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Decrease of SYNGAP1 in GABAergic cells impairs inhibitory synapse connectivity, synaptic inhibition and cognitive function

Martin H. Berryer1,2, Bidisha Chattopadhyaya1,2, Paul Xing1,2, Ilse Riebe1,3, Ciprian Bosoi2, Nathalie Sanon2, Judith Antoine-Bertrand4, Maxime Lévesque5, Massimo Avoli5, Fadi F. Hamdan2, Lionel Carmant1,2, Nathalie Lamarque-Vane4, Jean-Claude Lacaille1,3,*, Jacques L. Michaud1,2,6,• & Graziella Di Cristo1,2,•

Haploinsufficiency of the SYNGAP1 gene, which codes for a Ras GTPase-activating protein, impairs cognition both in humans and in mice. Decrease of Syngap1 in mice has been previously shown to cause cognitive deficits at least in part by inducing alterations in glutamatergic neurotransmission and premature maturation of excitatory connections. Whether Syngap1 plays a role in the development of cortical GABAergic connectivity and function remains unclear. Here, we show that Syngap1 haploinsufficiency significantly reduces the formation of perisomatic innervations by parvalbumin-positive basket cells, a major population of GABAergic neurons, in a cell-autonomous manner. We further show that Syngap1 haploinsufficiency in GABAergic cells derived from the medial ganglionic eminence impairs their connectivity, reduces inhibitory synaptic activity and cortical gamma oscillation power, and causes cognitive deficits. Our results indicate that Syngap1 plays a critical role in GABAergic circuit function and further suggest that Syngap1 haploinsufficiency in GABAergic circuits may contribute to cognitive deficits.

1Department of Neurosciences, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal, Quebec, Canada H3C 3J7. 2CHU Sainte-Justine Research Center, 3175 Côte-ste-Catherine, Montréal, Quebec, Canada H3T 1C5. 3Groupe de Recherche sur le Système Nerveux Central, Université de Montréal, C.P. 6128, succ. Centre-ville, Montréal, Quebec, Canada H3C 3J7. 4Department of Anatomy and Cell Biology, Cancer Research Program of the Research Institute of MUHC, McGill University, 2155 Guy Street, Suite 500, Montreal, Quebec, Canada H3H 2R9. 5Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, 3801 University Street, Montreal, Quebec, Canada H3A 2B4. 6Department of Pediatrics, Université de Montréal, CHU Sainte-Justine, 3175, chemin de la Côte-Sainte-Catherine, 7th floor, block 9, Montreal, Quebec, Canada H3T 1C5. * These authors contributed equally to this work. Correspondence and requests for materials should be addressed to J.L.-M. (email: jacques.michaud@recherche-ste-justine.qc.ca) or to G.D.C. (email: graziella.dicristo@recherche-ste-justine.qc.ca).
Long-term changes in the strength of synaptic transmission are thought to be critical both during brain development and for learning and memory throughout life. The Ras family GTPases, their downstream signalling proteins and upstream regulators are key biochemical cascades modulating synaptic plasticity. SYNGAP1 codes for a GTPase-activating protein (GAP) that physically interacts with the small GTPase Ras, which in turn acts in a cycle as a molecular switch with an active GTP-bound form and an inactive GDP-bound form. Ras has a slow intrinsic GTPase activity, and GAPs such as SYNGAP1 negatively regulate Ras by enhancing the hydrolysis of GTP to GDP. The importance of SYNGAP1 in synaptic plasticity is exemplified by the fact that de novo mutations in the SYNGAP1 gene cause moderate or severe intellectual deficiency (ID) 3–9. SYNGAP1 function has been mainly studied in excitatory neurons. For example, in primary neuronal cultures, SYNGAP1 functions to limit excitatory synapse strength by restricting the expression of the AMPA receptor (AMPAR) at the postsynaptic membrane. In mice, Syngap1 haploinsufficiency causes abnormal synaptic plasticity as well as behavioural abnormalities and cognitive deficits. Syngap1+/− mice are also characterized by enhanced excitatory synaptic transmission early in life and the premature maturation of glutamatergic synapses. Thus, it has been proposed that glutamatergic synaptic alterations represent the main contributing factor for the occurrence of cognitive and behavioural deficits.

During healthy cortical network activity, excitation is precisely balanced by GABAergic inhibition. Inhibitory activity not only regulates circuit excitability, but also restricts the temporal window for integration of excitatory synaptic inputs and resulting spike generation, thereby facilitating an accurate encoding of information in the brain. In addition, GABAergic cells are implicated in generating temporal synchrony and oscillations among networks of pyramidal neurons, which are involved in complex cognitive functions, such as perception and memory. Furthermore, GABAergic inhibition plays a critical role in modulating developmental plasticity in the young brain. Highlighting the importance of GABA interneurons in cognitive functions, cortical circuits in several mouse models of ID and autistic-like behaviour show excitation/inhibition imbalance, which is due to alterations in glutamatergic or GABAergic neurotransmission, or more often, in both. Whether and to what extent Syngap1 haploinsufficiency affects GABAergic cell circuits, thus contributing to excitation/inhibition imbalance and cognitive abnormalities remains unclear.

Here, we examined the specific contribution of Syngap1 to the formation of perisomatic innervations by parvalbumin-positive basket cells, a major population of GABAergic neurons, by single-cell deletion of Syngap1 in cortical organotypic cultures. In addition, we generated mice with specific deletion of Syngap1 in GABAergic neurons generated in the medial ganglionic eminence (MGE) to assess its role in the establishment of mature GABAergic connectivity and mouse cognitive function in vivo. We found that Syngap1 strongly modulated the formation of GABAergic synaptic connectivity and function and that MGE cell-type specific Syngap1 haploinsufficiency altered cognition.

**Results**

**Single-cell Syngap1 knockdown reduced PV + cell innervations.** Syngap1 expression peaks when the processes of synaptogenesis and developmental plasticity are heightened. While its expression in glutamatergic cell is well documented, few studies have also reported SYNGAP1 expression in GABAergic neurons. To confirm that SYNGAP1 is present in GABAergic neurons, we prepared dissociated neuronal cultures from E18 wild-type embryos and immunostained them for GAD67, which is the main GABA synthesizing enzyme, and SYNGAP1 at DIV21, after the peak of synapse formation. We found that GAD67-positive cells co-localized with SYNGAP1 (Supplementary Fig. 1a, 63 ± 5% co-localization), indicating that SYNGAP1 is indeed expressed by GABAergic neurons.

