7G, Supplementary Fig. 7E-G**).

For the analysis of activity bouts in females, we report no group x lighting condition interactions, but poly IC-exposed mice had increased number of bouts [Group, $F_{(1, 9)} = 24.11$, $p = .0008$], decreased average bout length [Group, $F_{(1, 9)} = 6.878$, $p = .0277$] and decreased counts per bout [Group, $F_{(1, 9)} = 12.03$, $p = .0071$] than controls (**Fig. 7H and Table 2**). These differences were not seen in females of the second cohort (**Supplementary Fig. 7H and Supplementary Table 2**).

Overall, in the first cohort, poly IC-exposed males had greater intradaily variability scores than controls, and in the second cohort, poly IC-exposed males showed a trend for greater average bout length and had a greater average peak rate than controls. Poly IC-exposed females showed a trend for greater intradaily variability scores, and significantly increased number of

bouts, decreased average bout length and decreased counts per bout compared to controls in the first cohort, which was not observed in the second cohort.

3.2.4. Evaluating Sex Differences

To confirm that the effects of MIA on wheel-running activity are sex dependent, circadian activity variables were analyzed using a three-way ANOVA with the following factors: sex (male, female), group (poly IC, saline exposure) and lighting condition (12:12LD, DD, LL) (**Supplementary Table 3**). We explored the group x sex interaction and the main effect of sex. Notably, in the first cohort, there was a significant group x sex interaction on (subjective) day activity (counts) (p =.0430), number of bouts (p =.0297) and average counts per bout (p =.0132). As well, there was a significant effect of sex on the majority of the parameters we tested including period (h) (p <.0001), alpha (h) (p =.0002), total activity (p =.0003), (subjective) day activity (%) (p =.0002), and intradaily variability (p =.0003). In the second cohort, we were able to reproduce the main effects of sex in all of the aforementioned parameters, except period (h), alpha (h) and (subjective) day activity (%). Data collected from the daily activity profiles were analyzed with two-way ANOVAs with the following factors: sex (male, female) and group (poly IC, saline exposure) (**Supplementary Table 4**). Notably, there was a main effect of sex under 12:12LD at the beginning of day in cohort 1 (p =.0004) and cohort 2 (p =.0276). In the first cohort only, we also observed a significant group x sex interaction at the end of the day activity under 12:12LD (p =.0014). Overall, the group x sex interactions found in the first cohort support poly IC exposure having a sex-specific effect on wheel-running parameters. Additionally, the

many statistically significant effects of sex in both cohorts supports sex differences across group and lighting conditions.

3.3. Maternal behavior of poly IC versus saline injected dams

It is known that mother-pup interactions have direct long-term effects in adult offspring (Walker et al., 2004). However, it is unknown whether a single poly IC injection during gestation (E9.5) alters maternal behavior. We aimed to explore postnatal maternal behavior in poly IC-injected dams compared to controls (protocol depicted in **Supplementary Fig. 1**). **Fig. 8** presents a general overview of maternal behavior, which we divided into 3 general categories: out of nest behavior, in nest behavior and nest building behavior. Maternal behavior did not differ between groups for out of nest behavior (day: p=.9084; night p=.4189), in nest behavior (day: p=.6567; night p=.5942) or nest building behavior (day: p=.2620; night p=.5976) during the day or night (**Fig. 8**). When maternal behavior was explored in more detail, there were still no differences between groups for grooming behavior (day: p=.4790; night p=.9639), wandering (day: p=1798; night p=.8622), nest rustling (day: p=.4339; night p=.8354), nest still (day: p=.7559; night p=.4681), eating (day: p=4345; night p=.5259) and drinking (day: p=.4579; night p=.2860) (**Supplementary Fig. 8**). Overall, these data imply that the differences that we observed in offspring behaviors are not due to differences in maternal behavior.

4. Discussion

Circadian rhythm disruption is a prominent feature in schizophrenia (SCZ) and autism spectrum disorder (ASD), thus there has been a growing interest in studying the role circadian rhythms play in these neurodevelopmental disorders. Here, we report that adult male offspring exposed *in utero* to poly IC exhibited altered circadian running behavior under a standard laboratory lighting condition (12:12LD), constant darkness (DD) and constant light (LL) compared to saline-exposed controls. In particular, poly IC-exposed male mice exhibited more (subjective) day activity than controls. This was driven mainly by increased activity in the beginning of the (subjective) day in 12:12LD and DD, and at the end of the day in 12:12LD. These were sex-dependent differences, as female offspring exposed to poly IC showed much fewer disturbances than males. Our data contributes to the current literature by showing that MIA, a risk factor for neurodevelopmental disorders, leads to alterations in circadian rhythms in mice, which has repeatedly been observed in individuals with SCZ and ASD. Additionally, circadian rhythms under different lighting conditions have only been characterized in animal models with mutations in risk genes for SCZ and ASD. Thus, our data can serve as a baseline for future experiments that aim to integrate both genetic and environmental risk factors for SCZ and ASD in animal models.

A number of sleep and circadian rhythm disruptions have been reported in genetic animal models used to study SCZ and ASD (Delorme et al., 2020; Wintler et al., 2020). Interestingly, the models display somewhat different sleep and circadian rhythms disruptions from one another. Phenotypes include decreased sleep time, more fragmented sleep and circadian activity, and increased free-running period. Some studies have even found a heterogeneity of disruption,

whereby a subset of SCZ and ASD animals exhibit more severe disruptions than their SCZ and ASD counterparts. In our study using a neurodevelopmental risk factor for SCZ and ASD, there are parallels in the disruptions we found to those reported in genetic animal models. For example, similar to our findings, other groups have also reported increased (subjective) day activity in 12:12LD (*Snap-25* mutant mice (Oliver et al., 2012)), DD (*Pallidin* mutant mice (Lee et al., 2018)) and LL (*Dysbindin-1* mutant mice (Bhardwaj et al., 2015)). Also, similar to Maple and colleagues (*Egr3* mutant mice), we found an extended *alpha* under 12:12LD (Maple et al., 2018). These parallels are possibly outlining a common mechanism through which factors that contribute to SCZ and ASD lead to disrupted circadian rhythms. Overall, each animal model captures important features of SCZ and ASD-related sleep and circadian disruptions, and the differences observed between models may speak to the heterogeneity of sleep and circadian disruptions observed in humans affected by SCZ and ASD. For example, some patients exhibit highly irregular 24 h patterns, while others exhibit delayed activity rhythms and even free-running rhythms (Wulff et al., 2012). A metanalysis comparing sleep parameters in psychiatric patients found that SCZ patients exhibited longer total sleep time, greater sleep latency and poorer sleep continuity (Meyer et al., 2020). Therefore, future studies should examine sleep differences in the MIA model, to explore if the increase (subjective) daytime running activity that we observed is due to disturbed sleep initiation and continuity. To our knowledge, only one prior study has reported behavioral rhythm alterations in the MIA model: Missig and colleagues who tested the combined effects of prenatal poly IC (E12.5) and post-natal lipopolysaccharide (LPS) treatment (postnatal day 9) (Missig et al., 2018). Using wireless telemetry transmitters, they reported that male mice exposed to prenatal poly IC only had more general home cage activity during the light phase under 12:12LD at 7 but not 12 weeks of age. Although we

administered poly IC earlier in gestation (E9.5), these findings are consistent with ours because we found more wheel running activity during the light phase in poly IC-exposed male mice. However, we showed that the effect is also seen under free-running conditions (DD, LL), and that appears to be sex-dependent (the previous report was only in males).

In this report, we provided evidence that both poly IC lots used induced an immune response in pregnant dams. Consistent with existing literature, we reported an increase in several pro- and anti-inflammatory cytokines and chemokines, including IL-6, 1L-1β, IL-10, MCP-1 and TNFα, following poly IC injection in pregnant dams (Arrode-Bruses and Bruses, 2012; Meyer et al., 2006). Interestingly, in the literature, IL-6 was shown to be critical in the adverse effects of *in utero* poly IC exposure. Namely, a single administration of IL-6 in a pregnant dam (but not IFNγ) led to similar deficits to those observed in the MIA model, and coadministration of anti-IL-6 antibody normalized MIA-induced changes (Smith et al., 2007). In our study, although IL-6 expression levels in maternal plasma from both lots of poly IC were significantly higher than controls, there was a 2-fold reduction in the second lot compared to the first. Perhaps this contributed to the milder effects on behavior observed in the second cohort. Further, varying potencies of poly IC per lot appear to be common in the literature (Meyer et al., 2006; Mueller et al., 2019). To further support our main findings in both cohorts of mice, we performed additional analyses, where we added cohort as a factor and performed 3-way ANOVAs on the data from the wheel running parameters. We consistently found a main effect of group for increased (subjective) day running, with no cohort x group interaction (data not shown). Thus, irrespective of the lot of poly IC, we do observe an increase in (subjective) day activity counts in both cohorts.

Intriguingly, our analyses indicate that this effect is sex-dependent. Namely, female offspring showed overall much milder effects of *in utero* poly IC exposure than males. This finding is consistent with reports of individuals with SCZ (Ochoa et al., 2012) and ASD (Ferri et al., 2018), where incident rate, age of onset and course of the disorder are all affected in a sex-dependent manner. An association has been reported between increased maternal cytokine levels during pregnancy and psychosis in adult offspring (Allswede et al., 2016; Brown et al., 2004b; Buka et al., 2001). However, exposure to different prenatal maternal cytokine levels may contribute to the development of psychosis in a sex-dependent manner. For example, in humans, when maternal serum was collected early in the third trimester, researchers found that higher levels of maternal IL-6 were more prevalent in male offspring with SCZ than male controls, and lower levels of maternal TNFα levels were more found among female individuals with SCZ than female controls (Goldstein et al., 2014). In rodent models of prenatal infection, sex-differences are apparent in various SCZ and ASD-like behaviors (Carney, 2019; Gogos et al., 2020; Xuan and Hampson, 2014), and across gene expression and cellular correlates (Braun et al., 2019; Hui et al., 2018). Thus, immunological processes may affect fetal brain development in a sex-dependent manner that perhaps leads to the sex differences observed in circadian behavior.

Despite altered sleep and circadian rhythms being documented in genetic animal models of SCZ (Delorme et al., 2020) and ASD (Wintler et al., 2020), and reported here in a neurodevelopmental model, the biological mechanisms that underly these alterations are largely unknown. Further, it is largely unknown how MIA specifically affects the circadian system, or how circadian rhythm disruption affects outcomes in offspring exposed to MIA. To our knowledge, the only data to test this were those of Spisska and colleagues, who simulated maternal infection using LPS in rats and measured clock gene expression in the suprachiasmatic

nucleus during postnatal development. Differences were mainly observed in *Nr1d1* expression, which was arrhythmic at P3 and had a significantly increased amplitude at P20 compared to controls. As well, in the night, pineal glands from LPS-treated animals had higher activity of alkylamine-N-acetyltransferase (AA-NAT), the rate-limiting enzyme in melatonin synthesis (Spisska et al., 2020). Overall, the higher amplitude of *Nr1d1* expression and AA-NAT were hypothesized to be an adaptive feature of the clock against immune perturbations (Spisska et al., 2020). For future studies, a possible mechanism that may be of interest involves altered synaptic transmission and connectivity, which have been suggested as key factors in the pathogenesis of SCZ and ASD (Fromer et al., 2014; Guang et al., 2018; Yin et al., 2012), and have also been linked to the circadian system (Hannou et al., 2020). MIA is also known to affect synaptic development and function in offspring (Coiro et al., 2015; Oh-Nishi et al., 2010). Moreover, in genetic animal models of SCZ and ASD that exhibit sleep and circadian disruptions, many of the susceptibility genes encode proteins that are implicated in neuronal communication, and/or protein and vesicle trafficking (Bhardwaj et al., 2015; Delorme et al., 2020; Ingiosi et al., 2019; Jaaro-Peled et al., 2016; Lee et al., 2018; Maple et al., 2018; Oliver et al., 2012; Pritchett et al., 2015). Altogether, further research is needed to uncover the biological mechanisms linking prenatal maternal inflammation to altered circadian rhythms.

