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LETTER TO THE EDITOR

# Forebrain elimination of *cacna1c* mediates anxiety-like behavior in mice

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The *CACNA1C* gene encoding the $Ca_v1.2$ subunit of the L-type calcium channel has emerged as a new candidate gene for neuropsychiatric disease, including bipolar disorder, major depression, schizophrenia and autism.[1–3] We report that global haploinsufficiency, forebrain-specific elimination and prefrontal cortex (PFC)-specific knockdown of *cacna1c* all increase anxiety-related behavior in mice, a prominent component of the forms of neuropsychiatric disease in which aberrations in *CACNA1C* have been implicated, without affecting compulsive behavior.

Constitutive *cacna1c* heterozygous mice (*HET*) were evaluated in three behavioral assays related to anxiety: open field test, light–dark conflict test and elevated plus maze (EPM). *HET*s displayed anxiety-like behavior in the EPM (Figure 1a), spending significantly less time exploring the open arms compared with wild-type littermate controls (WT; $F_{1,19} = 6.437$; $P < 0.05$). However, no differences were observed between *HET*s and WTs in the open field and light–dark conflict test, (Figures 1a and b, Supplementary Material). We also observed a similar statistically significant effect of increased anxiety-like behavior compared with WTs in EPM in adult female *HET*s (Figure 1d, Supplementary Material) and adolescent male *HET*s (Figure 1e, Supplementary Material). To more specifically investigate the function of *cacna1c* in the brain, we generated forebrain-specific conditional *cacna1c*-deficient mice (*forebrain-cacna1c cKO*) by crossing *cacna1c*-floxed mice with mice harboring alphaCaM Kinase II promoter-driven expression of Cre recombinase.[4] Relative to WTs, this strategy achieved ~70% elimination of *cacna1c* mRNA in the hippocampus, PFC, basolateral amygdala, striatum and nucleus accumbens, as assessed by quantitative PCR (Table 1, Supplementary Material). *Cacna1c* mRNA levels were unaffected in the ventral tegmental area and cerebellum. With this greater reduction in *cacna1c* in forebrain than could be achieved in *HET*s, significantly increased anxiety-like behavior was observed across all three behavioral assays. In EPM, *forebrain-cacna1c cKO* mice spent significantly less time exploring