GABAergic circuits comprise an astonishing variety of different cell types, exhibiting differences in molecular, morphological and electrophysiological properties. These differences are particularly important in the light of recent discoveries suggesting that different GABAergic cell types are recruited by different behavioural events. Among the different GABAergic neuron subtypes, the parvalbumin-expressing (PV+) basket cell comprise the largest subpopulation in cortical circuits. Each PV+ basket cell innervates hundreds of neurons, with large, clustered boutons targeting the soma and the proximal dendrites of postsynaptic targets, an optimal location to control timing and frequency of action potential generation. This distinct feature of PV+ basket cell innervations are achieved during the first postnatal month in rodents and are modulated by neural activity levels. We found that almost the totality of PV+ basket cells express SYNGAP1 in dissociated neuronal cultures (Supplementary Fig. 1b) and thus we sought to investigate whether Syngap1 plays a role in the formation of the innervation of PV+ basket cells, by inducing single-cell Syngap1 deletion in cortical organotypic cultures.

To reduce Syngap1 expression in isolated PV+ basket cells and simultaneously label their axonal arbours at synaptic resolution, we used a previously characterized promoter region P<sub>G67</sub> (ref. 37) to express either the Cre recombinease together with GFP (P<sub>G67</sub>-GFP/Cre) or GFP alone (P<sub>G67</sub>-GFP; control basket cells) in cortical organotypic cultures prepared from Syngap1<sup>flox/flox</sup> mice. In organotypic cultures, PV+ basket cells initially display very sparse and simple axons, which develop into complex and highly branched arbours in the following 4 weeks, recapitulating the in vivo situation. We chose to induce Syngap1 deletion in PV+ basket cells at Equivalent Postnatal day 10 (EP10 = cultured prepared at Postnatal day 4 + 6DIV) and collect the cultures at EP24 because extensive and stereotyped maturation of PV+ basket cell innervations occurs during this time window. We investigated two aspects of PV+ basket cell axon innervation: (1) the extent of perisomatic innervation around single neuronal somata (terminal branching and perisomatic bouton density) and (2) the fraction of potentially innervated cells in the field (percentage of innervation). By studying the localization of pre- and post-synaptic markers and performing electron microscopy, we have previously shown that the vast majority of GFP-labelled boutons in our experimental condition represent presynaptic terminals.

Whereas control basket cells at EP24 showed complex perisomatic innervations (Fig. 1a,b), Syngap1 knockout in single-basket cells from EP10-24 induced a significant reduction in the number of both axonal branching and synaptic boutons innervating the target neurons (NeuN-positive cells; Fig. 1c-f: boutons/soma, 7.4 ± 0.3 for control versus 5.2 ± 0.5 for basket cells transfected with P<sub>G67</sub>-GFP/Cre; Students t-test P = 0.002).

On the other hand, the percentage of potentially innervated neurons were not significantly different between the two groups (Fig. 1g: 71% ± 2% for control versus 66% ± 2% for basket cells transfected with P<sub>G67</sub>-GFP/Cre; Students t-test, P = 0.125), suggesting that Syngap1 deletion specifically affected local synapse formation of PV+ basket cells but not their overall axonal growth. Syngap1 knockout at later age (EP16-24) caused a similar decrease in perisomatic bouton density (Supplementary Fig. 2,
boutons/soma, 7.4 ± 0.3 for control versus 4.7 ± 0.5 for basket cells transfected with P_{G67}-GFP/Cre; Students t-test P = 0.00023). Altogether, these data demonstrate that SynGAP1 promotes the formation of PV+ basket cell innervations in a cell-autonomous manner.

**MGE-specific SynGAP1 knockdown impaired PV cell connectivity.** Our data suggest that reducing SynGAP1 expression has a direct impact on the formation of PV+ basket cell innervations in vitro. To investigate the role of SynGAP1 in PV+ cell circuits in vivo, we generated mice that were heterozygous or homozygous for the SynGAP1^{floxed} allele and hemizygous for the Tg(Nkx2.1-Cre) transgene. This approach allowed the conditional deletion of SynGAP1 in cortical, hypothalamic and mesencephalic (striatum) GABAergic interneurons originating from the MGE as early as embryonic day 10.5 and throughout adulthood. In mouse, Nkx2.1-expressing MGE precursors produce most of PV+ and Somatostatin (SST)+ cortical

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**Figure 1 | Single-cell Cre-mediated inactivation of SynGAP1 reduces the number of cortical GABAergic basket cell boutons and terminal branching.** (a) Control GABAergic basket cell transfected with pGAD67-GFP (green) in EP24 cortical organotypic cultures from SynGAP1^{floxed/floxed} mice showing (b) rich axonal branching and numerous clustered boutons (arrowheads) around NeuN-positive somata (blue). (c,d) Conversely, a basket cell transfected with pGAD67-Cre/GFP from EP16-24 shows markedly reduced axonal branching and perisomatic boutons (arrowheads). (e) Bouton density and (f) complexity of terminal axon branching are significantly decreased in pGAD67-Cre/GFP basket cells (e: Student’s t-test, *P = 0.002; f: Student’s t-test, P = 0.045 at 8 µm and P = 0.010 at 9 µm), whereas the percentage of innervated cells (g) is not affected compared with age-matched control basket cells (Student’s t-test, P = 0.125). n = 8 basket cells transfected with pGAD67-GFP from n = 5 mice; n = 6 basket cells transfected with pGAD67-Cre/GFP from n = 5 mice. Scale bar, (a,c) 50 µm; (b,d) 10 µm.
interneurons. To confirm the specificity of CRE expression, we fluorescently labelled the cells expressing Cre by crossing Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice with a GFPR reporter line (RCE mouse). We confirmed that cortical GFP+ cells expressed either PV or SST (Supplementary Fig. 3; percentage of GFP+ cells expressing PV or SST, 74 ± 7% and 25 ± 6%, respectively, n = 3 mice). Further, the large majority of cortical neurons immunopositive for PV also expressed GFP (85 ± 6%, n = 3 mice), as previously reported.

