Figures and figure supplements

HCN2 channels in the ventral tegmental area regulate behavioral responses to chronic stress

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Figure 1. CMS produced depressive- and anxiety-like behaviors. (A) The timeline of CMS, behavioral tests, and electrophysiology. (B) CMS significantly decreased body weight compared with non-stressed control mice (**p=0.004, control, n = 14 mice; CMS n = 12 mice from B to G). (C) CMS significantly decreased the center time (**p=0.007) without affecting total distance traveled in the OFT (p=0.615). (D) CMS significantly decreased sucrose preference compared to control (**p<0.001). (E) CMS did not affect entries into the open arms (p=0.919), but significantly decreased the time spent in the open arms (*p=0.036) in the EPM test. (F) CMS significantly increased the latency to feed in the novel environment (Novelty) in the NSF test (**p=0.008) but did not significantly affect the latency to feed in the home cage (Home) (p=0.345). (G) CMS significantly increased immobility time in the FST (**p<0.001).

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Figure 1—figure supplement 1. C57BL/6J and DAT-tdTomato mice do not significantly differ in body weight or behaviors at baseline or in response to CMS. Figure 1 was reanalyzed to compare C57BL/6J (C57) and DAT-tdTomato (tdTomato) reporter mice for control and CMS-induced effects on body weight (A) and behavior in OPT (B) SPT (C) EPM (D) NSF (E) and FST (F). Detailed statistical analysis is presented in Supplementary file 1. Control-C57, n = 7 mice; control-tdTomato, n = 6 mice; CMS-C57, n = 7 mice; CMS-tdTomato, n = 6 mice.

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Figure 2. CMS decreased single-unit AP firing in VTA dopamine neurons in vivo. (A) Sample traces of VTA dopamine neuron AP firing in control and CMS mice. Dopamine neurons were identified by a broad triphasic extracellular action potential of a width greater than 2 ms and a relatively slow firing rate (<10 Hz). (B) A recorded dopamine neuron was confirmed by neurobiotin (red) and TH (tyrosine hydroxylase, green) co-localization. (C–E) Population activity (C, ***p<0.001, control, n = 4 mice; CMS n = 5 mice), firing rate (D) *p=0.045, control, n = 15 cells from four mice; CMS, n = 17 cells from five mice), and the percent of spikes in burst (E, **p=0.006, control, n = 10 cells from four mice; CMS, n = 13 cells from five mice) were decreased in CMS mice.

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Figure 3. Retrobead labeling of VTA dopamine neurons that project to the lateral shell of the NAc. (A) DAT-Cre mice were bred with Ai9 reporter mice, which express tdTomato in the presence of Cre, to produce DAT-tdTomato mice. TdTomato and TH (green) were completely co-localized, indicating that tdTomato expression provides faithful reporting of dopamine neurons for slice physiology. (B) Green Retrobeads were injected into the lateral shell of the NAc (LAcbSh) in DAT-tdTomato mice. (C) The Retrobeads were retrogradely transported to the VTA and were predominantly co-localized with tdTomato-positive VTA dopamine neurons.

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Figure 4. CMS decreased $I_h$ currents in VTA dopamine neurons that project to the lateral shell of the NAc (LAcbSh). (A) Left: Voltage protocol for recording $I_h$ current. Right: Representative $I_h$ current recorded from NAc-projecting VTA dopamine neurons in control and CMS mice. (B) Compared with the control group, $I_h$ amplitude was significantly decreased in the CMS group at corresponding hyperpolarization potentials (*p<0.05, **p<0.01, ***p<0.001, control, n = 15 cells from five mice; CMS, 13 cells from three mice from B to G). $I_h$ amplitude was calculated by subtracting the instantaneous current from the steady-state current achieved during the voltage step. (C) The membrane capacitance ($C_m$) was not significantly different between control and CMS mice (p=0.273). (D) $I_h$ current density was significantly decreased in the CMS group compared with the control group (***p=0.009). (E) $I_h$ activation curves in the control and CMS groups generated by the tail current protocol. Tail current amplitudes were fitted with a Boltzmann function. (F) CMS led to a significant hyperpolarizing shift of the half-activation potential ($V_{1/2}$) compared with that of control (***p=0.007). (G) The resting membrane conductance ($G_{resting}$) was not significantly different between control and CMS mice (p=0.107).

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Figure 4—figure supplement 1. The measurement of resting membrane conductance. (A) The instantaneous inward current (I_{ins}) was measured as indicated by the dashed lines. (B) A representative example for calculating resting membrane conductance. I_{ins} was plotted against the hyperpolarizing voltage steps. The slope of these I-V curves is indicative of the resting membrane conductance shown in Figure 4G.

