Correspondence

Reply to: Microglia, Monocytes, and the Recurrence of Anxiety in Stress-Sensitized Mice

To the Editor:

We thank Kronenberg *et al.* (1) for their insightful comments on our article, "The Influence of Microglial Elimination and Repopulation on Stress Sensitization Induced by Repeated Social Defeat," recently published in *Biological Psychiatry* (2). We are happy to address some of the comments and clarify a few of our conclusions.

First, the transcriptomic analysis of microglia 24 days after repeated social defeat (RSD) [Figure 1 in (2)] has been submitted to the Gene Expression Omnibus data repository and will soon be publicly available. We agree that sharing these data with other investigators is important and valuable to the field.

Second, we would like to address the comments regarding monocyte engraftment in the brain after exposure to RSD. To clarify, we do not believe, nor do we claim in the current study, that the inflammatory monocytes engraft in the brain after RSD. We concur that our initial study using green fluorescent protein (GFP^+) bone marrow chimera mice revealed increased GFP^+/Iba-1^+ myeloid cells in the brain parenchyma after 6 cycles of repeated social defeat (3). It is important to highlight, however, that these studies were completed using chemoablation with a busulfan regimen and the re-establishment of bone marrow with GFP^+ donor stem cells. Based on our more recent studies (4–6), this chimeric model likely overrepresented the number of parenchymal monocytes in the brain with RSD. Nonetheless, RSD increases expression of cell adhesion molecules in the vascular endothelium in stress-responsive brain regions (4,5). Monocytes are recruited to the brain and adhere to endothelial cells. These adherent monocytes persist in the brain despite vascular perfusion at time of sacrifice. In fact, our subsequent studies indicate a complex relationship between microglia, monocytes, and brain endothelial cells. For example, in both the current study and in McKim *et al.* (5), we showed that microglia recruit inflammatory monocytes to the brain vasculature after RSD. McKim *et al.* (5) also showed that these inflammatory monocytes delivered interleukin-1β (IL-1β) to the IL-1 receptor type 1 on brain endothelial cells in a region-dependent manner. This activation of IL-1 receptor type 1 signaling in threat appraisal regions was essential for the augmentation of inflammation and anxiety-like behavior (5). In summary, engraftment of monocytes in the brain after RSD is limited, whereas vascular (or perivascular) recruitment of inflammatory monocytes by microglia after RSD is critical.

Related to the above comments, even in the GFP^+ chimeric mice, there was no evidence that monocyte-derived macrophages engrafted and persisted 24 days after RSD (7). This was also the case in wild-type mice: increased $CD45^+$ monocytes within the brain vasculature after RSD were no longer present 24 days later. In general, we have detected monocytes by flow cytometry and histology only for 8 to 10 days after RSD. There is also no engraftment of monocytes in the brain following one cycle of acute defeat in the stress-sensitized mice (24 days after initial RSD exposure). Again, monocyte recruitment to the brain vasculature is relevant, but these cells do not engraft in the brain. The 14-hour time point after acute stress (i.e., one cycle of RSD) in stress-sensitized mice represents the time at which inflammatory monocytes are being recruited to the brain vasculature and augmenting IL-1 signaling (7). We interpret these data to indicate that adherent and recruited monocytes augment neuroinflammatory signaling that reinforces the recurrence of anxiety in RSD-sensitized mice.

Third, we would like to address the comments on microglia repopulation in the brain after the cessation of PLX5622 in the $CX_3CR1^{CreER/+}$/$R26^{tdTOM/+}$ (tdTomato1) mice. Based on our data [Figure 3 in (2)], we conclude that the repopulating microglia are from the 3% to 5% of the resident microglia that remained after colony-stimulating factor 1 receptor antagonist–mediated elimination. We understand that bone marrow–derived myeloid cells can repopulate the brain in certain contexts (8,9). Addressing this issue was not the primary objective of our current study. Rather, our objective was to elucidate how microglia repopulated following colony-stimulating factor 1 receptor antagonist-mediated depletion to better understand RSD-induced stress sensitization and microglial priming. Kronenberg *et al.* (1) also correctly point out that we observed 46% of $CD11b^+$/$CD45^{hi}$ cells isolated from tamoxifen-injected mice after microglial elimination and repopulation lacked yellow fluorescent protein (YFP). We agree that other leukocytes are present in the brain and are involved in immune surveillance. The cells in question in the bivariate dot plots are CX_3CR1^{neg}/$CD11b^+$/$CD45^{hi}$. Based on these data, these cells are likely neutrophils [reviewed in (10)]. These cells, however, do not become microglia. For instance, all microglia after elimination and repopulation in the $CX_3CR1^{CreER/+}$/$R26^{tdTOM/+}$ mice were YFP^+/$TdTom^+$. We agree that the leakiness of the Cre recombinase is concerning. Nonetheless, if this confounded our experiments, we would expect to see a 50/50 mix of YFP^+/$TdTom^+$ microglia and YFP^+/$TdTom^{neg}$ microglia after Cre induction and elimination and repopulation. Here, we use tamoxifen-inducible Cre recombinase induction to drive tdTomato expression as a fluorescent reporter. We show that 99% of microglia were both $tdTomato^+$ and YFP^+ weeks after tamoxifen injection and microglial elimination and repopulation. Thus, whereas Cre leakiness is a technical concern (noted in the article), it does not affect the overall conclusion that microglia self-renew.

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