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

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Cho et al. (Reports, 2 October 2015, p. 82) report that gene repression after contextual fear conditioning regulates hippocampal memory formation. We observe low levels of expression for many of the top candidate genes in the hippocampus and robust expression in the choroid plexus, as well as repression at 4 hours after contextual fear conditioning, suggesting the inclusion of choroid plexus messenger RNAs in Cho et al. hippocampal samples.

In light of the substantial consequences that inadvertent ChP inclusion in the hippocampal samples would have on the interpretation of the data, we attempted to replicate the findings of Cho et al. Adult C57BL/6N male mice (the strain used by Cho et al.) were subjected to foot shock to contextually condition a fear response or were left untreated. After 4 hours, the hippocampus as well as the ChP in the lateral and fourth ventricles were concurrently isolated from trained and control mice. We performed quantitative polymerase chain reaction (qPCR) of 10 transcripts from the list of the top 15 DEGs at 4 hours after foot shock [see table S2 in (1)]. The expression of these 10 genes was 100- to 20,000-fold higher in the ChP compared to the hippocampus (Fig. 1B). As controls, we also measured Gapdh-normalized mRNA levels of three hippocampus-specific genes [hippocampal expression: Neurod6 (8.2%), Frzb (1.6%), and Trpc6 (0.5%) (6)] not highly expressed in the ChP [ChP expression: Neurod6 (0.005%), Frzb (0.1%), and Trpc6 (0.001%) (6)].

To compare the expression levels of the 10 ChP signature genes in our hippocampal samples with the Cho et al. hippocampal samples, we normalized the data to Neurod6 (6). The expression of these genes in our samples showed 0.02 to 1.4% enrichment, demonstrating their low expression in the hippocampus (Fig. 1C). In contrast, the hippocampal RNA expression values deposited by Cho et al. in the Gene Expression Omnibus (GSE72064) suggested enrichment of these ChP signature transcripts (7 to 36% of Neurod6 mRNA levels) (Fig. 1C). Together, these results point to the presence of ChP-derived mRNA in the Cho et al. hippocampal samples at 4 hours after contextual fear conditioning.

Given these gene enrichment patterns, we were compelled to evaluate the exciting but unexplored possibility that contextual fear conditioning causes rapid changes in gene expression in the ChP, possibly to a degree exceeding changes in the adjacent hippocampus. To test this prediction, we measured the mRNA levels of the 10 genes in lateral and fourth-ventricle ChP and found that their expression levels were suppressed in both tissues after foot shock compared with time-matched controls, with reduced expression of Sostdc1, Augurin (1500015010Rik, Erg4), and Kcene2 reaching statistical significance (Fig. 1, D and E). In agreement with Cho et al., the expression levels of several genes (Aply, Kcene2, Tmem72, and Ska5) were reduced in our hippocampal samples after foot shock (Fig. 1F). However, the overall expression levels of these genes were low with respect to Gapdh (Fig. 1F). Hippocampal expression of Sostdc1 did not change, whereas the expression of Cldn2 increased in response to foot shock (Fig. 1F). Taken together, our data suggest that the unintended presence of ChP mRNAs in the Cho et al. hippocampal samples resulted in robust gene expression changes at 4 hours after contextual fear conditioning, which cannot be attributed solely to the hippocampus. Indeed, our results suggest that the most robust responses are attributable to the ChP itself. We did not directly compare ChP and hippocampal gene expression at other times post-conditioning; therefore, it is unknown whether inclusion of ChP occurred for other time points.

Although it remains to be determined whether transcriptional repression of these genes in the ChP is functionally important for memory formation, it is clear that the ChP transcriptome rapidly responds to stressful stimuli, including contextual fear conditioning. Indeed, foot shock activates the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, with sudden increases in blood pressure, heart rate, and body temperature (7, 8), all stimuli known to affect the ChP at the blood-CSF barrier.

Recently, Stankiewicz et al. (9) reported acute stress-induced reduction in expression of a set of genes in hippocampal samples that overlap with those reported here (Fig. 1) and in the Cho et al. DEG set (1). The authors reported that these genes represent the ChP transcriptome and cautioned against ChP inclusion in hippocampal samples (9). Sárvári et al. (10) observed that administration of estradiol to ovariolectomized female rats increased levels of multiple ChP-signature transcripts in hippocampal samples. Consistent with Sárvári et al. (10), Cho et al. observed reduced expression of most of these genes, including Tr (figure 2D in (4)), after peripheral administration of an estrogen receptor antagonist. Although we did not repeat these pharmacological studies, previous investigations have demonstrated direct actions of estrogens on ChP transcription of Tr (11), its most highly expressed gene (3, 4).

In summary, the probable inclusion of ChP mRNAs in hippocampal samples in Cho et al. (and likely in studies from other groups) provides an alternative location for the source of the genes showing the greatest changes in expression after contextual fear conditioning. Our study reexamined gene expression 4 hours after fear conditioning; it is unknown whether the rapid translational and transcriptional changes observed at the other time points examined by Cho et al. also reflect contributions from the ChP. An...
interaction between the ChP and hippocampal cognitive function has been suggested (22). Changes in protein secretion by the ChP, due either to translational or transcriptional changes, could contribute to the mechanisms of hippocampal memory formation. Our study provides a reference guide for the research community with which enrichment of ChP signature genes in hippocampal samples can be used to evaluate the purity of the isolated starting material. Furthermore, our results reveal that the ChP rapidly responds to the environment by altering mRNA levels of some of its most highly expressed genes. These transcriptomic events may ultimately contribute to changes in CSF composition, thereby influencing brain function.

REFERENCES AND NOTES

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