A longstanding hypothesis states that neurodevelopmental disorders result from complex interactions between genetic and environmental risk factors that alter the development of brain structures and proper brain development and function. Future studies should explore a complementary angle to study the role of circadian rhythms play in neurodevelopmental disorders. There are various negative health and behavioral consequences observed when the sleep or circadian system is disrupted in healthy subjects or in laboratory animals. Emerging

studies on the effects of sleep and circadian disruption (induced through shiftwork or jetlag) on mental and physical health are becoming more numerous (Foster et al., 2013; Karatsoreos, 2012). However, such studies are largely correlative, and the underlying mechanisms are unknown. Due to the prevalence of sleep and circadian disruption observed in patients with SCZ or ASD, one could wonder what impacts environmentally disrupting circadian rhythms would have on the course of these disorders, especially in genetically vulnerable individuals. Such findings would highlight a role for sleep and circadian rhythms as a causal risk factor for SCZ and ASD, and not only a characteristic trait of the disorder.

Discovering more efficient therapeutic options for individuals with SCZ or ASD may rely on exploring novel risk factors and uncovering the complex mechanisms in which these risk factors interact to jointly exert their effect. The development of circadian-based therapies in conjunction with other therapies may be a promising avenue of research.

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Figure Legends

Fig. 1. Schematic for the maternal immune activation protocol. On E9.5, pregnant dams were intraperitoneally injected with poly IC (5 mg/kg) or saline. (A) Confirmation of inflammatory response in pregnant dams injected with poly IC. Pregnant dams were anesthetized 3 h after poly IC or saline injection and blood was collected. Plasma was tested for levels of pro- and anti-inflammatory cytokines and chemokines. (B) Another cohort of pregnant dams delivered their litters naturally after poly IC or saline injection. Resulting offspring were placed in running wheels at adulthood. Circadian locomotor activity was recorded under different lighting conditions: 12 h of light, 12 h of dark (12:12LD), constant darkness (DD) and constant light (LL).

Fig. 2. Pro- and anti-inflammatory cytokine and chemokine expression levels in plasma of pregnant dams injected with poly IC (lot: 086M4045V) (n=4) or saline (n=5). Expression levels of the following cytokine and chemokines: (A) IL-6, (B) IL-1β, (C) IL-10, (D) TNFα, (E) IL-12(p70), (F) MCP-1, (G) IL-2 and (H) IL-4. Samples that were below the detectable range were designated as 0 pg/mL. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, ****p<.0001, ***p<.001, and *p<.05. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 2.

Fig. 3. Circadian locomotor activity variables in male offspring. Representative actograms of (A-C) saline-exposed (n=11-12 from 4 litters) and (D-F) poly IC-exposed (n=10-12 from 5 litters)

male offspring under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in hours) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha* (h), (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction and Sidak's *post-hoc* tests. If no interaction was found, main effects were explored and presented in the graph. ****p<.0001, ***p <.001, **p <.01 and *p <.05. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 3.

Fig. 4. Circadian locomotor activity variables in female offspring. Representative actograms of (A-C) saline-exposed (n=5 from 4 litters) and (D-F) poly IC-exposed (n=6 from 5 litters) female offspring under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in h) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha*, (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. No significant group differences were found. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 4.

Fig. 5. Daily activity profiles averaged across all saline-exposed (n=11-12 from 4 litters) and poly IC-exposed (n=10-12 from 5 litters) male offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-3) and end (ZT (CT) 10.5-12) of the (subjective) day in (D, G) 12:12LD, (E, H) DD and (F, I) LL respectively. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, ****p <.0001 and **p <.01. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 5.

Fig. 6. Daily activity profiles averaged across all saline-exposed (n=5 from 4 litters) and poly IC-exposed (n=6 from 5 litters) female offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-3) and end (ZT (CT) 10.5-12) of the (subjective) day in (D, G) 12:12LD, (E, H) DD and (F, I) LL respectively. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 6.

Fig. 7. Activity rhythm characteristics and analysis of activity bouts. Analysis was done for (A-D) male offspring (saline-exposed n=11-12 from 4 litters; poly IC-exposed n=10-12 from 5 litters) and (E-H) female offspring (saline-exposed n=5 from 4 litters; poly IC-exposed n=6 from

5 litters) separately for the following measures: (A, E) intradaily variability, (B, F) relative amplitude, (C, G) onset variability and (D, H) number of bouts. Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. If no interaction was found, main effects were explored and presented in the graph. ***p <.001 and *p <.05. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 7.

Fig. 8. Maternal behavior of saline and poly IC-injected dams. Dams were observed between postnatal day 4 to 5 during both the (A, C, E) daytime and the (B, D, F) nighttime and scored for maternal behavior. Dams were scored on (A-B) in nest behavior, (C-D) out of nest behavior and (E-F) nest building. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found.

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Table 1. Summary of wheel running parameters from poly IC and saline-exposed male offspring.

	12:12LD		DD		LL	
	Saline (n=12)	Poly IC (n=10)	Saline (n=12)	Poly IC (n=12)	Saline (n=11)	Poly IC (n=11)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.6 ± 0.02	23.7 ± 0.02	24.9 ± 0.04	25.0 ± 0.04
Alpha (h)	12.0 ± 0.08	12.6 ± 0.08	13.7 ± 0.36	14.9 ± 0.28	10.3 ± 0.60	10.8 ± 0.43
(Subjective) Day Activity (counts)	627 ± 84	1992 ± 339	2499 ± 306	4123 ± 321	781 ± 138	1652 ± 286
(Subjective) Night Activity (counts)	26081 ± 1509	26181 ± 1243	18276 ± 1563	16265 ± 1301	9489 ± 1151	9087 ± 1339
Total Activity (counts)	26707 ± 1457	28174 ± 1434	20564 ± 1486	20180 ± 1263	10515 ± 1341	10494 ± 1468
(Subjective) Day Activity (%)	2.54 ± 0.43	6.88 ± 0.97	13.25 ± 2.09	21.18 ± 1.98	7.89 ± 0.83	14.43 ± 1.80
Intradaily Variability	0.481 ± 0.028	0.578 ± 0.029	0.812 ± 0.107	1.08 ± 0.083	0.892 ± 0.080	1.074 ± 0.101
Relative Amplitude	0.997 ± 0.001	0.986 ± 0.004	0.968 ± 0.012	0.970 ± 0.010	0.977 ± 0.011	0.975 ± 0.014
Interdaily Stability	0.834 ± 0.019	0.858 ± 0.013	N/A	N/A	N/A	N/A

Onset Variability	0.18 ± 0.02	0.18 ± 0.02	2.27 ± 0.21	2.22 ± 0.33	2.72 ± 0.40	2.76 ± 0.48
Number of Bouts	66.0 ± 4.79	73.0 ± 5.68	125.5 ± 11.03	128.5 ± 9.05	132.7 ± 6.65	147.1 ± 5.75
Average Bout Length (minutes)	84.6 ± 5.93	82.9 ± 7.95	52.9 ± 6.14	49.4 ± 4.41	27.8 ± 4.13	24.7 ± 3.77
Average Counts per Bout	4187 ± 438	4023 ± 534	2509 ± 308	2290 ± 253	1117 ± 223	1010 ± 176
Average Peak Rate	69.69 ± 2.09	71.41 ± 3.33	72.46 ± 2.34	74.11 ± 1.61	67.73 ± 1.82	69.67 ± 2.57

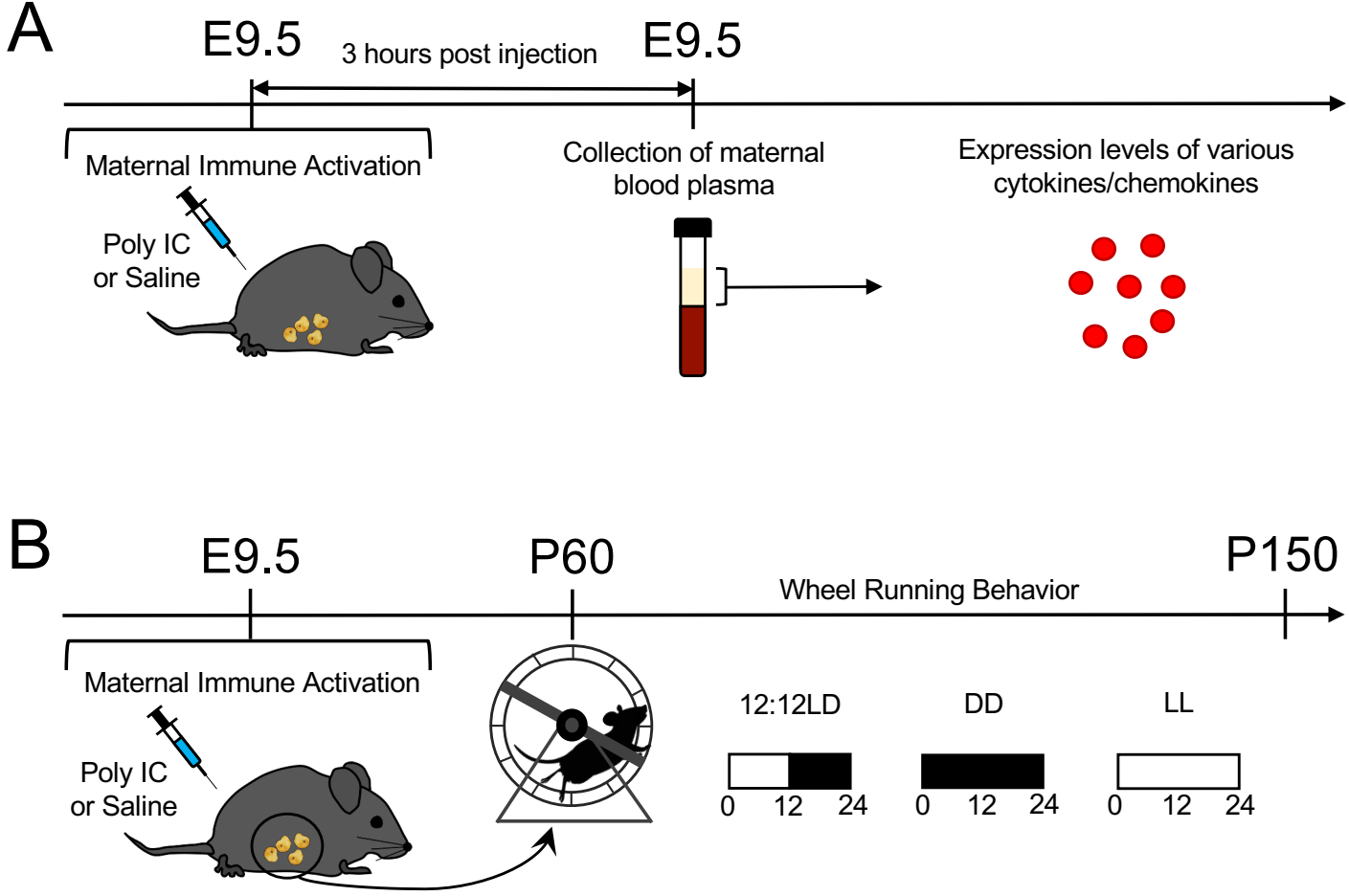
Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