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Figure 5. CMS decreased AP firing in VTA dopamine neurons in midbrain slices. (A) *Ih* currents recorded at −130 mV in NAC-projecting VTA dopamine neurons in both control (***p<0.001, n = 6 cells from three mice) and CMS groups (**p<0.001, n = 6 cells from three mice) were abolished by the *Ih* channel blocker ZD7288 (30 µM). (B) Representative AP firing in cell-attached recordings from NAC-projecting VTA dopamine neurons in control and CMS slices before and after ZD7288 (30 µM). (C) The AP firing rate was significantly decreased in the CMS group (n = 13 cells from three mice) compared with the control group (n = 12 cells from four mice; ***p<0.001). ZD7288 significantly decreased the firing rate in both control (n = 11 cells from three mice) and CMS (n = 12 cells from five mice) groups (*p<0.5, ***p<0.001). The mean firing rate was not significantly different between control (n = 11 cells from three mice) and CMS (n = 12 cells from four mice) groups following ZD7288 (p=0.382). (D) The firing rate (FR) suppression (%) by ZD7288 in the control group (n = 11 cells from three mice) was significantly higher than that of the CMS group (n = 12 cells from five mice; **p=0.004).

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Figure 6. AAV-mediated shRNA knockdown of HCN2 in the VTA. (A,B), Immunofluorescence labeling showing the expression of AAV2-HCN2-shRNA-eGFP (green), TH (dopamine neuron marker, red) and NeuN (neuronal marker, blue) in the midbrain under low magnification (A) and high magnification (B). Scale bars: 50 μm. (C), The percentage of TH⁺ VTA dopamine neurons that were infected with AAV2-HCN2-shRNA-eGFP or scramble-shRNA (n = 3 mice/group). (D), Maximal Ih current amplitude was significantly decreased in AAV2-HCN2-shRNA-eGFP-infected dopamine neurons (n = 14 from three mice) compared with AAV2-scramble-shRNA-eGFP-infected dopamine neurons (n = 15 from four mice; ***p<0.001). (E), AP firing frequency was decreased in AAV2-HCN2-shRNA-eGFP-infected neurons (n = 10 from three mice) compared with AAV2-scramble-shRNA-eGFP-infected neurons (n = 9 from four mice; *p=0.036).

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The resting conductance ($G_{\text{resting}}$) was significantly decreased in the HCN-shRNA group ($n = 14$) compared with the scrambled group ($n = 15$, $p=0.022$).

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Figure 7. ShRNA knockdown of HCN2 in the VTA produced anxiety- and depressive-like behaviors. (A) VTA-specific HCN2 knockdown did not significantly affect the body weight of mice (control, n = 7 mice; CMS, n = 7 mice, p=0.727). (B) HCN2 knockdown significantly decreased the center time (*p=0.042) without affecting the total distance traveled (p=0.197) in the OFT. (C) HCN2 knockdown significantly decreased sucrose preference (**p=0.003). (D) HCN2 knockdown did not affect entries into the open arms (p=0.104) but significantly decreased time spent in the open arms (*p=0.018) in the EPM test. (E) HCN2 knockdown increased the latency to feed in the novel environment (Novelty) in the NSF test (*p=0.017) but did not significantly affect the latency to feed in the home cage (Home; p=0.583). (F) HCN2 knockdown increased immobility time in the FST (*p=0.039).

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Overexpression of HCN2 in the VTA prevented the development of CMS-induced depressive-like behavior. (A) The maximal amplitude of Ih current in AAV2-HCN2-eGFP-infected VTA dopamine neurons (n = 15 from four mice) was significantly increased compared with that in AAV2-eGFP-infected VTA dopamine neurons (n = 14 cells from five mice; **p=0.002). (B) CMS significantly decreased the body weight of mice in the AAV2-eGFP injection group (***p<0.001, control, n = 9 mice; CMS, n = 10 mice from B to G), whereas CMS did not decrease the body weight of mice in the HCN2 overexpression group (p=0.419, control, n = 10 mice; CMS, n = 8 mice from B to G). (C) Neither CMS nor HCN2 overexpression affected the total distance traveled in the OFT test (p>0.05). Compared with non-stressed control mice, CMS significantly decreased the time spent in the center square of the open field in the AAV2-eGFP group (***p=0.002), and this decrease was prevented by HCN2 overexpression (**p=0.004). (D) CMS significantly decreased sucrose preference in the AAV2-eGFP injection group (***p<0.001), whereas HCN2 overexpression prevented this decrease (**p=0.001). (E) In the AAV2-GFP group, CMS significantly decreased time spent in the open arms (*p=0.023) but did not affect open arm entries (p>0.05). HCN2 overexpression did not significantly affect open arm time nor entries (p=0.057) compared to the AAV2-GFP group. (F) CMS induced a significant increase in the latency to feed in the novel environment in the NSF test (**p=0.002), which was prevented by HCN2 overexpression (**p=0.001). Neither CMS nor HCN2 overexpression affected the latency to feed in the home cage (p>0.05). (G) CMS produced a significant increase in immobility in the FST (***p<0.001), and this increase was prevented by HCN2 overexpression (**p=0.001).

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