We focused our study on Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice, since most of Tg(Nkx2.1-Cre);Syngap1<sup>flox/flox</sup> mice died at birth (9 out of 11, Tg(Nkx2.1-Cre);Syngap1<sup>flox/flox</sup> versus none of Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice) as it is the case for Syngap1<sup>−/−</sup> mice. Therefore, Syngap1 expression in MGE-derived cells may be required for survival. Alternatively, since both Syngap1 and Nkx2.1 are expressed in the developing lungs and heart, it is possible that the deletion of Syngap1 outside the brain is the cause underlying this early mortality.

First, we investigated whether Syngap1 haploinsufficiency restricted to MGE-derived GABAergic cells affected perisomatic innervations formed by PV+ basket cells in the cortex. In particular, we analyzed the intensity of PV immunostaining around layer 5 NeuN-positive neurons in the somatosensory cortex of P45 Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> and control littersmates as reported in. In control mice, distinct PV-positive perisomatic bouton rings enclosed a large portion of the pyramidal cell soma, while PV-positive perisomatic rings were significantly less developed in Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice (Fig. 2a,b,e; PV mean intensity (a.u.), 15.9 ± 0.9 for Syngap1<sup>flox/+</sup> mice versus 10.1 ± 0.8 for Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice; One-way ANOVA post hoc Dunn’s test, P = 0.00404), which suggests a decrease of perisomatic boutons made by PV+ basket cells. This effect was not restricted to the cortex, as we found a similar reduction in PV staining intensity around hippocampal CA1 pyramidal cells (PV mean intensity (a.u.), 17 ± 2, n = 52 cells from three Syngap1<sup>flox/+</sup> mice versus 9.9 ± 0.9, n = 56 cells from four Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice; one-way ANOVA, post hoc Dunn’s test, P = 0.0183).

To evaluate whether germ-line Syngap1 haploinsufficiency affects GABAergic perisomatic innervation formed by PV+ basket cells in the cortex, we performed the same analysis in Syngap1<sup>+/−</sup> and control littersmates. We found a reduction in perisomatic PV-positive rings in the somatosensory cortex of Syngap1<sup>+/−</sup> mice, which was comparable to what we observed in Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice (Fig. 2c–e; PV mean intensity (a.u.), 12 ± 1, versus 8.6 ± 0.7 from Syngap1<sup>+/+</sup> and Syngap1<sup>+/−</sup> mice, respectively; one-way ANOVA, post hoc Dunn’s test P = 0.0274). Thus, Syngap1 haploinsufficiency both in germ line and MGE-derived interneurons reduced cortical PV+ basket cell bouton density in vivo.

To further investigate how Syngap1 haploinsufficiency in MGE-derived GABAergic cells affects the formation of the PV+ basket cell axonal arbour and synaptic boutons, we prepared cortical organotypic cultures from P4 Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> and their control littersmates. PV+ basket cells were labelled by biolistic transfection of P<sub>GAD</sub>-GFP. In EP24 organotypic cultures from control mice (Syngap1<sup>flox/+</sup>), NeuN-positive somata were surrounded by highly branched basket cell axonal terminals and numerous perisomatic boutons (Fig. 3a,b); on the contrary, in age-matched cultures from Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup> mice, we found a significant decrease of the complexity of the terminal axonal branching (Fig. 3c,d,f) as well as of the perisomatic bouton density formed by basket cell around targeted neurons (Fig. 3e; boutons/soma, 10 ± 1 for Syngap1<sup>flox/+</sup> versus 4.7 ± 0.3 for Tg(Nkx2.1-Cre); Syngap1<sup>flox/+</sup>, Students t-test P = 0.02). Similarly to what we observed in single-basket cell knockout of Syngap1 performed at EP10-24, the fraction of innervated somata was not affected by Syngap1 knockdown in MGE-derived PV+ basket cells at the embryonic stage (Fig. 3g, percentage of innervation, 69% ± 3% for Syngap1<sup>flox/+</sup> versus 67% ± 3% for Tg(Nkx2.1-Cre);Syngap1<sup>flox/+</sup>; Students t-test, P = 0.623). All together, these data show that Syngap1 haploinsufficiency in MGE-derived neuron affects the formation of PV+ basket cell innervations in vivo.
MGE-specific Syngap1 knockdown impaired GABA transmission. The observation that PV⁺ basket cells in Tg(Nkx2.1-Cre); Syngap1flox/+ formed less boutons around their potential postsynaptic targets raises the possibility of an impairment in GABAergic synaptic transmission. Thus, we examined the effects of MGE-specific Syngap1 knockdown on spontaneous inhibitory synaptic transmission in acute cortical and hippocampal slices from adult Tg(Nkx2.1-Cre); Syngap1flox/+ mice and their respective control littermates. We found that mean inter-event interval (IEI) of miniature inhibitory postsynaptic currents (mIPSCs) recorded from pyramidal neurons in L2-3 somatosensory cortex was significantly longer in Tg(Nkx2.1-
Synap1flox/+ mice relative to the controls (Fig. 4a,c; IEI: 92 ± 7 ms for Synap1flox/+ versus 143 ± 17 ms Tg(Nkx2.1-Cre);Synap1flox+/-; linear mixed model (LMM), P = 0.004186). Similarly, inter-event intervals of mIPSCs recorded from CA1 pyramidal neurons were significantly longer in Tg(Nkx2.1-Cre);Synap1flox+/- mice relative to the controls (Fig. 4e; IEI: 184 ± 26 ms for Synap1flox+/- versus 789 ± 157 ms Tg(Nkx2.1-Cre);Synap1flox+/-; LMM, P = 0.00109). Amplitude of mIPSCs was not affected in Tg(Nkx2.1-Cre);Synap1flox+/- mice compared with controls (Fig. 4a,c,e,g; LMM, P > 0.5). Finally, mIPSC kinetics and mIPSC charge transfer were not significantly different for cortical and hippocampal neurons between the different genotypes (Supplementary Fig. 4). Therefore, Synap1 haploinsufficiency in MGE-derived interneurons in vivo strongly affects GABAergic transmission in adult mice.