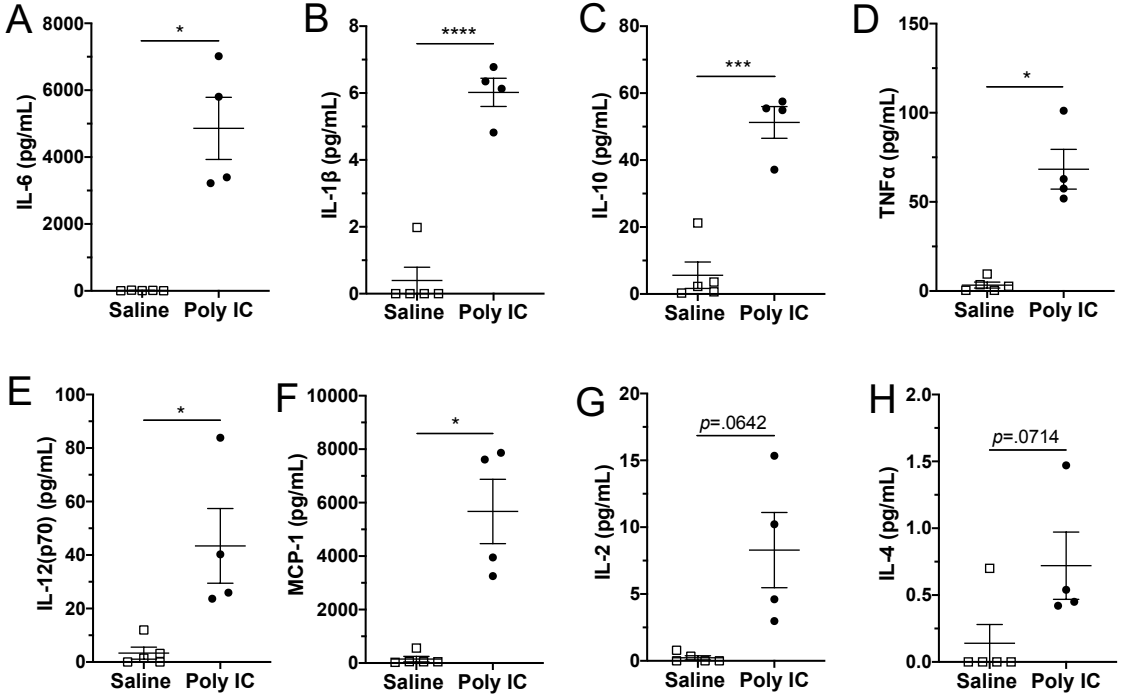
Table 2. Summary of wheel running parameters from poly IC and saline-exposed female offspring.

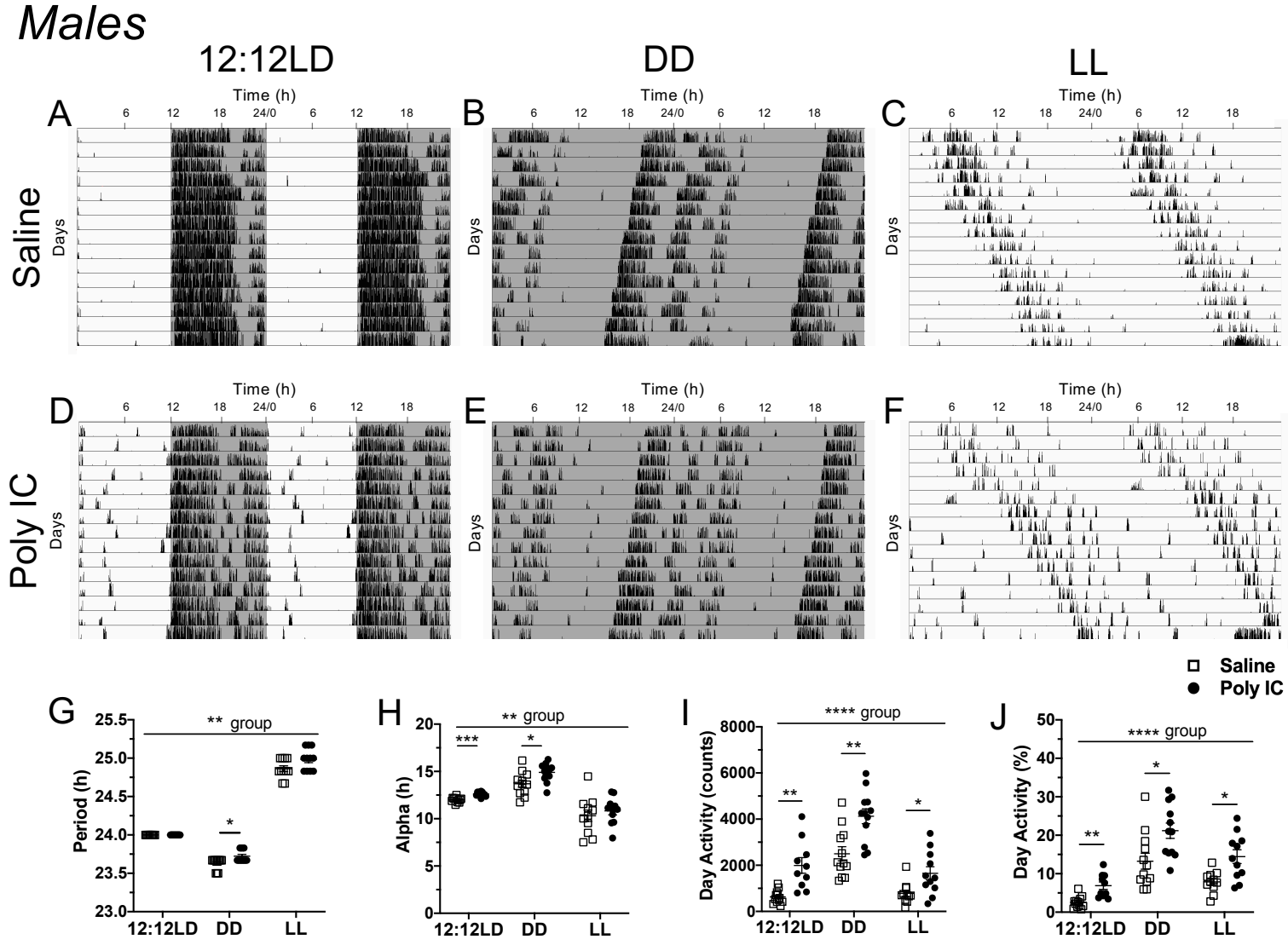
	12:12LD		DD		LL	
	Saline (n=5)	Poly IC (n=6)	Saline (n=5)	Poly IC (n=6)	Saline (n=5)	Poly IC (n=6)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.8 ±0.06	23.9 ± 0.07	25.1 ± 0.03	25.1 ± 0.12
Alpha (h)	10.4 ± 0.12	11.1 ± 0.12	12.1 ± 0.47	11.9 ± 0.77	9.7 ± 1.30	11.6 ± 0.76
(Subjective) Day Activity (counts)	66 ± 24	193 ± 64	2825 ± 958	2500 ± 566	2285 ± 1081	1450 ± 227
(Subjective) Night Activity (counts)	31928 ± 2142	26558 ± 915	28133 ± 2536	26451 ± 2025	10721 ± 3487	11254 ± 1324
Total Activity (counts)	31994 ± 2151	26751 ± 873	30958 ± 2859	28951 ± 2366	11786 ± 4001	13237 ± 1542
(Subjective) Day Activity (%)	0.20 ± 0.08	0.75 ± 0.26	8.94 ± 2.31	8.45 ± 1.73	8.00 ± 1.86	14.45 ± 3.55
Intradaily Variability	0.297 ± 0.046	0.405 ± 0.022	0.423 ± 0.028	0.527 ± 0.063	0.831 ± 0.156	1.059 ± 0.115
Relative Amplitude	0.999 ± 0.001	0.999 ± 0.001	0.988 ± 0.010	0.979 ± 0.005	0.969 ± 0.023	0.897 ± 0.057
Interdaily Stability	0.863 ± 0.014	0.867 ± 0.021	N/A	N/A	N/A	N/A

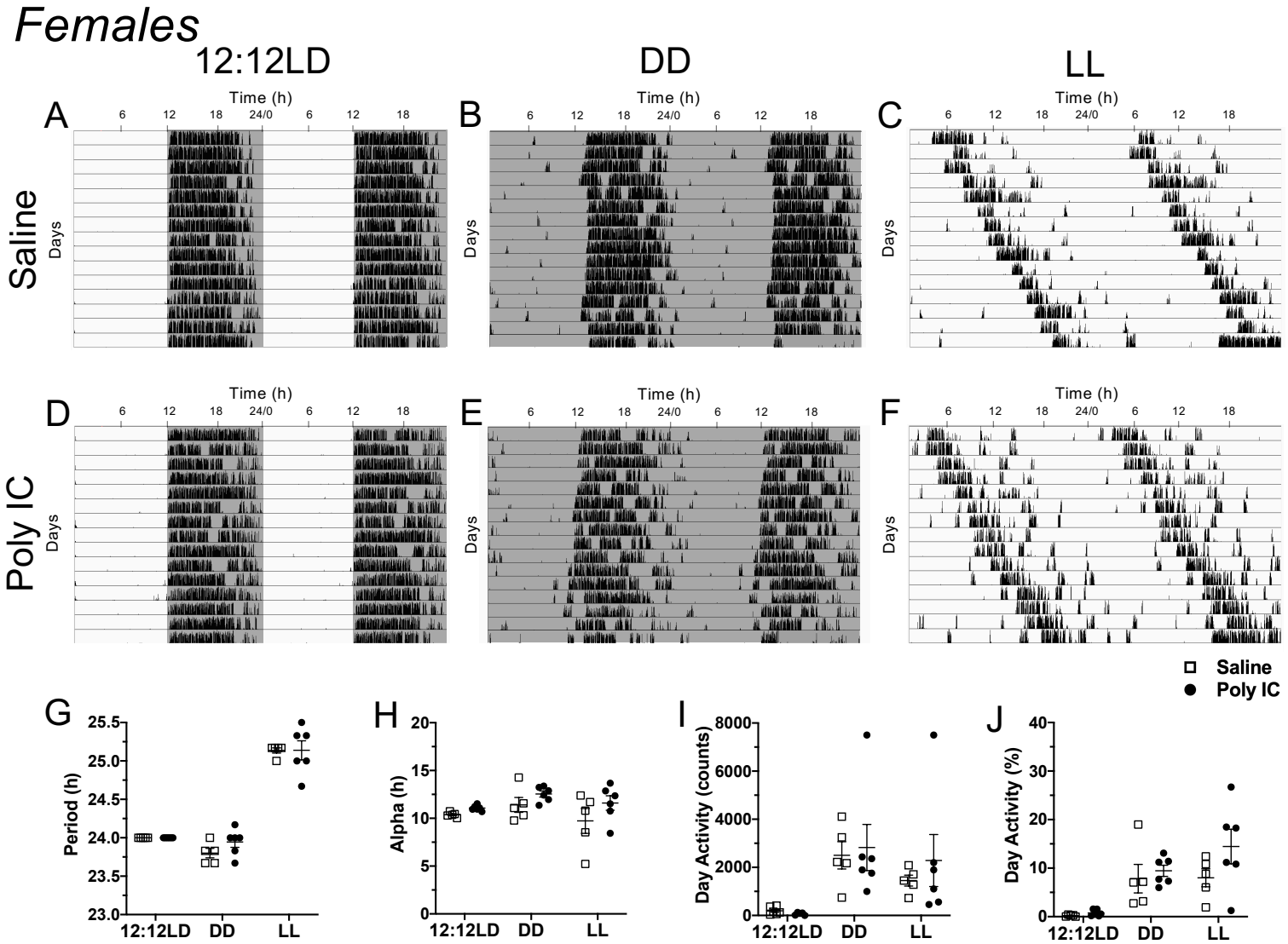
Onset Variability	0.33 ± 0.06	0.29 ± 0.07	2.46 ± 0.64	1.95 ± 0.38	1.55 ± 0.43	1.91 ± 0.54
Number of Bouts	77.6 ± 6.31	106.2 ± 6.12	88.8 ± 10.26	119.0 ± 5.56	98.6 ± 12.00	149.0 ± 8.67
Average Bout Length (minutes)	107.1 ± 13.44	76.6 ± 4.70	97.8 ± 18.34	66.2 ± 3.15	34.2 ± 8.19	25.72 ± 3.08
Average Counts per Bout	6045 ± 702	3602 ± 204	5516 ± 1094	3196 ± 280	1618 ± 473	1116 ± 149
Average Peak Rate	69.40 ± 3.20	63.85 ± 1.86	72.12 ± 1.06	69.50 ± 2.73	65.55 ± 6.58	67.91 ± 2.35

