Controls for the TetTag-DREADD method

We did several controls for the specificity of the TetTag-DREADD system. First, CNO given alone to mice that had not received any AAV injections produced no behavioral effects, no change to the EEG, as also noted by others\textsuperscript{27}, and no change in body temperature. Second, we found a low basal expression of the $P_{TRE\text{-tight}}$-$hM3Dq$ transgene in the PO hypothalamic area of AAV-injected mice on doxycycline (Fig. 3c). But even when we unlocked the transgene system by removing doxycycline for 2 days from the diet, and then gave CNO without any other treatments, nothing happened to the mice (Fig. 4c,d,g,h). Evidently, strong stimulation of the relevant neurons is needed to induce the TetTag genes. Third, we co-injected the AAV-$P_{cFox}\text{-tTA}$ and AAV-$P_{TRE\text{-tight}}$-$hM3D\tau$-$mCHERRY$ viruses bilaterally into the superior colliculi (SC) (Supplementary Fig. 8a), a region unlikely to be involved in either dexmedetomidine-induced sedation or recovery sleep after sleep deprivation. These SC-TetTag-$hM3D\tau$ mice were then given the full experimental procedure (Fig. 3b): doxycycline repression for one month, doxycycline removal, dexmedetomidine-sedation followed by 4 days recovery and then CNO injection; and then subsequently the sleep deprivation and recovery sleep treatments, followed by CNO administration. No behavioral or EEG change was found (Supplementary Fig. 8b), and there was little basal or induced $hM3D\tau$-$mCHERRY$ expression seen in the colliculi, suggesting that the sleep-recapitulating effect of CNO is specific for the PO hypothalamus.