To investigate whether inhibitory neurotransmission is also affected by germ-line deletion of Synap1, we performed the same analysis in Synap1+/−/− and control littermates. We found no difference in inter-event intervals of mIPSC recorded from either L2-3 or CA1 pyramidal neurons in Synap1+/−/− compared with control mice (Fig. 4b,d,f,h; L2-3: IEI: 111 ± 15 ms for Synap1+/−/− versus 106 ± 11 ms for Synap1+/+; CA1: IEI: 153 ± 21 ms for Synap1+/−/− versus 191 ± 28 ms for Synap1+/+; LMM, n = 11 neurons from Synap1+/−/− mice; n = 12 neurons from Synap1+/+ mice; n = 15 neurons from Synap1+/+ mice; n = 14 neurons from Synap1+/−/− mice. CA1: n = 9 neurons from Synap1+/−/− mice; n = 5 Tg(Nkx2.1-Cre);Synap1flox+/- mice; n = 10 neurons from Synap1+/+ mice; n = 9 neurons from Synap1+/+ mice. n = 3 Synap1+/−/− mice.

**Figure 4** | Synap1 haploinsufficiency in MGE-derived interneurons reduces mIPSC frequency. (a,b) Representative traces of mIPSCs from L2-3 pyramidal neurons in acute slices from Synap1flox/+ versus Tg(Nkx2.1-Cre);Synap1flox+/- (a) and Synap1+/−/− versus Synap1+/+ (b) young adult mice. (c,d) mIPSC inter-event intervals are significantly longer in Tg(Nkx2.1-Cre);Synap1flox+/- compared with Synap1flox+/- mice (c, LMM, genotype effect = −49.117, P = 0.004186), but not in Synap1+/−/− versus Synap1+/+ mice (d, LMM, genotype effect = −1.952, P = 0.5703). mIPSC amplitude is not affected in either mouse line (c,d; LMM, P > 0.5). (e,f) Representative traces of mIPSCs from CA1 pyramidal neurons in acute slices from Synap1flox+/- versus Tg(Nkx2.1-Cre);Synap1flox+/- (e) and Synap1+/−/− versus Synap1+/+ (f) young adult mice. (g,h) mIPSC inter-event intervals are significantly increased in Tg(Nkx2.1-Cre);Synap1flox+/- compared with Synap1flox+/- mice (g, LMM, genotype effect = 650.15, P = 0.00109 for IEI), but they are not significantly affected in neurons from Synap1+/−/− mice compared with control littermates (h, LMM, genotype effect = 0.1203, P = 0.9974 for IEI). mIPSC amplitude is not affected in either mouse line (LMM, P > 0.5). mIPSC frequency and amplitude are represented with Tukey boxplots (median ± 1.5 × interquartile range; box represents 1st-3rd quartile). The cross represents mean values. Individual cell values from the same animal are represented with the same symbols.
mIPSC amplitude, kinetics and charge transfer were also not affected (Fig. 4b,d,f,h and Supplementary Fig. 4). The differences observed between Synap1+/− and Tg(Nkx2.1-Cre); Synap1floxt/cm mice suggest that the concurrent Synap1 haploinsufficiency in glutamatergic and/or in GABAergic neurons, which do not originate from the MGE alters neuronal circuit development in a manner that compensates, at least partly, inhibitory synaptic deficits originating from Synap1 haploinsufficiency in MGE-derived GABAergic neurons.

To further confirm that Synap1 haploinsufficiency in MGE-derived interneurons impairs functional cortical GABAergic connectivity in vivo, we targeted expression of the light-sensitive channel channelrodopsin-2 (ChR2) to MGE-derived GABAergic interneurons, by injecting a Cre-dependent adenovirus-associated virus in somatosensory cortex [47] (Fig. 5a). Two-weeks post-transfection, we recorded inhibitory synaptic responses evoked by blue light pulses of different duration (input-output function) in layer 5 pyramidal cells. The amplitude of light-evoked IPSCs was significantly lower in Tg(Nkx2.1-Cre); Synap1flox/+ mice compared with those recorded in Tg(Nkx2.1-Cre); Synap1+/+ littermates (Fig. 5b; two-way Repeated Measure ANOVA with Bonferroni’s multiple comparison post hoc test, \( P < 0.0001 \)). Together with the observation that the number of cortical perisomatic boutons formed by PV+ baskets was reduced in Tg(Nkx2.1-Cre); Synap1flox/+ mice (Figs 2 and 3), this result demonstrated that Synap1 haploinsufficiency impaired GABAergic synapse formation and function of MGE-derived interneurons.

MGE-specific Synap1 knockdown affected gamma oscillations. PV+ cells represent the large majority of cortical GABAergic interneurons generated by MGE-derived precursors. Correlative, causal and computational evidence indicates that gamma oscillations in the 30–80 Hz range depend on synchronous activity of PV+ cells. Since we found that PV+ cell connectivity was reduced in both cortex and hippocampus of Tg(Nkx2.1-Cre); Synap1flox/+ mice (Fig. 2), we hypothesized that PV+ cell synaptic impairment would lead to changes in gamma oscillatory activity in these mice.

To test this hypothesis, we measured specific oscillatory patterns by video-electroencephalogram (EEG) recordings during active exploratory behaviour. We found that in the cortex, average power in the gamma frequency band was significantly lower in Tg(Nkx2.1-Cre); Synap1flox/+ animals compared with control littermates (Fig. 6; 0.27 ± 0.02 versus 0.32 ± 0.02 for Tg(Nkx2.1-Cre); Synap1flox/+ and Synap1flox/+ mice, respectively; Wilcoxon rank sum test, \( P = 0.01 \)). Similar results were observed in the hippocampus (Fig. 6c; 0.29 ± 0.02 versus 0.33 ± 0.005 for Tg(Nkx2.1-Cre); Synap1flox/+ and Synap1flox/+ mice, respectively; Wilcoxon rank sum test, \( P = 0.001 \)). Taken together, these data demonstrate that Synap1 haploinsufficiency in MGE-derived interneurons alters brain network activity, which in turn may contribute to cognitive deficits.

MGE-specific Synap1 knockdown caused cognitive deficits. While Synap1−/− mice die within the first days after birth, adult Synap1+/− mice showed behavioural and cognitive deficits, including hyperactivity, anxiety, abnormal reference, working and social memories [12, 14–17]. We have previously proposed that Synap1−/− mice represent an appropriate model to study the cognitive and behavioural abnormalities associated with the disruption of Synap1 in humans, as pathogenic mutations documented up to now in this gene are predicted to cause a loss-of-function.

