Response to Comment on “Multiple repressive mechanisms in the hippocampus during memory formation”

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Mathew et al. propose that many candidate genes identified in our study may reflect the events in the choroid plexus (ChP) potentially included in hippocampal samples. We reanalyze our data and find that the ChP inclusion is unlikely to affect our major conclusions regarding the basal suppression of translational machinery or the early translational repression (at 5 to 10 minutes). As Mathew et al. examined for a subset of genes at 4 hours, we agree that the late suppression may partly reflect the events in the ChP. Although the precise contribution of anatomical sources remains to be clarified, our behavioral analyses indicate that the late-phase suppression of these genes may contribute to memory formation.

We have previously identified numerous regulatory mechanisms during long-term memory formation by analyzing transcriptome and translatome in mouse hippocampus. In particular, we found three types of repressive events: basal translational suppression of ribosomal biogenesis, learning-induced early translational repression of genes such as Nrsn1 (at 5 to 10 min after learning), and late transcriptional suppression of a subset of genes in the estrogen receptor signaling pathway (at 30 min to 4 hours after learning).

Mathew et al. (1) performed reverse transcription polymerase chain reaction (RT-PCR) on the several candidate genes at the 4-hour time point and proposed that choroid plexus (ChP) might have been included in our hippocampal samples and that the ChP inclusion might affect the changes detected at 4 hours (2). It is important to note that an immediate and speedy isolation of tissues is known to degrade rapidly after death. Our protocol enabled us to perform an accurate time course study with short time intervals using ribosome profiling (RPF) and RNA sequencing (RNA-seq). As Mathew et al. and another recent study (3) indicate, this conventional method of simple and quick isolation inevitably involves the inclusion of the ChP that is closely attached to the hippocampus (3).

So as to evaluate the effect of ChP inclusion on our transcriptome (RNA-seq) and translatome (RPF) data, we sought to examine the expression patterns of the genes that are enriched in the ChP and depleted in the hippocampus. To our knowledge, the transcriptome of the ChP has not been profiled in direct comparison with that of the hippocampus, so it is actually impossible to properly define the “ChP signature genes.” Therefore, first, we examined the genes reported to be expressed relatively highly in ChP cells—compared with neurons, oligodendrocytes, and astrocytes—by in situ hybridization experiments (4) (categorized here as “ChP cell signature,” 109 genes). We determined their relative abundance ranks among the genes detected in our RNA-seq data from hippocampal tissue and primary culture, and those in the published ChP transcriptome data, independently (5) (deposited in Gene Expression Omnibus, GSE66312). These genes showed strong enrichment in the ChP transcriptome, whereas they were expressed at relatively low levels in hippocampal primary culture (Fig. 1, A and B).

In our previous study, we observed a low level of translation efficiency of the ribosomal protein-coding genes from hippocampal primary culture as well as hippocampal tissue (2). Considering that the ribosome protein-coding genes are repressed translationally in the ChP cell-depleted culture, the basal regulation observed in our findings is unlikely to be affected by ChP inclusion.

Although the ChP cell signature genes were detected at very low levels in the hippocampal primary culture, they showed a relatively moderate level of RNA expression in hippocampal tissue (Fig. 1, A and B). These results indicate that, as Mathew et al. and another study pointed out (3), there might be a chance of inadvertent ChP inclusion in hippocampal tissue preparation. Even though the relative proportion of the adjacent ChP is tiny compared to that of the hippocampus as the source for the libraries, several genes that are highly expressed in the ChP may influence the transcriptome and translatome data.

To further evaluate the contribution of the transcripts abundant in the ChP, we generated another list of genes that are expressed highly in the ChP transcriptome (top 15%) and expressed at low levels in the primary culture transcriptome (below 50%) (dubbed as “ChP enriched (vs Hippo PC), 369 genes”). We then examined how many differentially expressed genes (DEGs) that we identified at different time points belong to the two ChP-specific signature sets. Only one gene (Dnah6) of the “ChP enriched (vs Hippo PC)” and none of the “ChP cell signature” genes was included among the DEGs at early phases (among 25 DEGs at 5 min and 16 DEGs at 10 min) (Fig. 1C). Notably, Nrsn1, which we investigated as an example of the early repressed genes, is not highly expressed in ChP. We previously showed that Nrsn1 was down-regulated by neuronal activity in hippocampal primary culture and that the mice with Nrsn1 overexpression in hippocampal neurons exhibited deficits in long-term memory formation (2). Therefore, most of the significant changes detected at early phases are likely to be solely attributed to the hippocampus.

Unlike the early events, the DEGs at 30 min and at 4 hours after learning included many genes that belong to the two ChP-specific signature sets. Especially, a large proportion of the DEGs that showed transcriptional repression were members of either one or both of the sets (24 out of 42 DEGs at 30 min and 37 genes out of 55 DEGs at 4 hours) (Fig. 1C), whereas none of the activated DEGs are enriched in the ChP. We therefore do not exclude the possibility that the late persistent down-regulation may reflect at least partly the changes in the ChP rather than those in the hippocampus.

However, Mathew et al. show that seven out of the nine ChP abundant genes they tested by qRT-PCR were down-regulated in the hippocampus as well as in the ChP. Four of them reached statistical significance, which is the same number of significantly decreased genes in the ChP. Among the down-regulated DEGs at 4 hours, many genes were also previously identified as repressed genes in the hippocampus after learning (6). These reports, including ours, consistently show the gene repression after conditioning in the hippocampal transcriptomes. To conclusively clarify whether the learning-induced gene regulatory events occur in the hippocampus or the ChP or both, we must await subsequent studies using advanced methods.

Last, one of the fundamental purposes of our recent study was to find and suggest unknown mechanisms that are important for long-term memory formation. Our behavioral analyses upon the activation of ESR1, a potent upstream regulator
for a large portion (about half) of the repressed DEGs at late phase, underpins the importance of the late persistent down-regulation of these genes for long-term memory formation: We have validated that a large number of DEGs at 4 hours are under the control of the ESR1 signaling pathway and that ESR1 agonist injection into the hippocampus impaired hippocampus-dependent memory formation in an object location task with nonstressful training, as well as contextual fear conditioning (2). ESR1 agonist may have directly acted on the hippocampus, but we cannot exclude the possibility that the agonist affected the ChP cells by diffusion via cerebrospinal fluid or blood vessels. However, regardless of the routes of ESR1 agonist action, these results indicate the functional importance of ESR1 signaling-mediated repression in memory formation. The possible involvement of the ChP leads to an intriguing hypothesis that the altered transcriptomic changes in the ChP may affect the composition of the cerebrospinal fluid, eventually regulating the hippocampal plasticity during memory formation, which remains to be investigated.

To summarize, a small amount of ChP could have been included in our hippocampal samples, because it is technically very difficult to perfectly separate the two regions by using the widely used dissection method. Given that much research on hippocampal transcriptome to date has shared the common isolation method we used (2), we agree that Mathew et al. raised an important issue to be addressed in future studies. However, our data set is a rich source of valuable information on transcriptomic and translomic changes after learning. Most of the gene expression changes reflect the response from the hippocampus, which constitutes most of the sample volume unless the genes are highly specific to the ChP. The potential inclusion of the ChP does not affect our major conclusions regarding the basal translational suppression of translation machineries or the early translational repression of specific genes after learning. For some of the down-regulated genes at the late phase after contextual fear conditioning, we should consider the possibility that their changes in the transcriptomes may have been affected by ChP inclusion. Further analysis may be required to determine whether these regulations occur in the hippocampus, ChP, or both, and which event is critical for long-term memory formation.

REFERENCES
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