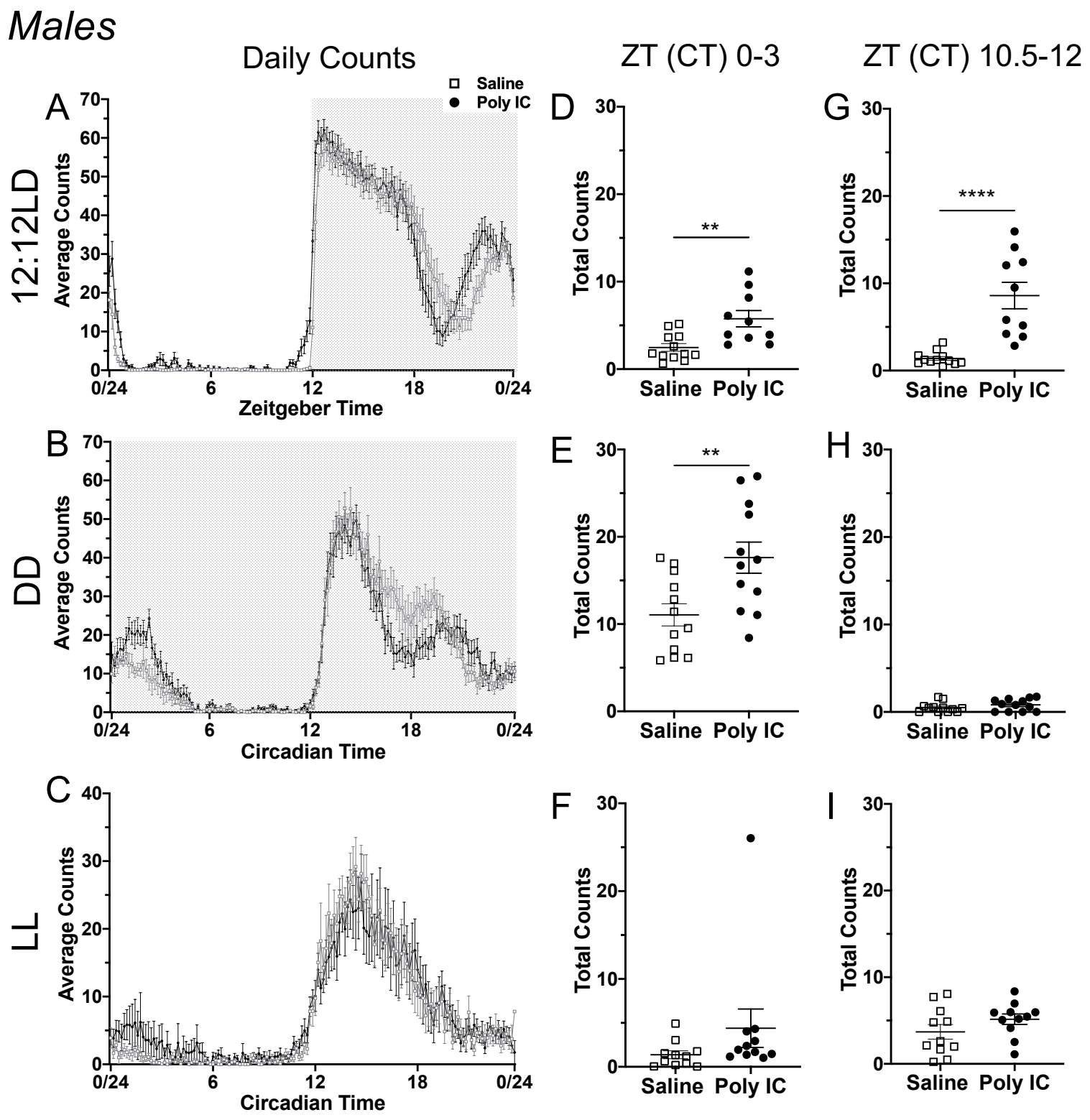
Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

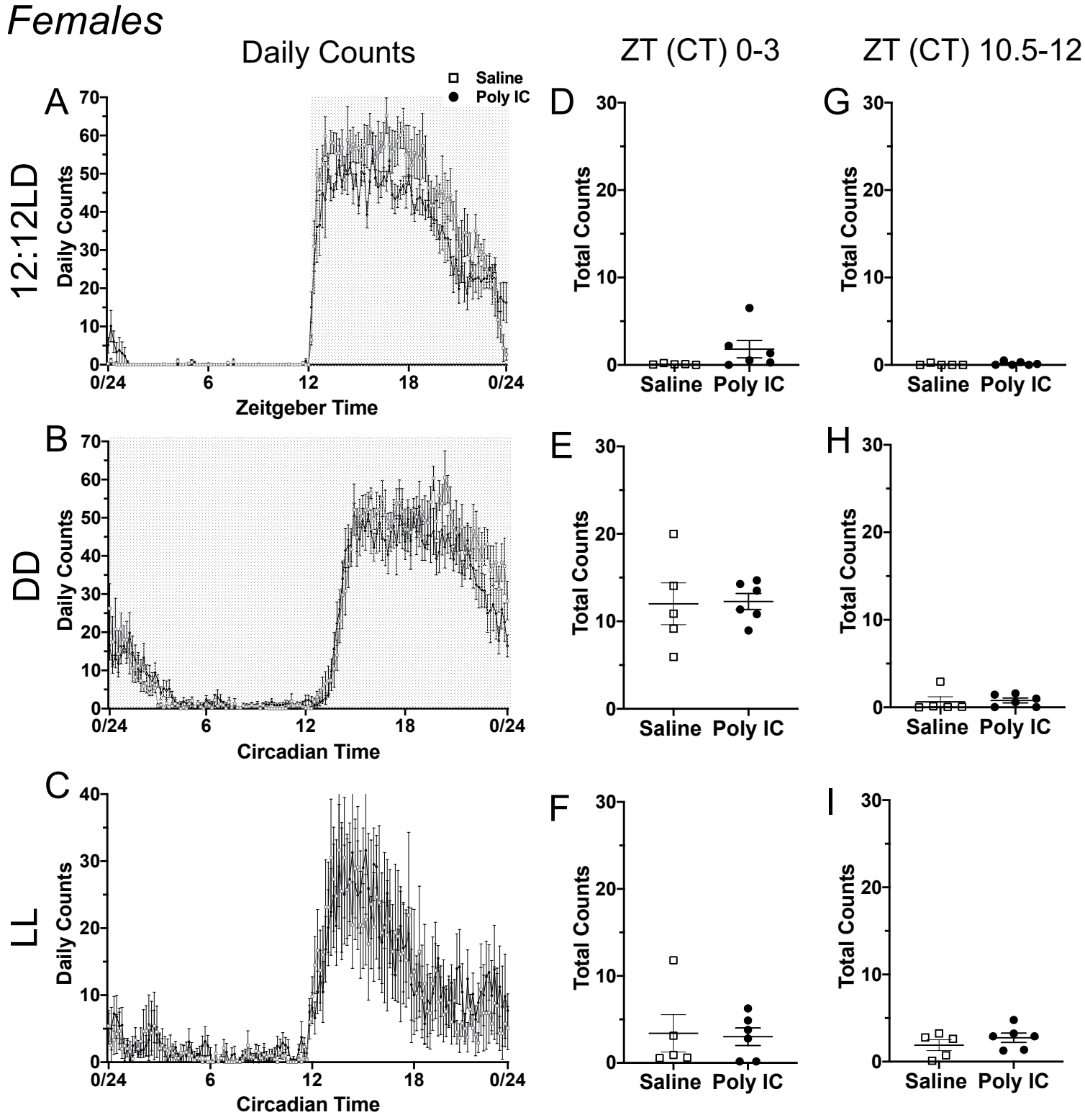

A
E9.5
3 hours post injection
E9.5
Maternal Immune Activation
Poly IC
or Saline
Collection of maternal
blood plasma
Expression levels of various
cytokines/chemokines
B
E9.5
P60
P150
Wheel Running Behavior
Maternal Immune Activation
Poly IC
or Saline
12:12LD
DD
LL
0
12
24
0
12
24
0
12
24