To understand to what extent Synap1 haploinsufficiency in MGE-derived GABAergic neurons may contribute to the cognitive and behavioural deficits observed in Synap1+/− mice, we compared the performance of young adult Synap1+/−, Tg(Nkx2.1-Cre); Synap1flox/+ mice and their respective control littermates in several behavioural tests. We first assessed the locomotion activity using the open-field arena. Relative to their wild-type littermates, Synap1+/− mice travelled more during a 10 min session (Fig. 7a; 40 ± 4 m for Synap1+/− versus 57 ± 6 m for Synap1+/+, Mann–Whitney rank-sum test, \( P = 0.0032 \)), which is consistent with previously published observations [12, 14–16].

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**Figure 5** | Synap1 haploinsufficiency in MGE-derived interneurons reduces their synaptic output to pyramidal cells. (a) Schematic of the experimental approach. pAAV.CAGGS.flex.ChR2.tdTomato.SV40 is bilaterally injected in somatosensory cortex of either Tg(Nkx2.1-Cre); Synap1flox/+ or Tg(Nkx2.1-Cre); Synap1+/− young adult mice. This strategy allows ChR2 and tdTomato expression only in cells expressing Cre. (b) Upper panel. Traces of IPSCs recorded in response to light pulses of different duration in representative somatosensory cortex layer 5 pyramidal neurons in acute slices from Tg(Nkx2.1-Cre); Synap1flox/+ (black) and Tg(Nkx2.1-Cre); Synap1flox/+ (red) mice. Lower panel. Summary graph showing data from input-output function of inhibitory responses evoked by light stimulation. The IPSC amplitudes are significantly lower in Tg(Nkx2.1-Cre); Synap1flox/+ compared with Tg(Nkx2.1-Cre); Synap1+/−, starting from the third point of the input-output function (two-way Repeated Measure ANOVA with Bonferroni’s multiple comparison post hoc test, \( P < 0.0001 \)). n = 4 Tg(Nkx2.1-Cre); Synap1+/− mice (7 neurons) and n = 4 Tg(Nkx2.1-Cre); Synap1flox/+ mice (9 neurons).
These results indicate that *Syngap1*/*−/−* mice are hyperactive and that they do not display major locomotor deficits. Next, we tested anxiety-like behaviour using the elevated plus-maze test. *Syngap1*/*−/−* mice spent significantly more time in the open arms than the wild-type littermates (Fig. 7b; 10 ± 2% for *Syngap1*/*+/−* versus 25 ± 4% for *Syngap1*/*+/+*, Student’s *t*-test, *P* = 0.01), suggesting a reduced level of anxiety-like behaviour in the germ-line *Syngap1* heterozygous mice, as reported in previous studies.14,16 Conversely, *Tg(Nkx2.1-Cre);*Syngap1*/*+/* mice showed normal levels of locomotion and anxiety-like behaviour compared with *Syngap1*/*+/* mice (Fig. 7a; for open field: 45 ± 5 m for *Syngap1*/*+/* versus 51 ± 6 m for *Tg(Nkx2.1-Cre);*Syngap1*/*+/*, Mann–Whitney rank sum test, *P* = 0.503; for elevated plus maze: 15 ± 3% for *Syngap1*/*+/* versus 21 ± 3% *Tg(Nkx2.1-Cre);*Syngap1*/*+/*, Student’s *t*-test, *P* = 0.136).

Several mouse models of ID and autism exhibit reduced social interaction behaviour.17,23 We tested social interactions in *Syngap1*/*+/* and *Tg(Nkx2.1-Cre);*Syngap1*/*+/* and their respective littermates. After a habituation period, we evaluated sociability by assessing the preference of the tested mice for an
**Figure 7** | Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> mice show cognitive deficits that partially recapitulate those observed in Syngap1<sup>+/−</sup> mice. 

(a,b) Syngap1<sup>+/−</sup> mice show hyperactivity (a; Mann--Whitney rank sum test, *P = 0.0032) and altered stress levels (b; Student’s t-test, *P = 0.01), while Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> mice behave similarly to their control littermates in the open field (a) and elevated plus maze (b) tests (a; Mann–Whitney rank sum test, *P = 0.503, b; Student’s t-test, *P = 0.136, NS, not significant). (c,d) Syngap1<sup>+/−</sup> mice (c) and Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> (d) mice show social novelty preference impairment and spend similar amount of time in the chambers with either the novel (Stranger 2) or the familiar mouse (Stranger 1), contrary to their control littermates, which spend more time with the novel mouse (Stranger 2/C2/Stranger 1/C2) Genotype, Two-way ANOVA with Sidak’s multiple comparison post hoc test, *P < 0.05). (e) Both Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> and Syngap1<sup>+/−</sup> mice perform worse than their littermates on the T-maze test (Student’s t-test, *P = 0.035 for Syngap1<sup>+/−</sup> versus Syngap1<sup>+/+</sup> and *P = 0.038 for Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> versus Syngap1<sup>+/−</sup>). 

