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# Role for the Membrane Receptor Guanylyl Cyclase-C in Attention Deficiency and Hyperactive Behavior

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**Midbrain dopamine neurons regulate many important behavioral processes and their dysfunctions are associated with several human neuropsychiatric disorders such as Attention Deficit Hyperactivity Disorder (ADHD) and schizophrenia. Here, we report that these neurons in mice selectively express guanylyl cyclase-C (GC-C), a membrane receptor previously thought to be expressed mainly in the intestine. GC-C activation potentiates the excitatory responses mediated by glutamate and acetylcholine receptors via the activity of cGMP-dependent protein kinase (PKG). GC-C knockout mice exhibit hyperactivity and attention deficits. Moreover, their behavioral phenotypes are reversed by ADHD therapeutics and a PKG activator. These results indicate important behavioral and physiological functions for the GC-C/PKG signaling pathway within the brain and suggest new therapeutic targets for neuropsychiatric disorders related to the malfunctions of midbrain dopamine neurons.**

Dopamine neurons in the midbrain ventral tegmental area and substantia nigra compacta (VTA/SNc) project their axons extensively to the forebrain and release dopamine to regulate diverse behavioral processes, such as motor activity, cognition, motivation and learning (*1, 2*). Numerous dopamine receptor agonists, antagonists, and reuptake inhibitors have been used to treat the symptoms of Parkinson's disease, schizophrenia, and ADHD (*3*). Studying how the activity of midbrain dopamine neurons is selectively regulated not only can contribute to our understanding of the neurobiological mechanisms of behavioral control but also may provide insight into developing more effective treatments of psychiatric disorders.

While studying the functions of membrane guanylyl cyclases in the nervous system, we observed that GC-C, thought to be expressed principally on intestinal mucosal cells (*4, 5*), is strongly and selectively expressed throughout the VTA/SNc in mice (*6, 7*). GC-C mRNA co-localized with tyrosine hydroxylase (TH) (Fig. 1A-C and figs. S1 and S2), an enzyme critical for dopamine synthesis (*1*). Dual-color immuostaining revealed that GC-C protein was expressed predominantly on the somata and dendrites of dopaminergic neurons in the VTA/SNc (Fig. 1D-I and fig. S1C). The GC-C expression pattern was further confirmed by GC-C immunostaining in TH-GFP transgenic mice, which express green fluorescent protein (GFP) in dopamine neurons (fig. S2A-F) (*8*). Strong GC-C immunoreactivity was detected in the GFP+ neurons in the VTA/SNc but not other brain areas (figs. S2G-L and S3).

GC-C is a membrane receptor for the gut peptide hormones guanylin (G) and uroguanylin (UG) (*9, 10*). Upon activation it increases the production of intracellular cyclic guanosine monophosphate (cGMP) and finally opens CFTR channels to stimulate electrolyte and water secretion. Its over-activation by *Escherichia coli* heat-stable enterotoxin causes acute secretory diarrhea (*4*). Although several membrane GCs are implicated in behavioral regulations in animals across taxa (*11*), the functions of GC-C remain unexplored in the nervous system.

As the anatomical organization of the midbrain dopamine system appears normal in GC-C knockout (KO) mice (figs. S1D and S4), we examined the physiological functions of GC-C signaling. Dopamine neurons in adult mouse brain slices were recorded using the perforated whole-cell patch-clamp method (fig. S5). Initial recordings revealed no obvious effects of GC-C activation that were predicted by known physiological functions of cGMP-stimulated signaling pathways (*12*). Applying GC-C ligands G/UG did not affect the intrinsic physiological properties of midbrain dopamine

neurons (fig. S6). G/UG application also did not produce any effects on the responses carried by AMPA-type glutamate receptors or $GABA_A$ receptors (figs. S7 and S8), suggesting minimal roles of GC-C in directly opening ion channels or modulating synaptic responses mediated by ionotropic glutamate or GABA receptors.

