A

gene	control	poly I:C
Shh	1.00±0.03	0.94±0.08
Fgf8	1.00±0.03	1.03±0.11
Gata2	1.00±0.12	1.00±0.11
Pet-1	1.00±0.03	1.01±0.29

B

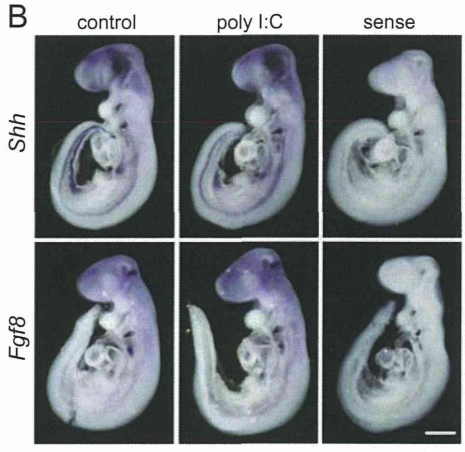

Fig. 2. Relative expression level of genes involved in serotonergic development between the control and poly I:C groups. We intraperitoneally injected poly I:C or vehicle (control) on GD9 and evaluated the relative expression levels of *Shh*, *Fgf8*, *Gata2*, and *Pet-1* in whole fetal brain at GD12 (A). Cranial regions of GD12 embryos were exactly cut just posterior to the fourth ventricle, and five heads of the embryos were collected from four of each pregnant mothers in the two groups. (B) The expression of *Shh* and *Fgf8* mRNA in whole embryos at GD12 were compared by whole-mount *in situ* hybridization. No staining was observed when *Shh* or *Fgf8* sense-strand probes were used. Eight embryos from each group were analyzed. Scale bar represents 500 μm.

To further investigate whether increased number of serotonergic neurons in the rostral cluster in the poly I:C group was caused by abnormal expression of genes such as *Shh*, *Fgf8*, *Gata2*, and *Pet-1*, which are involved in the differentiation and maturation of serotonergic neurons [14,27,28], we used quantitative PCR to determine the levels of transcription in the control and poly I:C groups at GD12. No difference was observed in the mRNA levels between the two groups (Fig. 2A). Consistent with this, analysis of *Shh* and *Fgf8* expression using whole-mount *in situ* hybridization with digoxigenin-labeled cRNAs probe did not detect differences between two groups upon microscopic observation (Fig. 2B). No staining was observed when the *Shh* or *Fgf8* sense strands were used as probes.

4. Discussion

In the present study, we demonstrate that maternal administration of poly I:C induces an increased number of serotonergic neurons in rostral raphe by GD15 and decreased 5-HT content in the hippocampus of offspring by P50. Because poly I:C administration mimics viral infection, these results suggest that maternal viral infection during pregnancy induces lasting perturbations in the serotonergic system, which may subsequently cause developmental disorders such as schizophrenia or ASD.

Effects of poly I:C on nutritional status during pregnancy or postnatal period could modify the brain development. In order to solve this issue, we compared the weight of hippocampus as well as striatum between control and poly I:C-injected groups. The weight of hippocampus and striatum did not differ between two groups, which suggest that no nutritional effects were observed by poly I:C injection.

Adult hippocampal 5-HT levels decreased following prenatal poly I:C injection, which is consistent with the data reported using mice [29]. However, our data are novel and important because we found that poly I:C injection on GD9 induced abnormal fetal serotonergic development. The mechanisms responsible for these effects remain to be determined. However, our analysis on fetuses using flat whole-mount immunohistochemistry may provide a new understanding of the mechanisms that determine the risk of maternal viral infection. Further studies aside from flat whole-mount preparation should be necessary to elucidate the effect of poly I:C during embryonic period.

Decreased hippocampal 5-HT levels in the adult following the administration of poly I:C to fetuses further suggests that GD10 viral infection may cause behavioral and cognitive abnormalities in the adult because behavioral and cognitive functions are closely influenced by 5-HT levels. This conclusion is supported by our previous findings that chemicals that perturb early serotonergic development induce ASD-like phenotypes, which represent behavioral and cognitive dysfunction. Therefore, we hypothesize that viral infection during pregnancy as well as chemicals that may perturb early serotonergic development induce behavioral and cognitive abnormalities such as ASD. Moreover, viral infection during pregnancy increases the risk of ASD in offspring [30–32]. Further investigation will be necessary to determine the precise mechanisms by which maternal viral infection causes behavioral and cognitive dysfunction.

We measured monoamines and their metabolites content only in the striatum and hippocampus. In our previous papers, we have found that embryonic effects on serotonergic system are exclusively occurred in hippocampus [13,22]. From our reports, hippocampus might be vulnerable by embryonic exposure such as chemical (e.g. thalidomide). Along these lines, we focused on hippocampus following poly I:C administration. The reason why we also saw striatum is that dopamine is well known to be rich especially in striatum.

Viral infection activates the immune system. We found that poly I:C administration increased the expression of cytokine genes such as *interferon-γ* (*IFN-γ*),

interleukine-1β (*IL-1β*) *IL-6*, and *tumor necrosis factor-α* (*TNF-α*)(data not shown). Moreover, elevated cytokine levels and activated microglia and astrocytes are present in postmortem brains of autistic people aged 5–44 years [33], and elevation of cytokine levels are observed in the cerebral spinal fluid of autistic children [34], which suggest that immune activation induced by poly I:C may cause ASD in offspring. Evidence indicating that exposure to chemicals such as valproic acid or mercury is a risk factor for ASD [35,36], and findings indicating that exposure to chemicals such as valproic acid or mercury induces immune activation [37,38] support our hypothesis that some chemicals which are known to be risk factors for ASD cause ASD in offspring through immune activation during pregnancy. Interestingly, chronic mercury exposure results in a male-specific increase in TNF-α expression in the cerebellum and hippocampus, which are two of the main areas that are affected in ASD [38]. This may explain the male-oriented bias in ASD. Further studies are required to resolve these issues.

5. Conclusion

Maternal immune activation induced by poly I:C administration increases the number of serotonergic neurons of offspring in the rostral raphe and decreases the 5-HT level in adult rat hippocampus in offspring. These results suggest that maternal viral infection during pregnancy influences fetal development of the serotonergic system. Perturbations in the early development of serotonergic neurons may cause developmental disorders after birth.

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