Open field and Elevated T-Maze: n = 12 Syngap1<sup>+/+</sup>, n = 11 Syngap1<sup>+/−</sup>, n = 12 Syngap1<sup>flo</sup>/<sup>+</sup>, n = 11 Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> mice. For three-chamber test: n = 10 Syngap1<sup>+/+</sup>, n = 10 Syngap1<sup>+/−</sup>, n = 10 Syngap1<sup>flo</sup>/<sup>+</sup>, n = 16 Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> mice. For T-maze, n = 15 Syngap1<sup>+/+</sup>, n = 9 Syngap1<sup>+/−</sup>, n = 13 Syngap1<sup>flo</sup>/<sup>+</sup>, n = 9 Tg(Nkx2.1-Cre);Syngap1<sup>flo</sup>/<sup>+</sup> mice.
unknown wild-type conspecific mouse matched for age, sex and background versus an empty wire cage (Supplementary Fig. 5). Mice of all genotypes spent significantly more time with an unfamiliar mouse (stranger 1) relative to the empty wire cage (Supplementary Fig. 5, two-way ANOVA,  \( P < 0.05 \)), indicating that social approach behaviour was normal in Syngap1\(^{+/+}\) and Tg(Nkx2.1-Cre);Syngap1\(^{+/+}\) relative to their respective control littermates. Next, we tested whether social novelty preference was affected in these mice by assessing the preference for a second stranger mouse. Syngap1\(^{+/+}\) and Syngap1\(^{+/+}\) mice spent significantly more time in the chamber containing the novel mouse (stranger 2) than in the chamber with the familiar one (stranger 1; Fig. 7c,d; stranger 2 vs stranger 1 (in s): 271 ± 29 versus 184 ± 22 for Syngap1\(^{+/+}\); 297 ± 27 versus 187 ± 18 for Syngap1\(^{+/+}\). two-way ANOVA post hoc Sidak’s multiple comparisons test \( P < 0.05 \)). In contrast, both Syngap1\(^{+/+}\) and Tg(Nkx2.1-Cre);Syngap1\(^{+/+}\) spent similar amount of time with the novel versus the familiar mice (Fig. 7c,d; stranger 2 versus stranger 1 (in s): 245 ± 23 versus 203 ± 20 for Syngap1\(^{+/+}\); 279 ± 26 versus 216 ± 32 for Tg(Nkx2.1-Cre);Syngap1\(^{+/+}\); two-way ANOVA post hoc Sidak’s multiple comparisons test \( P > 0.05 \)), indicating deficits in social novelty preference in both Syngap1\(^{+/+}\) and Tg(Nkx2.1-Cre);Syngap1\(^{+/+}\) mice.

Finally, we tested spatial working memory by measuring spontaneous alternation in the T-maze. Spontaneous alternation was strongly altered in both Syngap1\(^{+/+}\) and Tg(Nkx2.1-Cre); Syngap1\(^{+/+}\) relative to their respective control littermates (Fig. 7e; 67 ± 6% for Syngap1\(^{+/+}\) versus 41 ± 11% for Syngap1\(^{+/+}\); Student’s t-test \( P = 0.035 \), and 64 ± 7% for Syngap1\(^{+/+}\) versus 33.33 ± 11% for Tg(Nkx2.1-Cre); Syngap1\(^{+/+}\), Student’s t-test \( P = 0.038 \), suggesting impaired spatial working memory in both mutant lines. On the other hand, we found that Tg(Nkx2.1-Cre) mice showed no statistical difference in locomotion, anxiety, spontaneous alternation and social recognition behaviour (Supplementary Fig. 6). All together, these data show that specific inactivation of Syngap1 in MGE-derived interneurons recapitulates at least in part the behavioural and cognitive alterations observed in mice with germ-line Syngap1 haploinsufficiency.

**Discussion**

Our data show that Syngap1 is required for the proper terminal axonal branching and bouton formation of cortical GABAergic PV+ basket cells, both in organotypic cortical cultures and in vivo. Further, MGE-restricted Syngap1 haploinsufficiency causes deficits in cortical evoked-inhibitory synaptic transmission and gamma oscillations and specific impairments in social novelty preference and working memory, in adult mice. In contrast, a recent study did not observe any cognitive deficits in Gad2-cre; Syngap1\(^{+/+}\) mice\(^{17}\). One possible explanation for this discrepancy is that Nkx2.1 and Gad2 may be expressed at different developmental time points. In fact, Nkx2.1 is already expressed in all MGE-derived GABAergic interneurons by E10.5. (ref. 45) On the other hand, while Gad2 transcription starts early during embryogenesis\(^{50}\) (around E11.5), it is possible that not all GABAergic cells express it at the same time. For example, GAD65, which is coded by Gad2, is barely expressed before P6 in rodent somatosensory cortex\(^{16}\). Syngap1 haploinsufficiency appears to disrupt the function of excitatory circuits mainly by affecting their early synaptic development\(^{16,17}\). Our observations suggest that Syngap1 is also required during the early phases of the development of GABAergic circuit connectivity.

Another major difference is that, while Gad2 is expressed by most GABAergic interneurons, Nkx2.1 is expressed only by interneurons derived from the MGE, which include PV+ and SST+ interneurons but not, for example, those positive for the vasoactive intestinal polypeptide (VIP). Recent results demonstrate that VIP-expressing neurons form a disinhibitory microcircuit that is conserved across cortical regions\(^{52}\). In particular, VIP interneurons tend to inhibit most SST+ and a fraction of PV+ interneurons. Inhibition of these interneurons in turn disinhibits principal cells in vivo, providing a form of gain control.\(^{53}\) It is possible that removing Syngap1 in all interneurons may allow homeostatic synaptic adjustments that are not otherwise engaged when Syngap1 is removed only in MGE-derived interneurons. It will therefore be important to assess whether and how Syngap1 regulates the synaptic connectivity of distinct GABAergic interneuron types other than PV+ basket cells.

Disruption of excitatory/inhibitory balance is emerging as a common theme for many neurodevelopmental disorders.\(^{53,54}\) However, the critical challenge is to understand how specific circuit alterations resulting from mutations affect the development of cognitive abilities. Our study suggests new avenues of research for the understanding of mechanisms involved in SYNGAP1-related ID. For example, our results showed that Syngap1 plays a role in the formation of perisomatic synapses by PV+ basket cells. GABAergic neurotransmission from these cells promotes network gamma oscillations.\(^{48,55}\) Cortical gamma oscillations increase in power during tasks that require complex processing of sensory information, attention, working memory and cognitive control, suggesting that gamma oscillations are crucial for cognition.\(^{56}\) Interestingly, substantial evidence indicates that individuals with specific psychiatric disorders, such as schizophrenia or autism, show lower power of gamma oscillations induced during the performance of cognitive tasks compared with healthy individuals.\(^{57,58}\) Here, we showed that MGE-specific Syngap1 haploinsufficiency leads to reduced gamma oscillation power during exploration, which in turn may contribute to the occurrence of cognitive deficits. It will be interesting to explore the presence of alterations in brain oscillations in patients carrying SYNGAP1 mutations. Furthermore, cortical GABAergic inhibition, in particular the one mediated by PV+ cells, has been suggested to play a key role in critical period plasticity in the developing brain.\(^{21,59}\) Critical periods represent heightened epochs of brain plasticity during childhood, during which experience can produce permanent, large-scale changes in neuronal circuits. By regulating critical period plasticity, PV+ cell-mediated inhibition may influence how experience shapes brain wiring during early life. As a consequence, altered PV cell connectivity and function could drive cortical neuronal circuits along abnormal developmental trajectories, which could then contribute to cognitive dysfunction. Ultimately, the understanding of SYNGAP1 function in specific inhibitory and excitatory circuits might lead to the development of tailored therapies.