Unexpectedly, G/UG potentiated the responses evoked by (*S*)-3,5-Dihydroxyphenylglycine (DHPG), a ligand of group 1 metabotropic glutamate receptors (mGluRs) (Fig. 2A-D). Bath application of G/UG dramatically increased the firing frequency of action potentials evoked by DHPG (Fig. 2A). When neurons were recorded in the voltage-clamp mode, G/UG substantially and reversibly enhanced DHPG-evoked inward currents (Fig. 2B-D) The potentiatory effect of G/UG was resistant to the application of TTX, a sodium channel blocker, and 1H-(1, 2, 4) oxadiazolo [4, 3-a] quinoxalin-1-one (ODQ), a soluble GC (sGC) blocker (Fig. 2D), indicating that this effect is intrinsic to the recorded dopamine neurons and independent of sGC activity in surrounding GABAergic neurons (*13*). Muscarine is an agonist of muscarinic acetylcholine receptors (mAChRs) and excites midbrain dopamine neurons (*14*). G/UG drastically amplified the firing frequency of action potentials induced by muscarine and significantly potentiated the muscarine-evoked currents (Fig. 2E-H), indicating a similar potentiatory effect of G/UG on mAChR-mediated responses.

We next examined the signaling components downstream of G/UG. In GC-C KO mice, midbrain dopamine neurons exhibited apparently normal intrinsic properties (fig. S9). The potentiatory effect of G/UG on DHPG-evoked responses was abrogated in these mice, demonstrating a critical role of GC-C (Fig. 3A-C). GC-C activity increases the production of cGMPs that can in turn activate PKG. We tested whether PKG activity plays any role in downstream signaling of GC-C. The potentiation of DHPG-evoked currents by G/UG was abolished by Rp-8-pCPT-cGMPS and KT5823 (Fig. 3D-I), two membrane-permeable PKG inhibitors that act on the PKG regulatory subunit and catalytic domain respectively (*15, 16*). Conversely, the potentiatory effect of G/UG was mimicked by 8-Br-cGMP (Fig. 3J-L), a PKG activator (*12*). Consistent with the ineffectiveness of G/UG on directly opening channels, neither Rp-8-pCPT-cGMPS nor 8-Br-cGMP evoked any significant currents (fig. S10). These recordings thus strongly suggest that PKG mediates the potentiatory effect of GC-C activity.

As dopamine is involved in organizing or regulating animal behaviors, we asked whether the GC-C/PKG signaling pathway affects animal behavior by modulating brain dopamine levels. We first analyzed the locomotor activity of GC-C KO mice, which are physically healthy and show apparently normal intestinal fluidity and body weight (*17, 18*). Long-term monitoring revealed that the locomotor activity of GC-C KO mice was more than twice that of wild-type mice during dark but not light phases after one day in the test arena (Figs. 4A and S11A). In a novel open field, these mice manifested clear hyperactivity only after they were familiarized with the environment for about 100 minutes (Fig. 4B). The reduction of habituatory effect on locomotion was further confirmed by reintroducing GC-C KO mice into the open field a second time (fig. S11B, C).

We performed olfactory habituation test to examine whether GC-C KO mice had impaired response habituation in behaviors other than locomotion (fig. S12A). Wild-type mice reduced their interests in chemo-investigation when the same odorant was presented repetitively, but increased again in response to a novel odorant (Figs. 4C and S12B). GC-C KO mice spent significantly more time investigating odorant stimuli, suggesting a higher level of novelty-seeking (Fig. 4C). More importantly, GC-C KO mice displayed impairment in olfactory habituation following repetitive presentation of the same odorant (Figs. 4C and S12B).

