All behavioral parameters were recorded by observers who were blind to the treatment. In addition, all behavioral tests were conducted in a quiet room during the light period (between 13:00 and 18:00) under bright and moderate illumination, and the mice were kept in the room for at least 1 h before the assessment. Depression-related behavior was separately studied in adolescent (PND 35: tail suspension test [TST]; PND 40: forced swimming test [FST]) and adult (PND 85: TST; PND 90: FST) male mice.

TST: The TST was performed according to the previously described procedure.[6] At the beginning of the experiment, each mouse was individually suspended by its tail using a clamp, 2 cm from the distal end, for 5 min in a gray wooden box (40 cm high, 30 cm wide, and 20 cm deep), with the head about 25 cm above the floor. The total duration of immobility was recorded (in seconds). All animals that climbed their tails during the TST were excluded from the further analyses. Immobility was defined as the lack of whole-body motion, whereas mobility was defined as hind leg movement.[16]

FST: The FST remains one of the most widely used tools for measuring behavioral despair in rodents. To describe this behavioral model in mice, the following procedure was adopted: mice were individually placed into the transparent glass cylinders (height: 25 cm, diameter: 10 cm) filled with water to a height of 15 cm and maintained at 25 ± 1°C. The water was replaced between each test. The total duration of immobility was recorded during the last 4 min of the 6-min testing period. At the end of swimming session, the animals were removed from the cylinder, dried with towels, and gently placed near an electric heater for 15–30 min. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility time is considered indicative of depression-like behavior in mice.[6,10]

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences software (Version 21, IBM, Armonk, NY, USA). The depression results were analyzed using three-way analysis of variance, with age, treatment, and neonatal infection timing as the main factors. All data are presented as the mean ± standard error of the mean. Further analysis was carried out using Tukey's honest significant different *post-hoc* tests for multiple comparisons. $P < 0.05$ was considered as statistically significant.

RESULTS

Effects of early and late neonatal immune activation on depression-related behaviors during adolescence and adulthood in the TST

The three-way analysis revealed the significant effects of the time of neonatal immune activation ($F_{1,84} = 5.65$, $P < 0.03$), age ($F_{1,84} = 43.03$, $P < 0.001$), and treatment ($F_{2,84} = 11.57$, $P < 0.001$) on the immobility time in the TST. Significant interactions existed between age × treatment ($F_{2,84} = 4.66$, $P < 0.02$) and the time of neonatal immune activation × age × treatment ($F_{2,84} = 3.37$, $P < 0.04$). However, there was no significant interaction between the time of neonatal immune activation × age and the time of neonatal immune activation × treatment. These results indicate that neonatal immune activation with Poly I:C can influence depression-related behaviors in dose-, age-, and time-dependent manner in mice. Therefore, the dose of immunogen, the timing of immune activation, and age may be important factors for evaluating the consequences of neonatal immune activation on affective disorders, like depression, later in life.

The data analysis indicated that early neonatal immune activation with Poly I: C increased the total duration of immobility at the dose of 4 mg/kg in adolescence [Figure 2a; $P = 0.037$] and at both doses in adulthood [Figure 2b; $P = 0.042$ and $P = 0.002$], indicating high levels of depression-related behaviors in Poly I:C-treated mice in comparison with the saline-treated group.

As shown in Figure 3, late neonatal immune challenge with Poly I:C resulted in an increase in the total duration of immobility time at the dose of 4 mg/kg in adulthood [$P = 0.03$], but not in adolescence [Figure 3].

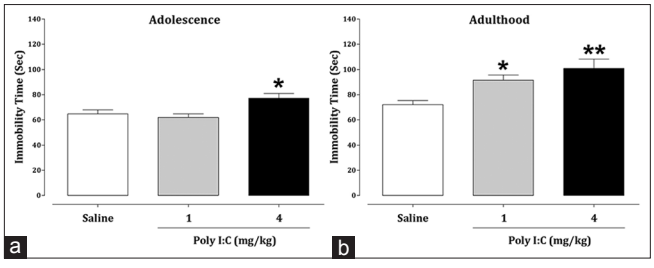

Figure 2: Effects of early neonatal immune activation on depression-like behavior during adolescence (a) and adulthood (b) in the tail suspension test. The data are presented as mean ± standard error of the mean ($n = 8$). *$P < 0.05$ and **$P < 0.01$ compared with the saline-treated group

In addition, we found high levels of depression-related behaviors in adulthood in Poly I:C-exposed mice compared with saline-injected mice.

Effects of early and late neonatal immune activation on depression-related behaviors during adolescence and adulthood in the FST

The three-way analysis indicated the significant effects of the time of postnatal immune activation ($F_{1,84} = 34.69$, $P < 0.001$), age ($F_{1,4} = 20.37$, $P < 0.001$), and treatment ($F_{2,84} = 20.53$, $P < 0.001$) on the immobility time in the FST. Considerable interactions existed between the time of neonatal immune activation × treatment ($F_{2,84} = 5.02$, $P < 0.009$) and age × treatment ($F_{2,84} = 5.77$, $P < 0.005$). However, there was no interaction between the time of neonatal immune activation × age and the time of neonatal immune activation × age × treatment. These data demonstrate that immune activation with Poly I:C during postnatal brain development can affect depression-related behaviors in dose-, age-, and time-dependent manner in adult mice. Thus, these different factors may affect the effects of neonatal immune activation on affective disorders later in life in animal models.

The results of the FST assessment showed that early postnatal immune activation with Poly I:C increased immobility time at the dose of 4 mg/kg during adolescence [Figure 4a; $P = 0.015$] and at both doses in adulthood [Figure 4b; $P = 0.002$ and $P = 0.000$]. Higher levels of depression-related behaviors were measured in Poly I:C-treated mice in comparison with the saline-treated group.