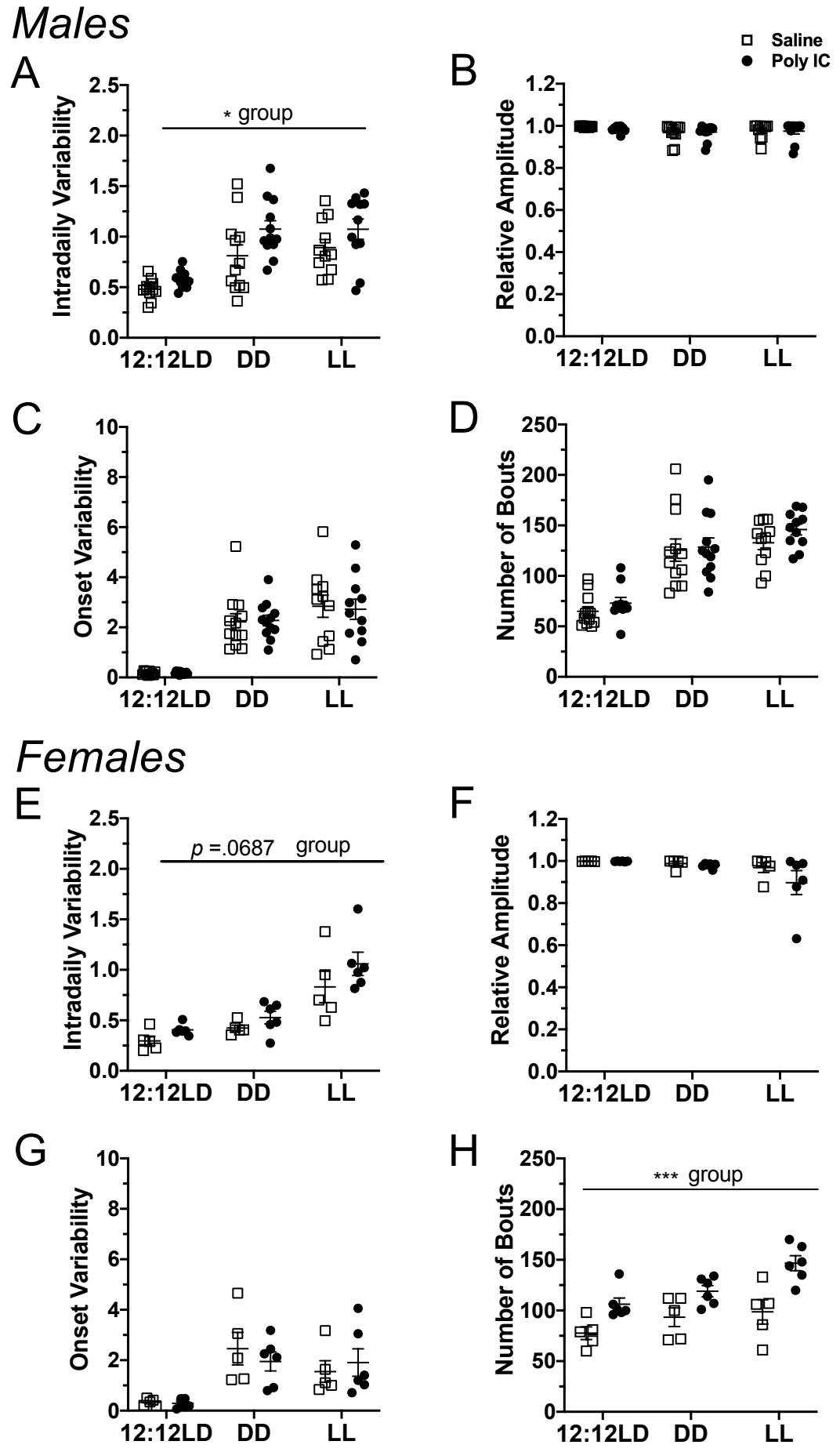

A
IL-6 (pg/mL)
8000
6000
4000
2000
0
*
Saline
Poly IC
B
IL-1β (pg/mL)
8
6
4
2
0
****
Saline
Poly IC
C
IL-10 (pg/mL)
80
60
40
20
0
***
Saline
Poly IC
D
TNFα (pg/mL)
150
100
50
0
*
Saline
Poly IC
E
IL-12(p70) (pg/mL)
100
80
60
40
20
0
*
Saline
Poly IC
F
MCP-1 (pg/mL)
10000
8000
6000
4000
2000
0
*
Saline
Poly IC
G
IL-2 (pg/mL)
20
15
10
5
0
p=.0642
Saline
Poly IC
H
IL-4 (pg/mL)
2.0
1.5
1.0
0.5
0.0
p=.0714
Saline
Poly IC

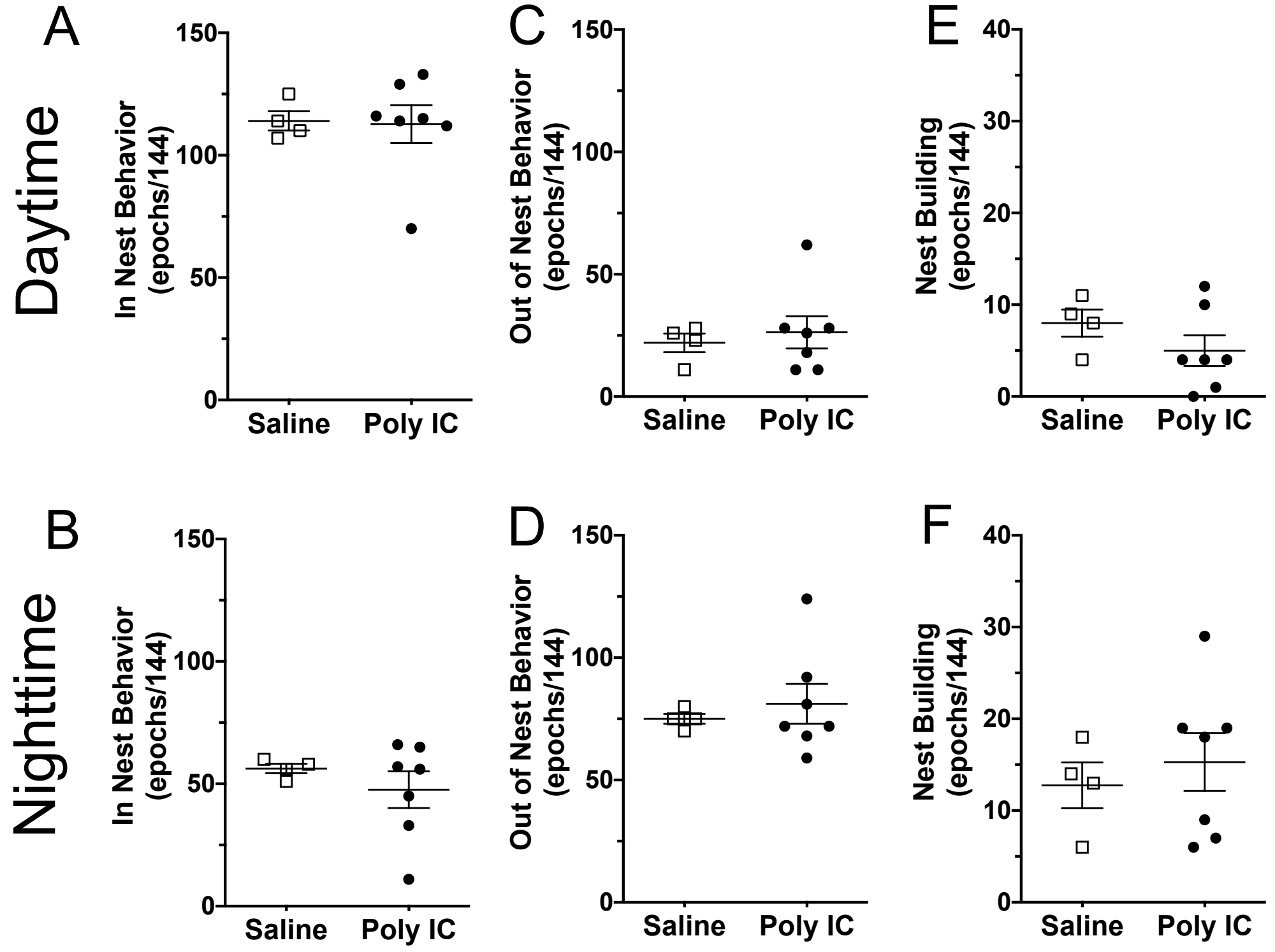

Males
12:12LD
DD
LL
Saline
Poly IC
A
B
C
D
E
F
Time (h)
6
12
18
24/0
Days
Saline
Poly IC
G
H
I
J
** group
**** group
Period (h)
Alpha (h)
Day Activity (counts)
Day Activity (%)
12:12LD
DD
LL


Females
12:12LD
DD
LL
Saline
Poly IC
A
B
C
D
E
F
Time (h)
6
12
18
24/0
Days
Saline
Poly IC
G
H
I
J
Period (h)
Alpha (h)
Day Activity (counts)
Day Activity (%)
12:12LD
DD
LL


Males
Daily Counts
ZT (CT) 0-3
ZT (CT) 10.5-12
12:12LD
DD
LL
A
B
C
D
E
F
G
H
I
Saline
Poly IC
Average Counts
Zeitgeber Time
Circadian Time
Total Counts
0/24
6
12
18
**
****


Females
Daily Counts
ZT (CT) 0-3
ZT (CT) 10.5-12
Saline
Poly IC
A
B
C
D
E
F
G
H
I
12:12LD
DD
LL
Daily Counts
Zeitgeber Time
Circadian Time
Total Counts
0/24
6
12
18
0/24
Saline
Poly IC

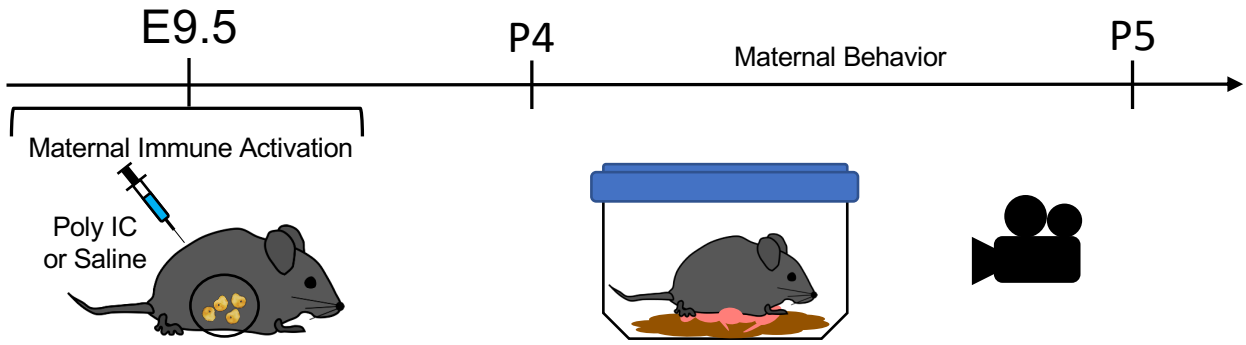

Males
Saline
Poly IC
A
Intradaily Variability
* group
12:12LD
DD
LL
B
Relative Amplitude
C
Onset Variability
D
Number of Bouts
Females
E
Intradaily Variability
p =.0687 group
F
Relative Amplitude
G
Onset Variability
H
Number of Bouts
*** group


A
Daytime
In Nest Behavior (epochs/144)
150
100
50
0
Saline
Poly IC
C
Out of Nest Behavior (epochs/144)
150
100
50
0
Saline
Poly IC
E
Nest Building (epochs/144)
40
30
20
10
0
Saline
Poly IC
B
Nighttime
In Nest Behavior (epochs/144)
150
100
50
0
Saline
Poly IC
D
Out of Nest Behavior (epochs/144)
150
100
50
0
Saline
Poly IC
F
Nest Building (epochs/144)
40
30
20
10
0
Saline
Poly IC