Reduced response habituation is associated with impaired attention in humans and animals (*19, 20*). We assessed the attention deficit of GC-C KO mice by challenging them with a go/no-go attention task (fig. S13A). Water-deprived mice were trained to lick a water port within a short time window after a 3 kHz auditory tone (CS+) to receive water rewards and to inhibit licking following a 15 kHz tone (CS-) to avoid the penalty of foot-shock and timeouts (fig. S13B). During the initial training phase, mice were provided with a 200-ms period after the onset of tone stimuli to judge the stimulus identity before action (Phase 1 in Figs. 4D and S13C). Both wild-type and GC-C KO mice could be trained to respond correctly with similar learning curves (fig. S13D). While wild-type mice typically initiated licking only after the onset of CS+, KO mice often started licking before stimulus onset and stopped only after the onset of CS- (Fig. 4D). Consequently, the no-go stop reaction time of GC-C KO mice was three times as long as that of wild-type mice (Fig. 4E), suggesting impaired behavioral inhibition to no-go signals and thus impulsivity for GC-C KO. When the 200-ms time window for judgment was removed (Phase 2 in fig. S13C), GC-C KO mice could be trained to start licking only after the CS+ onset. However, when the task difficulty was further increased by inserting a random delay of up to 2 s after trial initiation (test phase in fig. S13C), GC-C KO exhibited a significantly higher ratio of aborting the trial before tone stimuli (Fig. 4F) and consequently significantly lower ratio of correct responses (Fig. 4G), suggesting deficits in sustained attention.

Thus, the behavioral phenotypes of GC-C KO mice mimic the core symptoms of ADHD (*21, 22*), one of the most prevalent human psychiatric disorders. It is believed that ADHD pathophysiology involves the physiological

dysfunction of the midbrain dopamine system (*21–23*). Using in vivo microdialysis we found that GC-C KO mice have significantly lower levels of the basal extracellular dopamine than wild-type mice (Fig. 4H and fig. S14). ADHD symptoms can be treated by low but not high doses of psychostimulant amphetamine or its derivatives, which enhance extracellular dopamine concentrations by facilitating dopamine release and inhibiting its reuptake (*24, 25*). The dose-dependency of amphetamine on treating hyperactivity is commonly used to determine the validity of ADHD animal models (*22, 26*). Applying amphetamine at the dose comparable to that for treating human ADHD (1 mg/Kg) significantly reduced the locomotor activity of GC-C KO mice but not wild-type mice in a habituated open field (Fig. 4I and fig. S15). In contrast, high doses of amphetamine stimulated locomotion for both wild-type and GC-C KO mice (figs. S15). As PKG mediates GC-C signaling, we examined whether a PKG activator can restore the behavior of GC-C KO mice. Consistently, infusing 8-Br-cGMP to activate PKG in the bilateral VTA/SNc areas reduced locomotion of GC-C KO mice (Fig. 4J).

In summary, we have identified the selective expression of the membrane receptor GC-C on midbrain dopamine neurons and revealed its role in regulating animal activity level and attention. In dopamine neurons, DHPG and muscarine activate Gq-coupled GPCRs and eventually lead to the opening of C-family transient receptor potential channels (TRPCs) (*27*). We hypothesize that upon GC-C activation, PKG modulates certain components within the signaling pathway downstream of mGluRs and mAChRs (fig. S16). G and UG are secreted by the gut with circadian rhythm and circulate in the blood (*28, 29*), raising the intriguing possibility that these two hormones from the gut may modulate the physiology of midbrain dopamine neurons. The ADHD-like behaviors of GC-C KO mice suggest that these mice may be used as an ADHD model (supporting online material (SOM) text). More importantly, the GC-C/PKG pathway may be targeted to selectively manipulate the activity of midbrain dopamine neurons. As dopamine is also released by cells outside of the midbrain, drugs targeting dopamine receptors and transporters can have undesirable effects (*3*). Efforts of developing activators or inhibitors of the GC-C/PKG signaling pathway may lead to novel treatments of neuropsychiatric disorders associated with the malfunctions of the midbrain dopamine system, such as ADHD, schizophrenia, Parkinson's disease and addiction (*30–32*).