Our data also showed that immune activation with 4 mg/kg Poly I:C during late neonatal brain development increased the total duration of immobility in adulthood ($P = 0.019$), but not in adolescence [Figure 5], indicating high levels of depression-related behaviors during adulthood in Poly I:C-exposed mice relative to the saline-treated group. These results confirmed that time of postnatal immune challenge, age and the dose of immunogen can be important factors for evaluating depression-related behaviors in mice.

DISCUSSION

We recently showed that early postnatal immune challenge with the bacterial endotoxin and LPS can lead to increased levels of corticosterone (COR) and depression-like symptoms in adult male and female NMRI mice.[6] In addition, early postnatal immune challenge has been shown to have adverse outcomes on

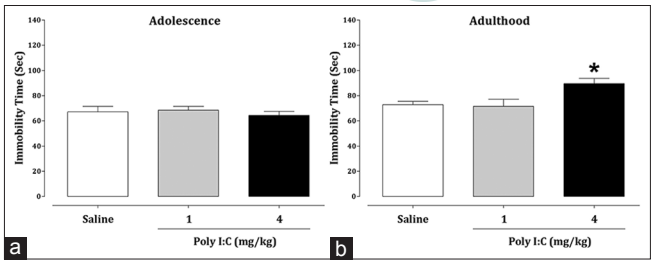

Figure 3: Effects of late neonatal immune challenge on depression-like behavior during adolescence (a) and adulthood (b) in the tail suspension test. The data are presented as mean ± standard error of the mean ($n = 8$). *$P < 0.05$ compared to the saline-treated group

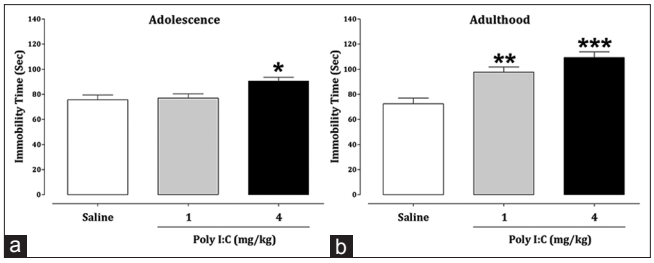

Figure 4: Impacts of early postnatal immune activation on depression-like behavior during adolescence (a) and adulthood (b) in the forced swimming test. The data are presented as mean ± standard error of the mean ($n = 8$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the saline-treated group

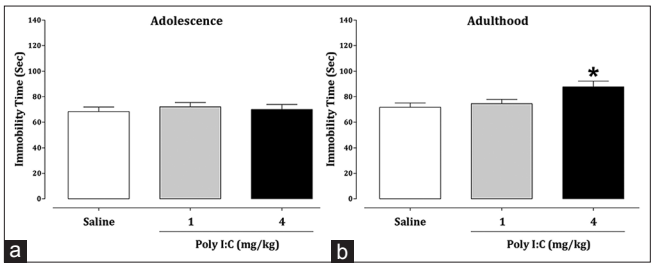

Figure 5: Effects of late neonatal immune activation on depression-like behavior during adolescence (a) and adulthood (b) in the forced swimming test. The data are presented as mean ± standard error of the mean ($n = 8$). *$P < 0.05$, compared to the saline-treated group

physiological, behavioral, and neuroendocrine systems in adulthood.[6,8,9] For instance, it has been reported that bacterial and viral infections during early[9,11] and late[15] neonatal periods results in increased anxiety-like behaviors and disrupted HPA axis activity in adult rodents. Our results demonstrated that early neonatal immune activation led to increased depression-related behaviors in both adolescent and adult mice, but late neonatal infection only increased depression in adult mice. In this regard, Konat *et al.*[15] showed that anxiety levels in rats following late postnatal immune activation were much larger than those observed by Ibi *et al.*[17] in mice following early neonatal immune challenge using a similar behavioral testing. Previous studies demonstrated that early postnatal immune challenge increased baseline COR levels during adolescence and adulthood, while late neonatal immune activation did not alter baseline COR levels in adulthood.[18] It was

also found that COR suppressed cell proliferation and neurogenesis in the hippocampus, which can induce depression-like behaviors.[19] Moreover, the important role of the hippocampus in depression-related behaviors has been shown in humans and rodents.[13] In line with this, we demonstrated that adolescent fluoxetine treatment reduced depression-like behaviors induced by early neonatal infection in adult mice.[6] It has been reported that chronic fluoxetine and imipramine treatments prevent the COR-induced reduction in cell proliferation and activates neurogenesis in the hippocampus.[19] It seems reasonable to speculate that an increase in depression-related behaviors or baseline COR during adolescence following early neonatal immune activation may further suppress cell proliferation and neurogenesis in the hippocampus in comparison with late neonatal immune activation, and these effects can increase the severity of depression in adulthood. Moreover, we observed an interaction between treatment, age, and the time of neonatal immune activation on depression-related behaviors in mice. Poly I:C at the dose of 4 mg/kg during early postnatal brain development increased depression-related behaviors in adolescent mice, while the same dose during late neonatal phase had no significant effect on depression in adolescent mice. Notably, the mice treated with Poly I:C at both doses during early neonatal period exhibited elevated depression-related behaviors as adults, while only the dose of 4 mg/kg during the late neonatal phase increased depression in adult mice. Taken together, the findings of this study suggest that the effect of neonatal immune activation on depression-related behaviors in mice is dependent on the timing of the immune challenge and the dose of immunogen.

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