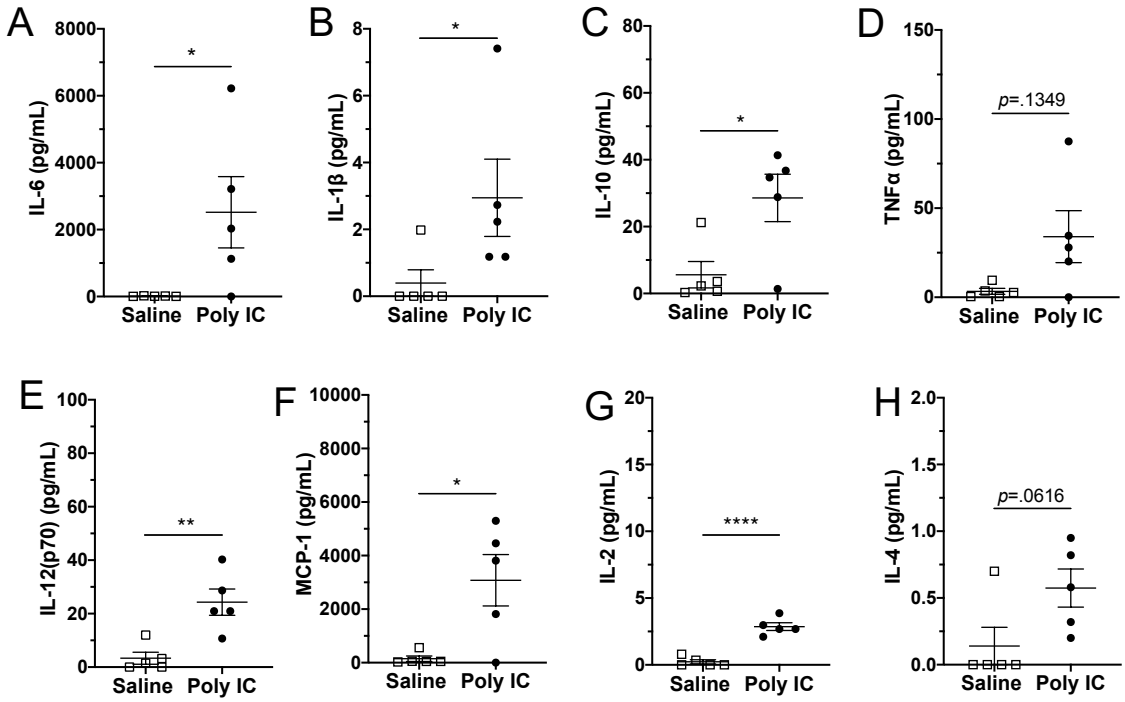
Altered Circadian Rhythms in a Mouse Model of Neurodevelopmental Disorders Based on Prenatal Maternal Immune Activation

Tara C. Delorme, Lalit K. Srivastava and Nicolas Cermakian

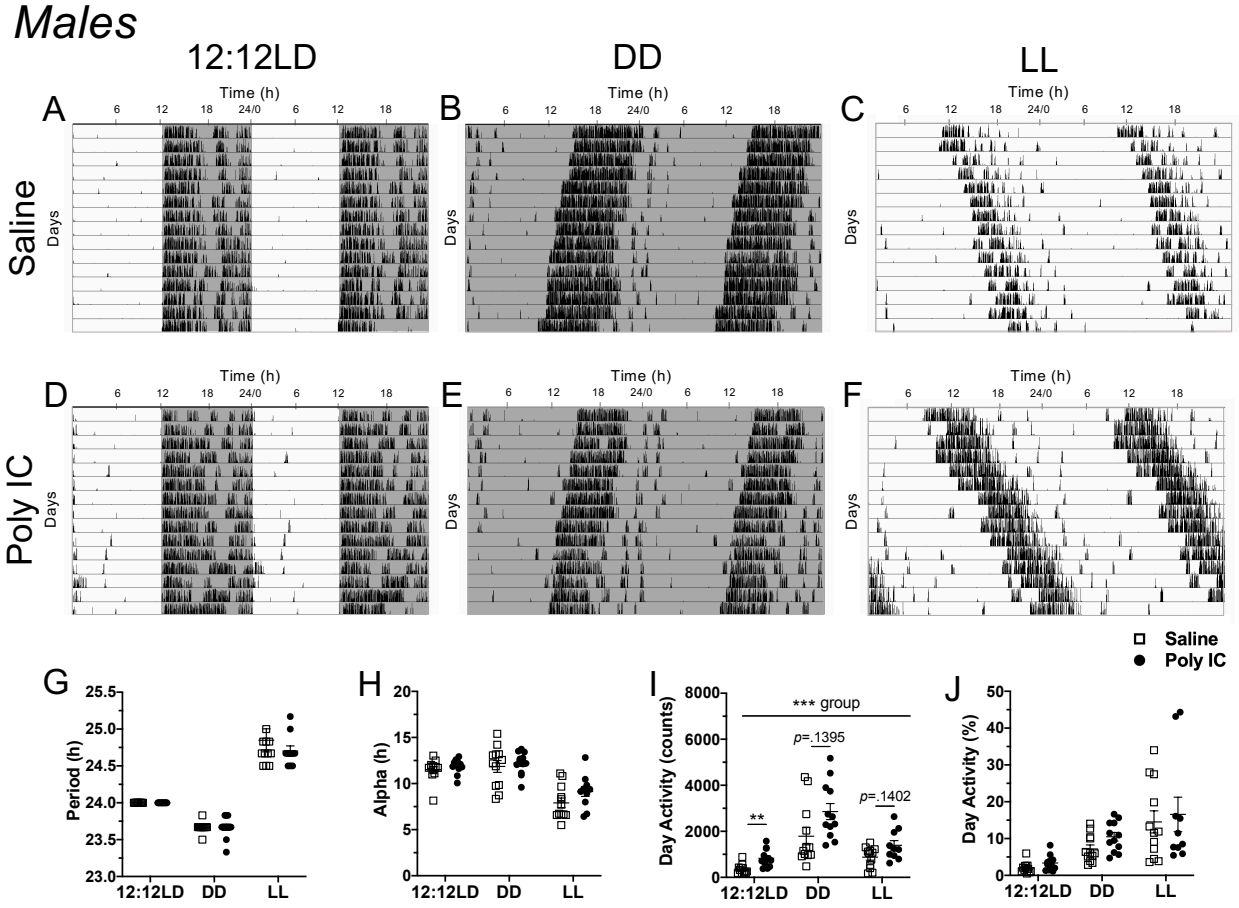
Supplementary Material



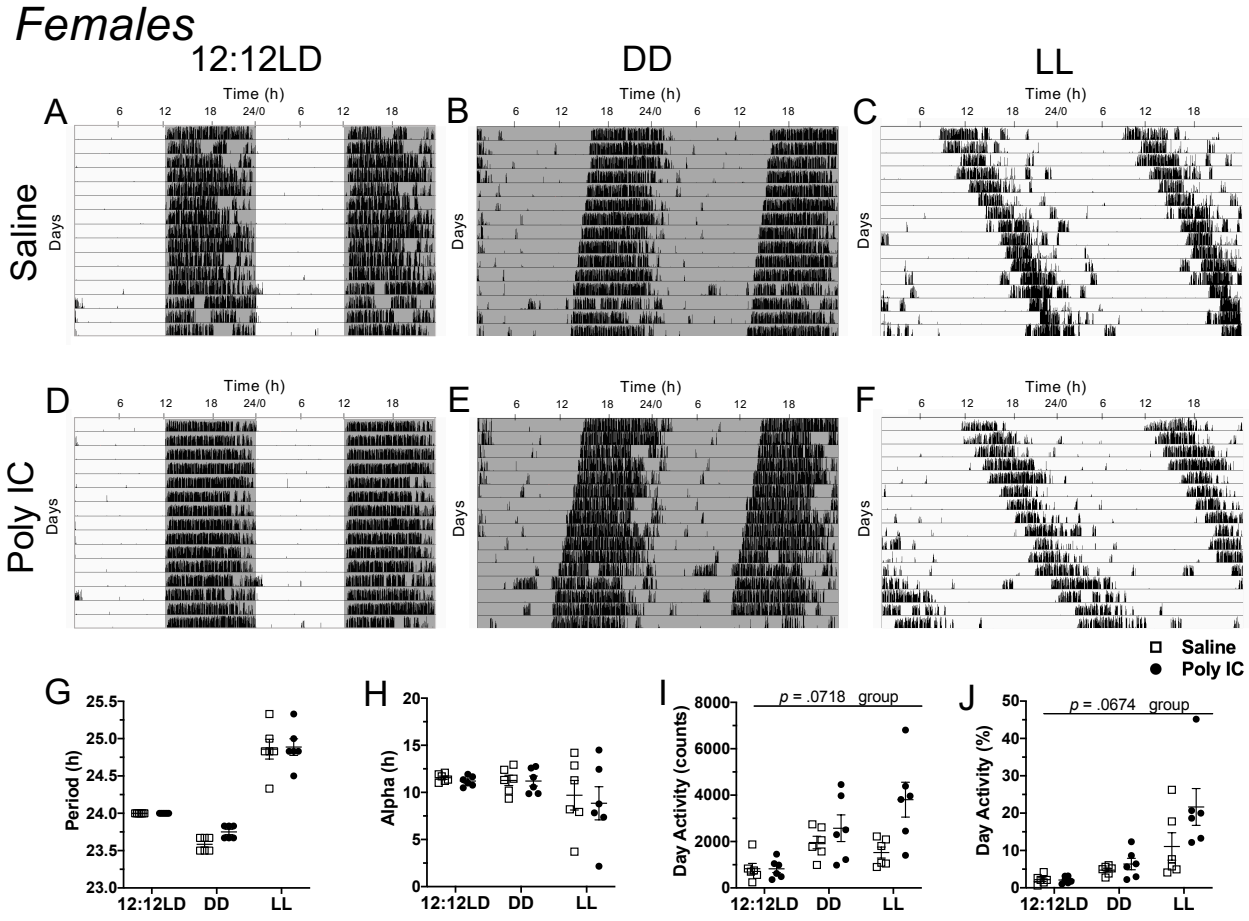
Supplementary Fig. 1. Schematic for scoring maternal behavior protocol. On E9.5, pregnant dams were intraperitoneally injected with poly IC (5 mg/kg) or saline. Pregnant dams delivered their litters naturally and maternal behavior was video recorded and scored between postnatal day 4-5.