**References and Notes**


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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/science.1207675
Materials and Methods
SOM Text
Figures S1 to S16
References (*33*–*51*)

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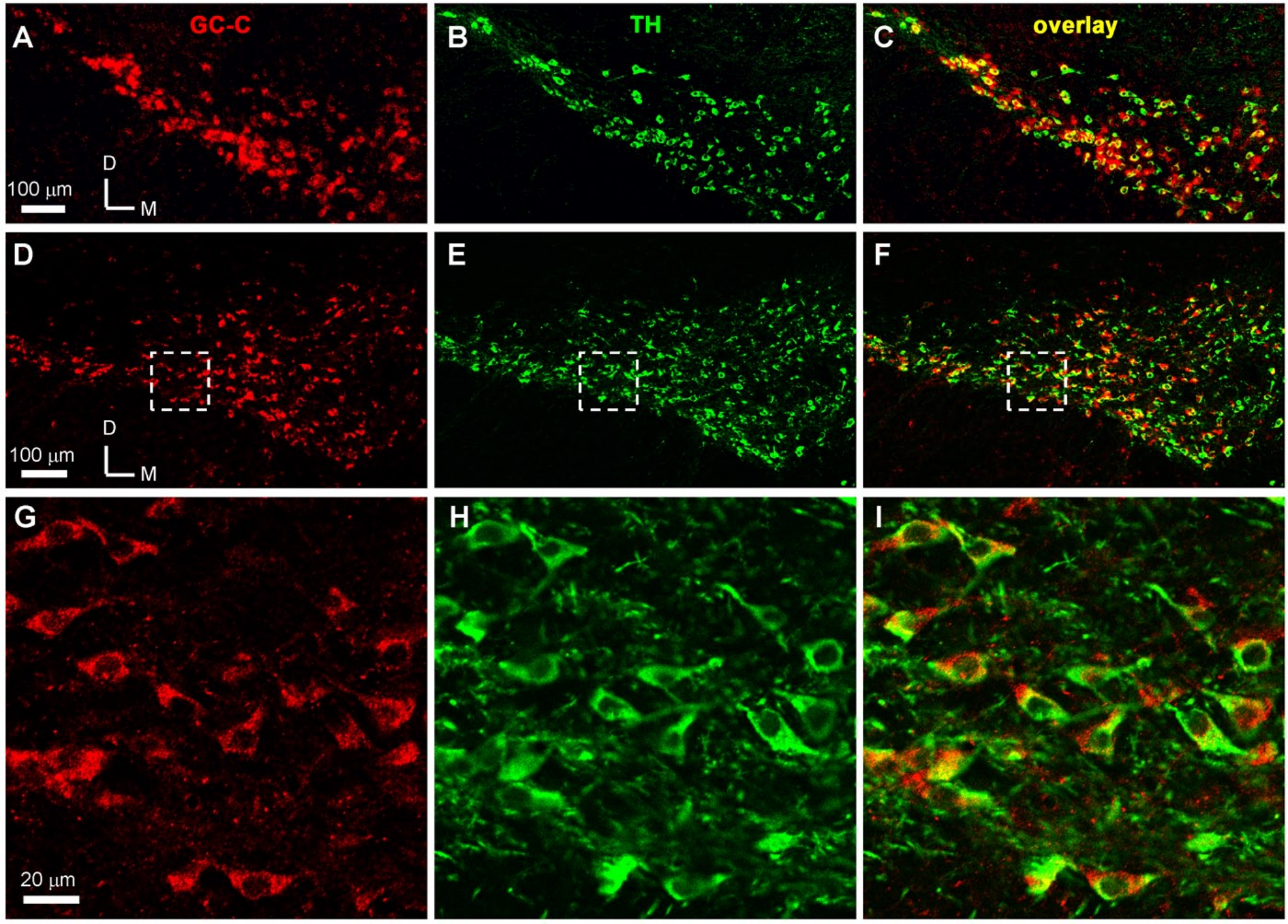
**Fig. 1.** GC-C is selectively expressed on midbrain dopamine neurons. (**A** to **C**) Similar patterns of GC-C mRNA expression and TH immunoreactivity in the mouse midbrain (n = 4 mice). (A) GC-C mRNA expression (red) in the VTA/SNc area is detected by in situ hybridization. M, medial; D, dorsal. (B) TH immunoreactivity (green) in the same coronal section as shown in (A). (C) Overlay of (A and B). (**D** to **F**) Dual-color immunostaining shows that GC-C (red) is expressed in midbrain dopamine neurons as marked by TH immunoreactivity (green) (n = 4 mice). (**G** to **I**) High-power view of the GC-C and TH immunoreactivity within the dashed box in (D-F).