MGE-derived interneurons include PV+ basket cells and SST+ interneurons,\(^{60}\) thus alterations in the synaptic connectivity of this latter group may also contribute to the cognitive deficits that were observed in Tg(Nkx2.1-Cre); Syngap1\(^{+/+}\) mice. Further, it is very likely that altered GABAergic signalling results in either causal or compensatory alterations in non-GABA circuits as well, which can further play a role in cognitive dysfunctions in mutant mice. The main challenges will be to understand when these alterations arise and whether and to what extent cognitive deficits are due to either altered neuronal circuit development leading to diverse changes in brain and behaviour and/or a state of continuous synaptic dysfunction in adulthood.

Haploinsufficiency of the NFI gene, which also codes for a RasGAP, causes neurofibromatosis type 1, a complex neuro-
cutaneous condition that is associated with cognitive impairment and autism spectrum disorders. Decrease of Nf1 induces deficits in hippocampal-dependent spatial learning, amygdala-dependent social learning and prefrontal cortex-dependent working memory by increasing GABA release in these different areas of the brain through the activation of the Ras–ERK pathway. The increase of Ras thus appears to produce opposite effects on GABA neurotransmission depending upon whether it is associated with Synap1 or Nf1 haploinsufficiency. How can we explain this paradox? One possibility is that Synap1 and Nf1 affect Ras activity at different time points during the development of GABAergic circuits. As discussed above, Synap1 haploinsufficiency might impact the establishment of GABAergic connectivity by acting during early synaptic development. Nf1 might on the other hand modulate GABA release in mature synapses. Alternatively, it is possible that SYNGAP1 and the Nf1 product, neurofibromin, function in distinct subcellular compartments of GABAergic neurons and thus regulate different processes. SYNGAP1 is associated with NMDA receptor complexes at the postsynaptic membrane of glutamatergic neurons, raising the possibility that it also interacts with such receptors in GABAergic neurons. In contrast, neurofibromin has been shown to localize to smooth cell bodies in the cell body and dendrites of Purkinje cells, but not with the plasma membrane. All together, these observations suggest that SYNGAP1 and neurofibromin modulate the development and function of GABAergic cells by regulating differently the spatial and/or temporal activity of Ras.

In summary, our study suggests that Synap1 haploinsufficiency affects GABAergic circuit connectivity and function, which in turn may contribute to cognitive alterations. Further studies will be required to determine whether pharmacological interventions during development can rescue these deficits.

Methods

Mice. All procedures described here had been approved by the Comité Institutionnel de Bonnes Pratiques Animales en Recherche (CIBPAR) of the Research Center of Sainte-Justine Hospital in accordance with the principles published in the Canadian Council on Animal’s Care’s Guide (to the Care and Use of Experimental Animals, Feb 91). Synap1+/- mice were kindly provided by Dr. Sebb Grant (Edinburgh University, United Kingdom) and Synap1fl/fl mice by Drs. Irene Knuesel and Mary Muhia (University of Zurich, Switzerland). They were generated and genotyped as described in refs 10,15. They were maintained as a wild-type background, organotypic slices from each mouse half of the organotypic cultures were transfected with pGAD67-Cre;Syngap1+/--; control basket cells, or pGAD67-Cre/pGAD67-GFP (Synap1+/--; basket cells). In particular, for each mouse half of the organotypic cultures were transfected with pGAD67-GFP and half with pGAD67-Cre;pGAD67-GFP, thus allowing us to control for inter-animal variance. Organotypic slices from Tg(Nkx2.1-Cre);Syngap1+/--; and Syngap1fl/fl mice were transfected with pGAD67-GFP to visualize PV+ basket cell morphology a minimum of 5 days before imaging.

Analysis of basket cells in vitro. Slices were fixed and immunostained as in ref. 40. To visualize basket cell targeted neurons, we used anti-NeuN (monoclonal anti-neuronal marker, Millipore Research Reagents, #04691) and anti-GFP (polyclonal anti-GFP antibody, 1:2000, Invitrogen A-21422) for 24 h at room temperature (RT), then mounted in Vectashield mounting medium (Vector). Confocal images were taken using a ×63 oil objective (Leica TCS SP2). Data are presented as mean ± s.e.m. Imaging and quantification were performed independently by two different operators. All quantification was done blind to the genotype or the experimental conditions.

Analysis of perisomatic innervation in vivo. P45 mice were anesthetized and perfused transcardially with 4% paraformaldehyde (PFA 4%) in phosphate buffer, pH 7.4. Brains were removed, immersed in PFA 4% overnight then cryoprotected in 30% sucrose in phosphate buffer pH 7.2) and finally embedded in optimal cutting temperature Tissue Tek. Coronal sections (40 µm thick) were incubated with Alexa Fluor 633-conjugated goat anti-mouse IgG (1:1000; Invitrogen A-11008) and Alexa 555-conjugated IgG goat anti-mouse;1:400, Invitrogen A-21422) for 0.5 h at room temperature (RT), then mounted in Prolong (Invitrogen P-8248) and stored at 4 °C. Images were acquired using a confocal microscope (LEICA TCS SPE or Leica TSC SPE).