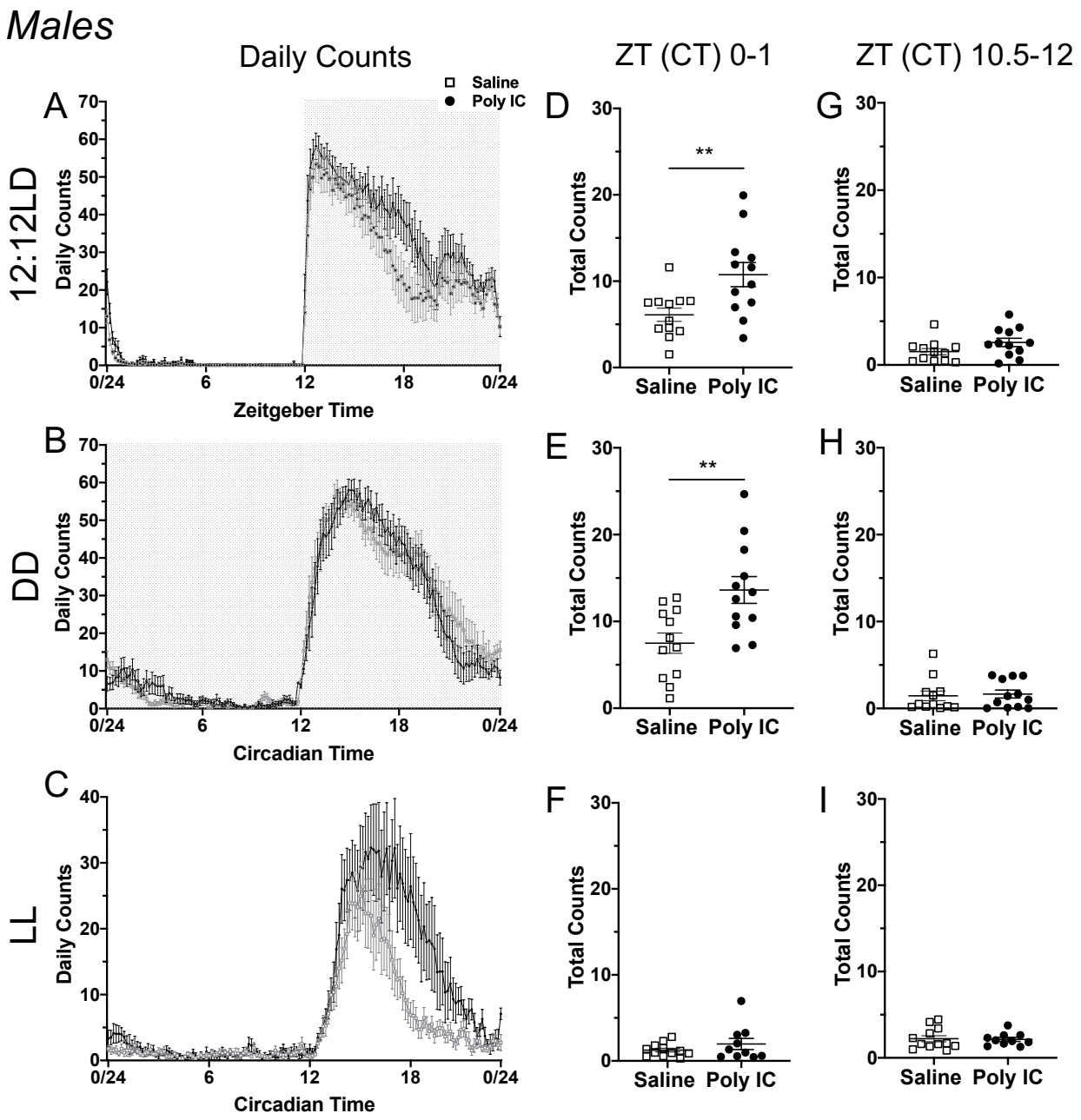
Supplementary Fig. 2. Pro- and anti-inflammatory cytokine and chemokine expression levels in plasma of pregnant dams injected with poly IC (lot: 096M4023V) (n=5) or saline (n=5). Expression levels of the following cytokine and chemokines: (A) IL-6, (B) IL-1β, (C) IL-10, (D) TNFα, (E) IL-12(p70), (F) MCP-1, (G) IL-2, (H) IL-4. Samples that were below the detectable range were designated as 0 pg/mL. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, ****p<.0001, **p<.01 and *p<.05.



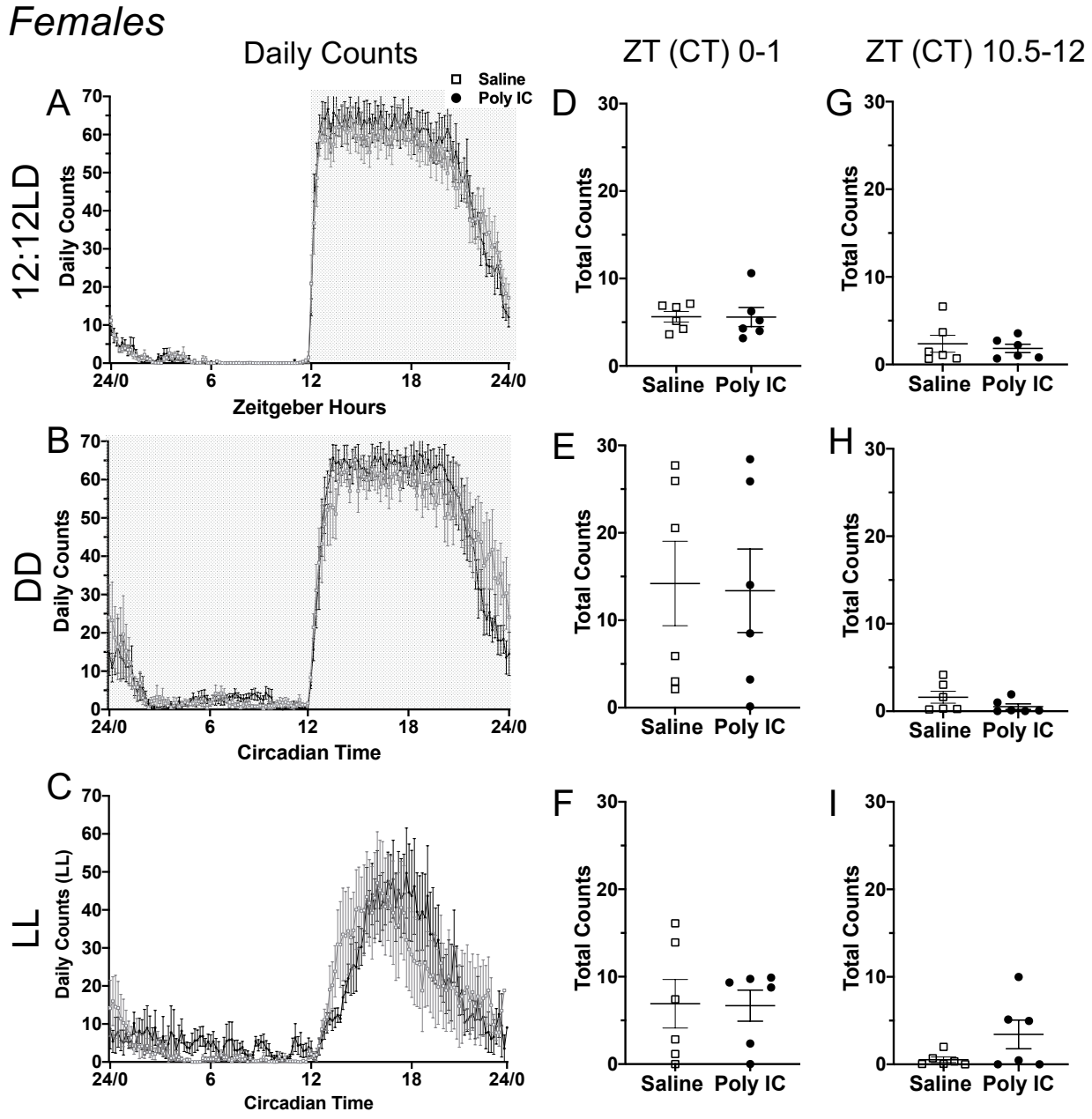
Supplementary Fig. 3. Circadian locomotor activity variables in male offspring. Representative actograms of (A-C) saline-exposed (n=12 from 6 litters) and (D-F) poly IC-exposed male offspring (n=10-12 from 6 litters) under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in hours) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha* (h), (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting, group) was used with Geisser-Greenhouse correction and Sidak's *post-hoc* tests. If no interaction was found, main effects were explored and presented in the graph. ***p <.001.



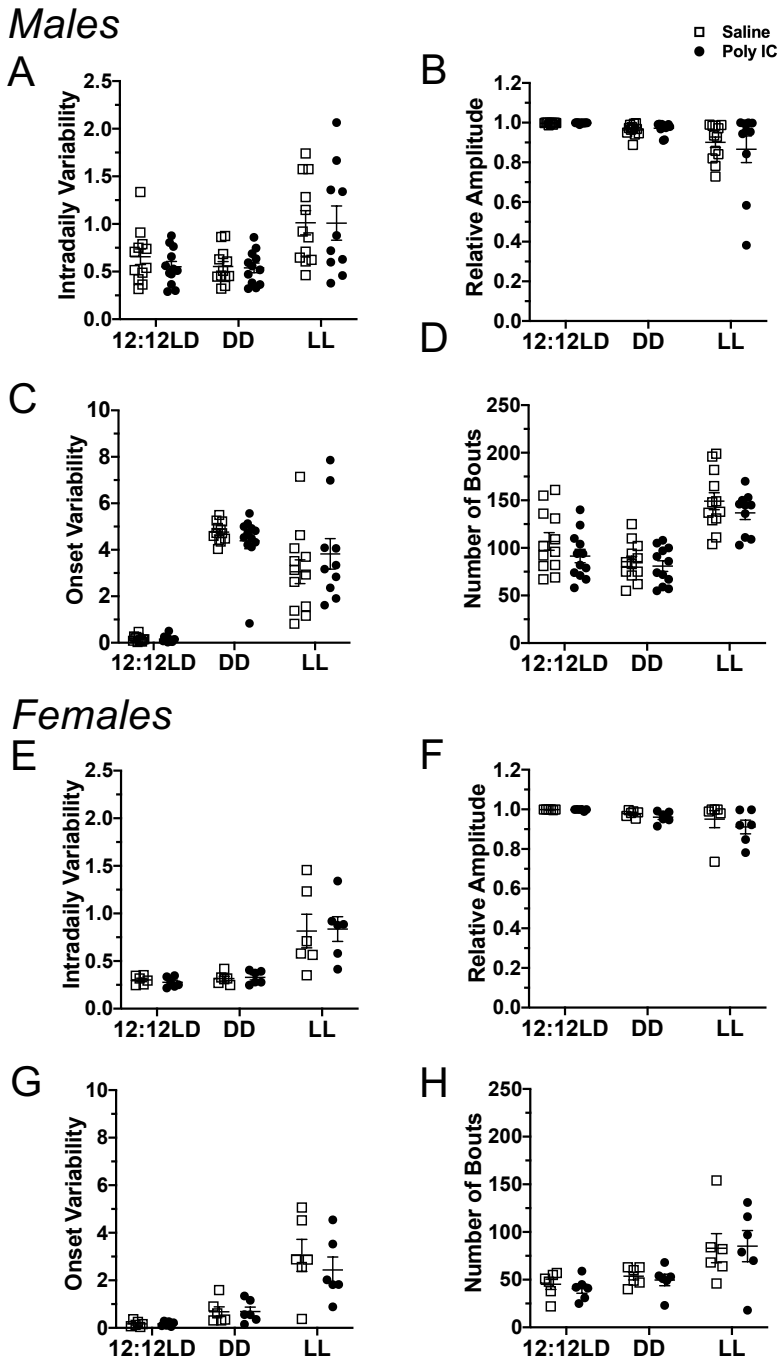
Supplementary Fig. 4. Circadian locomotor activity variables in female offspring. Representative actograms of (A-C) saline-exposed (n=6 from 6 litters) and (D-F) poly IC-exposed (n=6 from 6 litters) female offspring under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in h) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha*, (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. No significant group differences were found.