**Fig. 2.** Application of GC-C ligands potentiates the excitatory responses mediated by group 1 mGluRs and mAChRs on midbrain dopamine neurons. (**A** to **D**) G/UG potentiates the excitatory responses evoked by DHPG, a ligand of group 1 mGluRs. (A) Representative voltage traces show that G (1 μM) amplified DHPG-evoked action potential firing frequency. DHPG (10 μM) was applied by pressure ejection. Horizontal bars indicate drug application. (B) Representative current traces show that the DHPG-evoked inward currents were reversibly potentiated by G. (C) Plot of the amplitudes of DHPG currents over time for the same cell shown in (B). (D) Population data show a significant potentiatory effect of G/UG on the amplitude of DHPG currents. (**, $p<0.01$; paired t-test; n = 11 cells). This effect remains significant in the presence of TTX and ODQ (***, $p<0.001$; paired t-test; n = 9 cells). In this and following figures, thick black lines plot mean values; gray lines, the values of individual neurons; error bars, s.e.m.. (**E** to **H**) G/UG potentiates the responses to muscarine, an agonist of mAChRs. (E) UG (1 μM) increases the firing of action potentials induced by muscarine (50 μM). (F and G) Representative current traces and plot of muscarine-evoked current amplitudes show the reversible potentiatory effect of G. (H) Population data show the potentiatory effect of G/UG on muscarine currents (*, $p<0.05$; paired t-test; n = 5 cells).

**Fig. 3.** The potentiatory effect of G/UG depends on GC-C and PKG activity. (**A** to **C**) The potentiation of DHPG-evoked currents by G/UG requires GC-C. (A) Representative traces show the lack of potentiatory effect by UG on DHPG currents recorded from a midbrain dopamine neuron of a GC-C KO mouse. (B) Plot of DHPG-evoked responses for the same cell as in (A). (C) Group data show that G/UG does not affect DHPG-evoked responses in GC-C KO mice (n.s., not significant; p = 0.93; paired t-test; n = 11 cells). (**D** to **F**) The potentiatory effect of G/UG is abolished by pre-incubation of Rp-8-CPT-cGMPS, a PKG inhibitor. (D) Representative

current traces; (E) Plot of DHPG-evoked responses; (F) Population data ($p = 0.51$; paired t-test; $n = 9$ cells). (**G** to **I**) The potentiatory effect of G/UG is abolished by pre-incubation of KT5823, another PKG inhibitor ($p = 0.95$; paired t-test; $n = 8$ cells). (**J** to **L**) Representative current traces (J), plot of current amplitude (K), and group data (L) show that DHPG-evoked responses are significantly potentiated by 8-Br-cGMP (200 μM), a PKG activator (**, $p<0.01$; paired t-test; $n = 9$ cells).