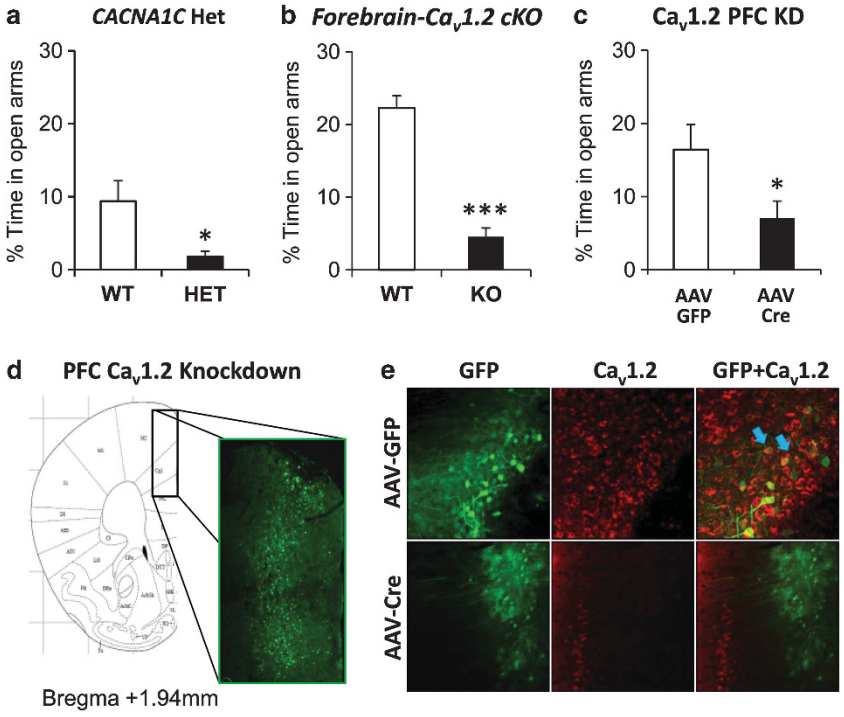


**Figure 1.** Anxiety-like behavior as measured in the elevated plus maze (EPM) assay is shown for (**a**) *cacna1c* haplosufficient (*cacna1c* HET; $n = 10$) and wild-type (WT; $n = 11$) littermates, (**b**) forebrain-specific *cacna1c* knockout (*forebrain-cacna1c* cKO; $n = 8$) and WT controls ($n = 10$), and (**c**) prefrontal cortex (PFC)-specific *cacna1c* knockdown (PFC-*cacna1c* KD; $n = 8$) and control virus ($n = 7$) microinjected mice. Decreased time in the open arm of the EPM reflects anxious-like behavior. Data are presented as mean (± s.e.m.) percent time spent in the open arms. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, *Bonferroni-Dunn posthoc* test. (**d**) Representative image of green fluorescent protein (GFP)-positive cells expressed by AAV-Cre-GFP stereotaxically microinjected into the PFC of *cacna1c*-floxed mice is shown. (**e**) Double immunohistochemical analysis with GFP and $Ca_v1.2$ antibodies is shown. Successful knockdown of $Ca_v1.2$ protein in the PFC was confirmed by the lack of co-localization of GFP and $Ca_v1.2$ in the same cells. Also shown is a representative image of GFP and $Ca_v1.2$ co-localization (blue arrows) in PFC neurons of control AAV-GFP microinjected mice.

the open arms compared with WTs (Figure 1b, $F_{1,16} = 68.587$; $P < 0.0001$ and Figure 2c, Supplementary Material). In the open field test, *forebrain-cacna1c cKO* mice spent less time exploring the center of the chamber compared with WTs (Figures 2a and 3a, Supplementary Material). In the light-dark conflict test, *forebrain-cacna1c cKO* mice spent significantly less time in the brightly lit side compared with WTs (Figures 2b and 3b, Supplementary Material).

Clinically, anxiety is often accompanied by compulsive behavior, such as in obsessive-compulsive disorder (OCD), in which patients seek alleviation from recurrent bouts of anxiety-inducing intrusive thoughts by engaging in compulsively repetitive behaviors. Experimental models for OCD, such as *SAPAP3*-[5] or *SLITRK5*-deficient[6] mice, display pathologically high compulsive grooming that is readily quantified by the spray-induced grooming test. Compared with respective WTs, we did not observe elevated grooming in either *HET*s or *forebrain-cacna1c cKO* mice (Figures 1c and 3c, Supplementary Material). Thus, the form of anxiety associated with *cacna1c* function is distinct from that associated with OCD spectrum illnesses.

Some genetic variations in *CACNA1C* have been associated with altered PFC function[7–9] in neuropsychiatric disease, so we next generated focal elimination of *cacna1c* in the PFC with adeno-associated viral (AAV) vector-expressing Cre recombinase (AAV-Cre).[10] AAV-Cre was stereotaxically delivered bilaterally into the PFC of floxed *cacna1c* mice, and regional elimination of $Ca_v1.2$ was immunohistochemically confirmed (Figures 1d and e). Following elimination of *cacna1c* in the PFC, mice showed no differences in basal locomotor activity compared with AAV-GFP control injected mice (Figure 4, Supplementary Material). However, selective elimination of *cacna1c* in the PFC was associated with less time spent exploring open arms of the EPM, compared with control AAV-GFP injected mice (Figure 1c, $F_{1,16} = 5.477$; $P < 0.05$). To evaluate the specificity of PFC *cacna1c* knockdown in mediating anxiety, we used AAV-expressing *cacna1d* siRNA[10] to selectively eliminate *cacna1d* in the PFC, the other L-type $Ca^{2+}$ channel isoform expressed in brain. Selective knockdown of *cacna1d* in the PFC had no effect on locomotor behavior (Figure 5a, Supplementary Material) or time spent in open arms in the EPM (Figure 5b, Supplementary Material).

In summary, we report here the first direct evidence for a role of forebrain *cacna1c* in regulating anxiety. Mice harboring forebrain-specific elimination of *cacna1c* may thus provide a useful tool for studying the pathophysiology of anxiety in forms of neuropsychiatric diseases in which *CACNA1C* is implicated.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)