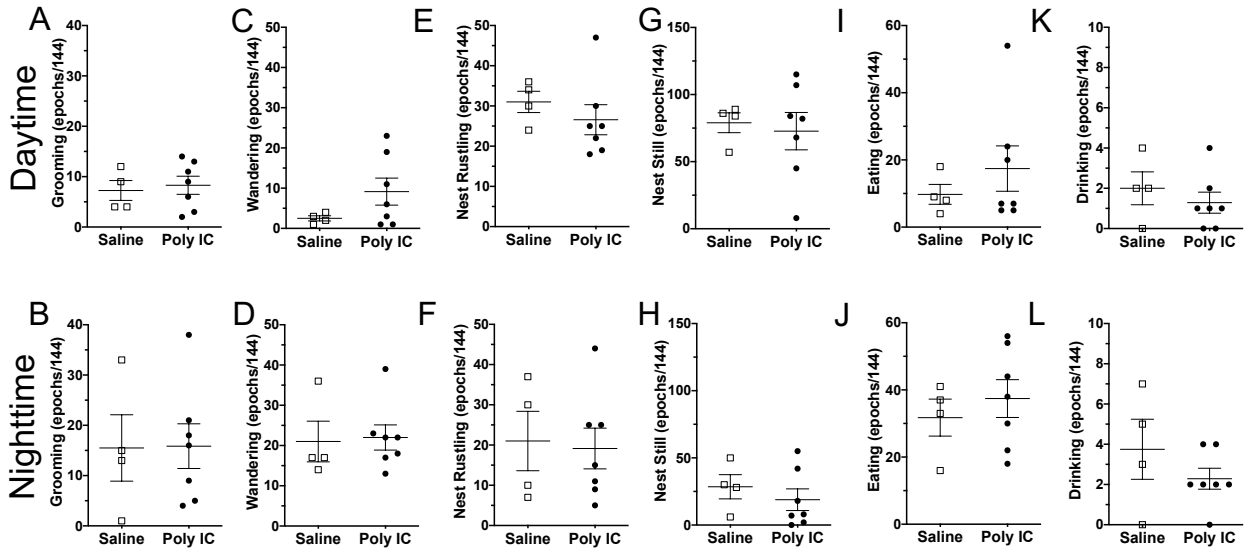
Supplementary Fig. 5. Daily activity profiles averaged across all saline-exposed (n=12 from 6 litters) and poly IC-exposed (n=10-12 from 6 litters) male offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-1) and end (ZT (CT) 10.5-12) of the (subjective) day in (D, G) 12:12LD, (E, H) DD and (F, I) LL respectively. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, $^{**}p < .01$.



Supplementary Fig. 6. Daily activity profiles averaged across all saline-exposed (n=6 from 6 litters) and poly IC-exposed (n=6 from 6 litters) female offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-1) and end (ZT (CT) 10.5-12) of the (subjective) day in (D, G) 12:12LD, (E, H) DD and (F, I) LL respectively. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found.



Supplementary Fig. 7. Activity rhythm characteristics and analysis of activity bouts. Analysis was done for (A-D) male offspring (saline-exposed n=12 from 6 litters; poly IC-exposed n=10-12 from 6 litters) and (E-H) female offspring (saline-exposed n=6 from 6 litters; poly IC-exposed n=6 from 6 litters) separately for the following measures: (A, E) intradaily variability, (B, F) relative amplitude, (C, G) onset variability and (D, H) number of bouts. Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. No significant group differences were found.



Supplementary Fig. 8. Maternal behavior of saline and poly IC-injected dams. Dams were observed between postnatal day 4 to 5 during both the (A, C, E, G, I, K) daytime and the (B, D, F, H, J, L) nighttime and scored for maternal behavior. Dams were scored on (A-B) grooming behavior, (C-D) wandering, (E-F) nest rustling, (G-H) nest still, (I-J) eating and (K-L) drinking. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found.

Supplementary Table 1. Summary of wheel running parameters from poly IC and saline-exposed male offspring from the second cohort.

	12:12LD		DD		LL	
	Saline (n=12)	Poly IC (n=12)	Saline (n=12)	Poly IC (n=12)	Saline (n=12)	Poly IC (n=10)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.7 ± 0.02	23.7 ± 0.04	24.8 ± 0.16	24.7 ± 0.07
Alpha (h)	11.5 ± 0.35	11.9 ± 0.23	11.9 ± 0.64	12.2 ± 0.35	7.9 ± 0.52	9.1 ± 0.53
(Subjective) Day Activity (counts)	350 ± 61	827 ± 110	1789 ± 375	2857 ± 349	876 ± 129	1392 ± 204
(Subjective) Night Activity (counts)	19826 ± 2695	26313 ± 2434	22527 ± 2097	25431 ± 2314	6695 ± 1164	11019 ± 2244
Total Activity (counts)	20175 ± 2725	27140 ± 2414	24316 ± 2271	28288 ± 2411	7571 ± 1207	12411 ± 2295
(Subjective) Day Activity (%)	2.07 ± 0.41	3.37 ± 0.58	7.19 ± 1.07	10.50 ± 1.17	14.53 ± 3.00	16.56 ± 4.68
Intradaily Variability	0.656 ± 0.082	0.551 ± 0.055	0.553 ± 0.053	0.539 ± 0.050	1.013 ± 0.127	1.009 ± 0.179
Relative Amplitude	0.997 ± 0.001	0.998 ± 0.001	0.963 ± 0.009	0.972 ± 0.008	0.901 ± 0.026	0.866 ± 0.067
Interdaily Stability	0.816 ± 0.032	0.867 ± 0.023	N/A	N/A	N/A	N/A
Onset Variability	0.17 ± 0.04	0.15 ± 0.04	4.76 ± 0.12	4.39 ± 0.35	3.06 ± 0.51	3.72 ± 0.60
Number of Bouts	106.8 ± 9.28	91.4 ± 7.08	85.5 ± 5.80	80.9 ± 5.58	149.1 ± 8.92	136.8 ± 6.94
Average Bout Length (minutes)	51.5 ± 7.60	70.7 ± 6.77	60.9 ± 6.37	68.3 ± 7.84	19.3 ± 2.04	28.6 ± 4.42
Average Counts per Bout	2359 ± 459	3481 ± 517	3078 ± 401	3585 ± 545	711 ± 116	1329 ± 270
Average Peak Rate	65.44 ± 3.06	72.15 ± 2.30	70.53 ± 2.78	71.94 ± 2.19	59.10 ± 3.13	69.92 ± 4.24

Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

Supplementary Table 2. Summary of wheel running parameters from poly IC and saline-exposed female offspring from the second cohort.

	12:12LD		DD		LL	
	Saline (n=6)	Poly IC (n=6)	Saline (n=6)	Poly IC (n=6)	Saline (n=6)	Poly IC (n=6)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.6 ± 0.04	23.8 ± 0.04	24.9 ± 0.13	24.9 ± 0.11
Alpha (h)	11.6 ± 0.17	11.2 ± 0.22	11.3 ± 0.55	10.9 ± 0.55	9.7 ± 1.60	9.1 ± 2.12
(Subjective) Day Activity (counts)	829 ± 226	826 ± 168	1957 ± 270	2573 ± 576	1526 ± 237	3805 ± 752
(Subjective) Night Activity (counts)	36594 ± 2359	38308 ± 2531	39126 ± 3188	39003 ± 2818	19326 ± 5668	17773 ± 3864
Total Activity (counts)	37423 ± 2451	39134 ± 2630	41083 ± 3367	41576 ± 2796	20852 ± 5734	21577 ± 4481
(Subjective) Day Activity (%)	2.19 ± 0.5	2.08 ± 0.4	4.74 ± 0.5	6.37 ± 1.5	11.08 ± 3.7	21.65 ± 4.9
Intradaily Variability	0.302 ± 0.018	0.277 ± 0.022	0.315 ± 0.024	0.329 ± 0.028	0.815 ± 0.176	0.836 ± 0.130
Relative Amplitude	0.999 ± 0.001	0.998 ± 0.002	0.979 ± 0.007	0.961 ± 0.012	0.950 ± 0.043	0.911 ± 0.035
Interdaily Stability	0.876 ± 0.030	0.928 ± 0.010	N/A	N/A	N/A	N/A
Onset Variability	0.19 ± 0.06	0.15 ± 0.04	0.68 ± 0.21	0.69 ± 0.19	3.05 ± 0.68	2.56 ± 0.65
Number of Bouts	46.3 ± 3.29	43.0 ± 4.95	53.7 ± 3.95	49.7 ± 6.00	83.0 ± 15.27	85.2 ± 16.30
Average Bout Length (minutes)	109.7 ± 14.68	117.7 ± 13.35	98.1 ± 9.18	107.97 ± 18.90	47.4 ± 16.71	42.51 ± 5.48
Average Counts per Bout	6425 ± 746	6992 ± 875	5912 ± 684	6745 ± 1328	2783 ± 1184	2317 ±322
Average Peak Rate	72.87 ± 3.32	62.49 ± 4.88	66.04 ± 3.23	67.47 ± 4.52	65.45 ± 4.03	80.29 ± 5.47

Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

Supplementary Table 3. Summary of three-way ANOVAs to explore sex differences in circadian locomotor variables.

	Cohort 1				Cohort 2			
	Group x Sex Interaction		Main Effect of Sex		Group x Sex Interaction		Main Effect of Sex	
	F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value
Period (h)	0.21	0.6540	58.38	0.0001	1.16	0.2900	0.35	0.5600
Alpha (h)	0.61	0.4400	18.22	0.0002	1.40	0.2458	0.07	0.7891
(Subjective) Day Activity (counts)	4.45	0.0430	4.58	0.0403	0.41	0.5279	7.0	0.0125
(Subjective) Night Activity (counts)	1.45	0.2385	24.71	0.0001	1.18	0.2847	35.46	0.0001
Total Activity (counts)	2.04	0.1636	16.46	0.0003	0.97	0.3327	35.50	0.0001
(Subjective) Day Activity (%)	2.47	0.1196	15.45	0.0002	0.35	0.5592	0.43	0.5172
Relative Amplitude	1.39	0.2469	0.48	0.4917	0.10	0.7576	0.90	0.3488
Intradaily Variability	0.09	0.7636	16.40	0.0003	0.09	0.7632	9.44	0.0043
Onset Variability	0.04	0.8365	2.36	0.1277	0.51	0.4765	44.69	0.0001
Number of Bouts	5.20	0.0297	0.77	0.3861	0.46	0.4997	56.16	0.0001
Average Bout Length (minutes)	3.59	0.0674	7.55	0.0099	0.22	0.6427	22.13	0.0001
Average Counts per Bout	6.91	0.0132	11.50	0.0019	0.18	0.6775	25.37	0.0001
Average Peak Rate	0.04	0.8365	2.36	0.1277	0.84	0.3654	0.08	0.7726

Notes. Results from three-way ANOVA for circadian locomotor variables, highlighting the group x sex interactions and main effects of sex analysis. ANOVA factors include sex (male, female), group (poly IC, saline exposure) and lighting condition (12 h of light, 12 h of dark (12:12LD), constant dark (DD) and constant light (LL)).

Supplementary Table 4. Summary of two-way ANOVAs to explore sex differences in data collected from daily profiles.

	Cohort 1				Cohort 2			
	Group x Sex Interaction		Main Effect of Sex		Group x Sex Interaction		Main Effect of Sex	
	F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value
Beginning of Day Activity (12:12LD)	0.96	0.3355	15.77	0.0004	3.66	0.0646	5.33	0.0276
Beginning of Subjective Day Activity (DD)	2.98	0.0942	1.46	0.2360	1.65	0.2089	1.42	0.2419
Beginning of Subjective Day Activity (LL)	0.91	0.3470	0.03	0.8588	0.13	0.7133	16.88	0.0003
End of Day Activity (12:12LD)	12.47	0.0014	23.60	0.0001	1.98	0.1693	0.01	0.9314
End of Subjective Day Activity (DD)	0.07	0.7930	0.03	0.8644	1.23	0.2767	0.72	0.4071
End of Subjective Day (LL)	0.14	0.7073	7.06	0.0127	4.91	0.0345	0.09	0.7623

Notes. Results from two-way ANOVA for data collected from daily activity profiles, highlighting the group x sex interactions and main effects of sex analysis. ANOVA factors include sex (male, female), group (poly IC, saline exposure). 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.