**Fig. 4.** GC-C KO mice exhibit ADHD-like behaviors. (**A**) Plot of travel distance shows hyperactivity of GC-C KO in the dark phase of the light/dark cycle (*, $p< 0.05$; t-test, $n = 5$ mice for GC-C KO and wild-type (Wt)). (**B**) In a novel open field, the locomotor activity of GC-C KO mice becomes about twice that of wild-type mice after ~100 minutes (genotype difference $p < 0.001$; ANOVA; $n = 14$ for GC-C KO and 22 for Wt). (**C**) In an olfactory habituation test, GC-C KO mice exhibit longer duration of odor investigation than wild-type mice (*, $p < 0.05$; **, $p < 0.01$; t-test; $n = 8$ for GC-C KO and 6 for Wt) and show clear reduction in habituation. AA: Amyl acetate; PMK: acetophenone. (**D** to **G**) GC-C KO mice exhibit impulsivity and deficits in sustained attention. (D) Schematics of training paradigm for Phase 1 training and representative raw traces of licking responses of wild-type and GC-C KO to tone stimuli after training. Up states indicate licking. (E) The stop reaction time of GC-C KO and wild-type mice to no-go stimuli (***, $p < 0.001$; t-test; $n = 6$ for GC-C KO and 10 for Wt). (F and G) When a variable delay of up to 2 s is inserted prior to stimulus onset, GC-C KO mice display a higher ratio of aborting the trial before stimulus onset (F; **, $p < 0.01$; t-test) and lower ratio of correct responses (G; **, $p < 0.01$; t-test; $n = 6$ for GC-C KO and 7 for Wt). (**H**) GC-C KO mice have lower concentrations of extracellular dopamine in the striatum of behaving mice measured in home cage environment (**, $p = 0.01$; t-test; $n = 15$ for GC-C KO and 12 for Wt). Data are normalized to the mean concentration of Wt. (**I**) Amphetamine reduces the locomotor activity of GC-C KO mice (drug difference for the first 70 min $p<0.001$; ANOVA; $n = 6$ mice). (**J**) Infusing 8-Br-cGMP (3 mM) into the VTA/SNc area reduces the locomotor activity of GC-C KO mice (drug difference for the first 70 min $p<0.001$; ANOVA; $n = 5$ mice respectively). Mice were tested in a novel chamber.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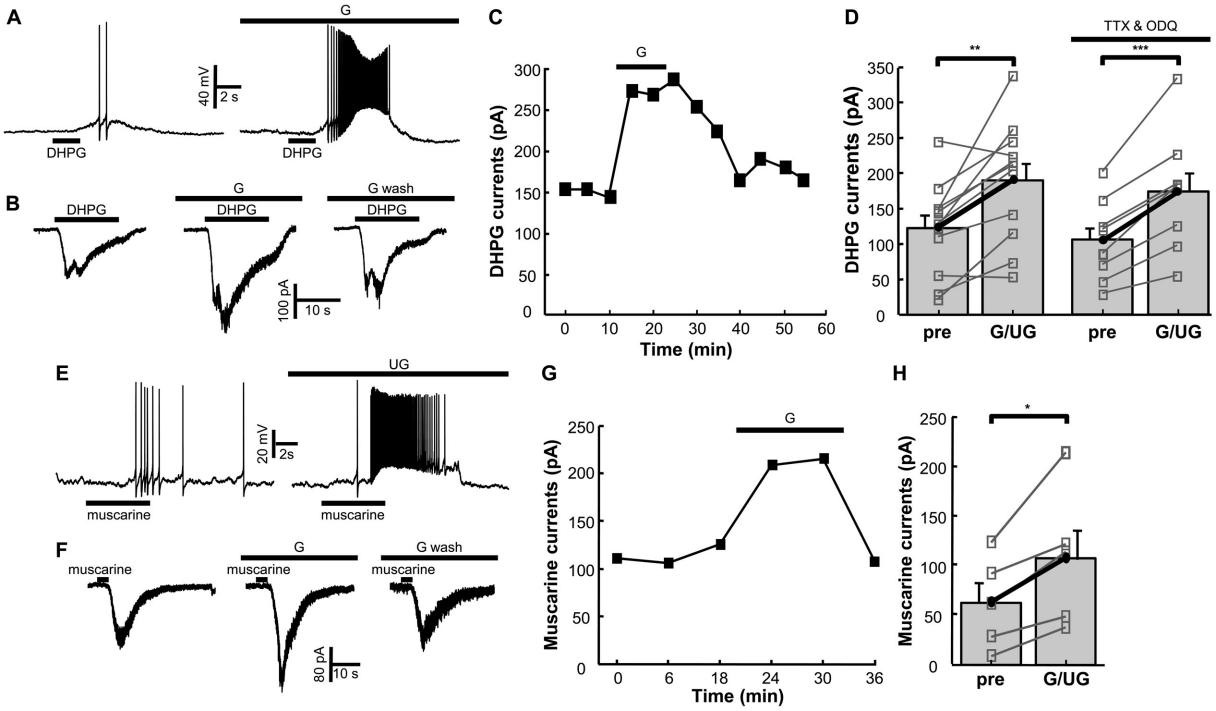
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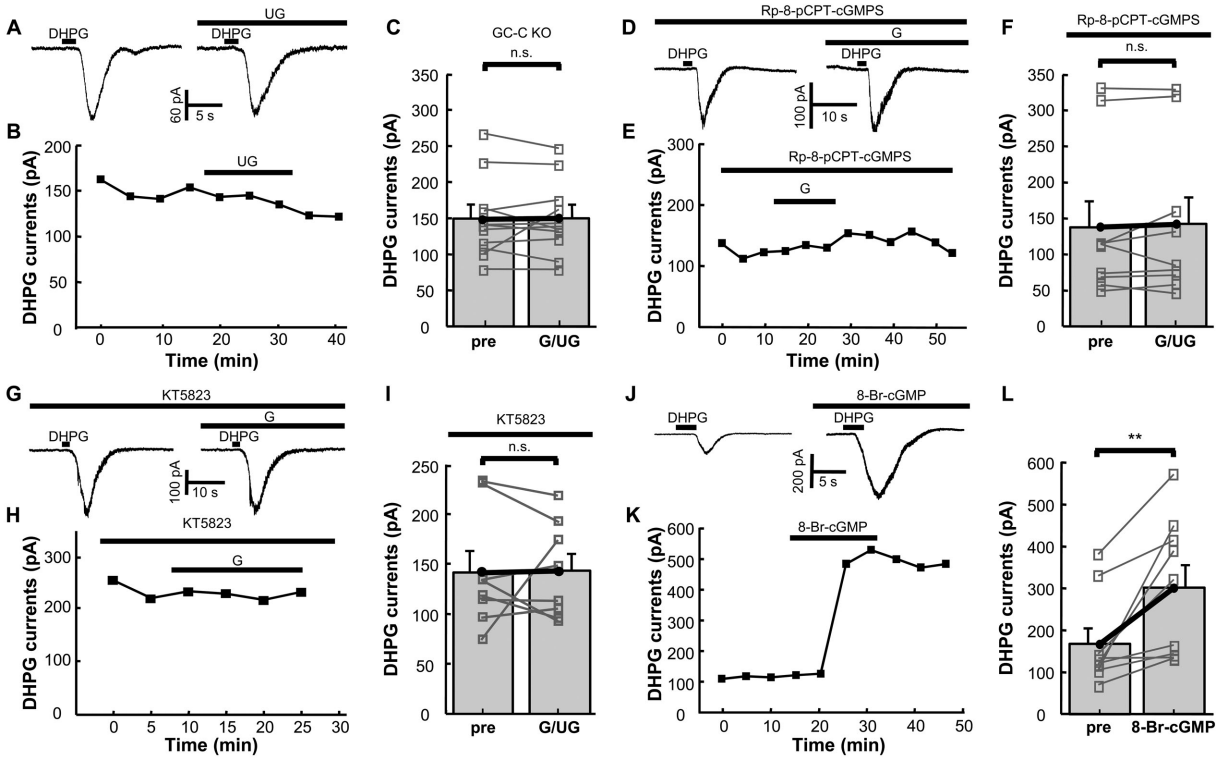
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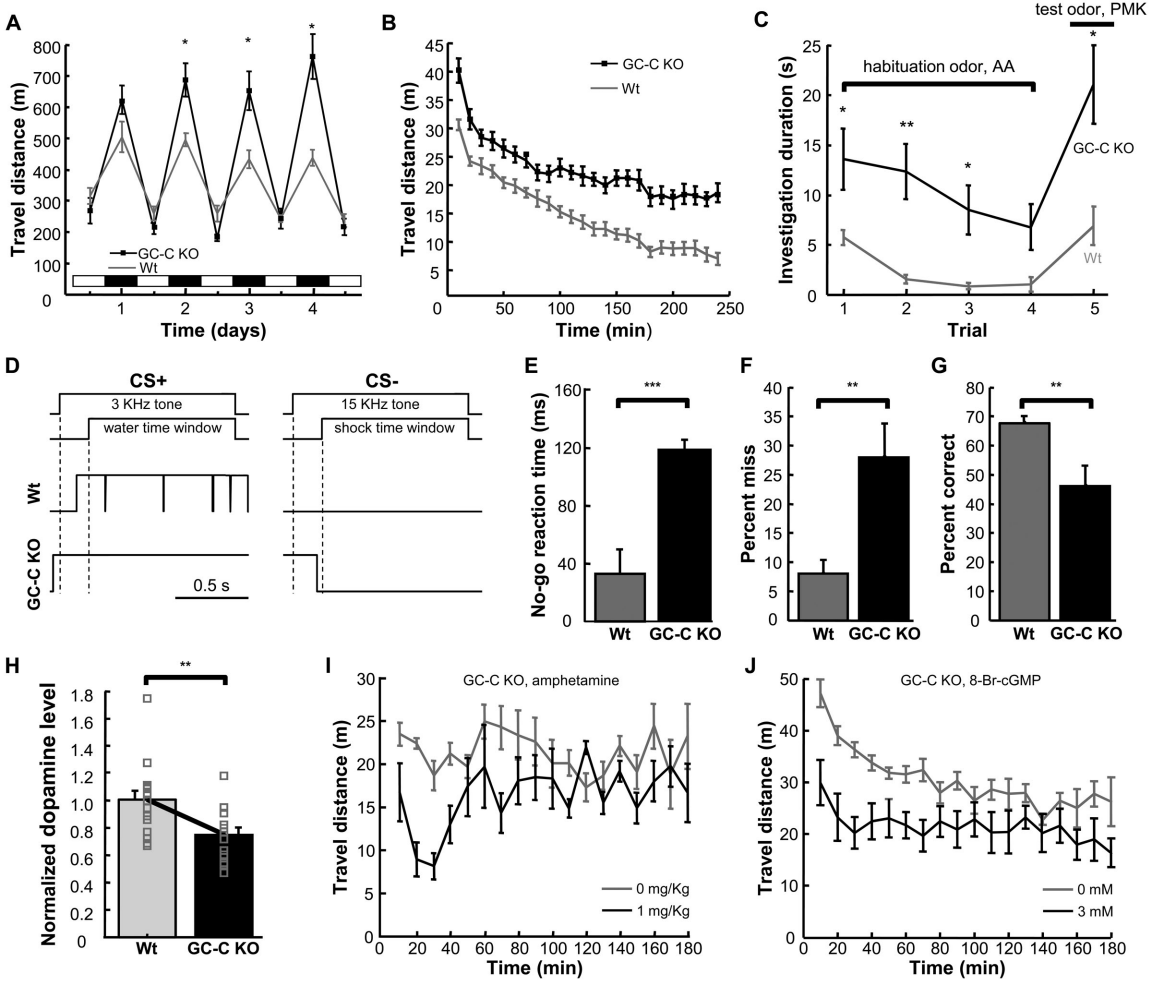
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