Cortical organotypic culture and biotinyl transfection. Slices culture preparation and gene gun transfection were performed as described in refs 74, 76. Briefly, brains from postnatal days 4–5 Syngap1fl/fl; Tg(Nkx2.1-Cre);Syngap1fl/fl+/- and Syngap1fl/fl+/- mice were sliced coronally (400 µm thick) using a chopper (Stoelting). Slices were plated on Millicell membrane inserts. Culture medium (containing DMEM, 20% horse serum, 1 mM glutamine, 15 mM glucose, 1 mM CaCl2, 2 mM MgSO4, 0.5 μM β-mercaptoethanol, 30 mM HEPES, 5 mM NaHCO3, and 0.001% ascorbic acid) was placed under the membrane inserts. Slices were incubated in a humidified incubator at 34°C with 5% CO2. Medium was changed three times per week. For biotin transfection, 1.6 µm gold particles (BioRad) were coated with 25–30 µg of each one of the plasmids of interest. DNA-coated gold particle were then shot using a gene gun (BioRad) at high pressure (1800) at the age specified in the result section, incubated for 8 or 14 days in the same culture medium as described above. In experiments where Syngap1 was deleted in PV+ single-basket cells in an otherwise wild-type background, organotypic slices from Syngap1fl/fl mice were transfected either with pGAD67-GFP (Synap1+/--; control basket cells), or pGAD67-Cre/pGAD67-GFP (Synap1+/--; basket cells). In particular, for each mouse half of the organotypic cultures were transfected with pGAD67-GFP and half with pGAD67-Cre/pGAD67-GFP, thus allowing us to control for inter-animal variance. Organotypic slices from Tg(Nkx2.1-Cre);Syngap1+/--; and Syngap1fl/fl mice were transfected with pGAD67-GFP to visualize PV+ basket cell morphology a minimum of 5 days before imaging.
mean ± s.e.m. Imaging and quantification were performed independently by two different operators. All quantification was done blind to the genotype.

**Viral vector and stereotaxic injections.** The pAAV.CAGGS.flex.ChR2.tETomato. SV40 was a gift from Scott Storrie (Addgene Plasmid # 18817) and was produced as AAV2/9 serotype by the UPenn Vector Core Facilities (University of Pennsylvania, Philadelphia). Mice aged postnatal day 24–28 (P24–P28) were used for all surgeries. Bilateral viral injections were performed at the following stereotaxic coordinates: 1.5 mm from bregma, 1.5 mm lateral from midline and 0.70 mm ventral from cortical surface. Surgery procedures were standardized to minimize the variability of AAV injections. To ensure minimal leak into surrounding brain areas, injection pipettes remained in the brain for ~5 min after injection before being slowly withdrawn. The final injected volume for the AAV was 0.5 μl. We waited 2 weeks for maximal viral expression. The titre for the viruses was ~2.69 ± 10^{12} genome copies per ml.

**Electrophysiology.** All the following experiments were done by investigators blind to the genotype until after data analysis was completed.

mIPSC recordings: Acute slices (300 μm thickness) were prepared from hippocampus or somatosensory cortex of 7–9 week old Syngap1+/−, TgNlk2.1-Cre;Syngap1fl/+ mice and their respective control littermates of both sexes27. Slices were cut on a Leica vibrotome in ice-cold oxygenated (95% O2, 5% CO2) cutting solution containing 87 mM NaCl, 2.5 mM KCl, 25 mM NaHCO3, 0.5 mM CaCl2, 1.25 mM NaH2PO4, 7 mM MgCl2, 25 mM glucose, 75 mM sucrose, 3 mM pyruvic acid and 1 mM ascorbic acid. Slices were then transferred to oxygenated (95% O2, 5% CO2) artificial cerebrospinal fluid containing: 124 mM NaCl, 2.5 mM KCl, 1.25 mM NaH2PO4, 2 mM MgSO4, 2 mM CaCl2, 26 mM NaHCO3, 10 mM D-glucose (pH 7.2–7.3; 300 mOsm) and allowed to recover at room temperature for at least 1 h before recording in artificial cerebrospinal fluid. Whole-cell patch clamp recordings were obtained from visually identified pyramidal neurons in hippocampal CA1 region or somatosensory cortex layer 2–3 overlying cortex. The insertion of electrodes was performed under isoflurane anaesthesia, while the head of the mouse was immobilized in a stereotaxic frame. A stainless steel bipolar electrode (Plastics-1 Inc., Roanoke, VA, USA) was inserted between the 1.5 and 0.5 mm for one electrode in the hippocampus and 0.5 mm for the one in the cortex. Following a 5-day recovery period, EEG was recorded with a Platform system 32-channel video-EEG recording unit for 2 weeks. Mice were monitored during 6-hour sessions (afternoon and night). An operator blind to the genotype extracted EEG traces during mouse exploratory behaviour spanning 30–120 s. A total of 10 min of active exploration was selected for each mouse.

EEG recordings from the cortex and hippocampus were first analyzed to detect time periods of strong theta oscillations, which were indicative of stable recordings during which the animal was exploring. To detect theta oscillations, we used the spectrogram method using discrete short-time Fourier transforms. More specifically, data sampled at 200 Hz were first low-pass filtered by a 100th order infinite impulse response filter. Spectrograms were then built from the data set and subtracted into 1 s intervals to the Hamming window function was applied. The discrete Fourier transforms were then evaluated over 214 points with zero padding. Finally, for each time slot, the algorithm identified oscillations with the highest intensity in the theta frequency band (4–12 Hz) and that lasted at least 1 s. The power spectral density was normalized in amplitude from 0 to 1 and equalized with an exponential window function with 0.13 to improve the content of low frequencies. Results were then presented in a list of theta oscillations for each extracted EEG recording. For each time period of theta oscillations, we then calculated the average power in the gamma frequency band. Analysis was performed in Matlab (7.11.0) (The Mathworks, Natick, MA, USA). Results are expressed as mean ± s.e.m. The investigator was blind to the genotype until after data analysis was completed.

**Behavioural studies.** All animals were kept under a light-dark cycle (12 h light–12 h dark) in a temperature and humidity controlled room. Food and water were available ad libitum. Room lights were kept low during all procedures. For all experiments, a camera was mounted above the arena; images were captured and transmitted to a computer running the software (Panlab, Harvard Apparatus). The sequence of animals tested was randomized by the genotype. Care was taken to test litters containing both the genotypes specific to the breeding. Results are presented as mean ± s.e.m.

**Spontaneous open-field activity.** The open-field test was performed as previously described44. Each subject (P25) was gently placed in the centre of the open-field, allowed to freely explore undisturbed for 10 min, after which the animal was removed from the arena cleaned with 70% ethanol and dried before testing the next animal. locomotor activity was indexed as the total distance travelled (m).

**Elevated plus maze.** Experiments were performed as described in ref. 67. The test session lasted 5 min during which the animals (P27) were allowed to explore the maze freely before being removed and returned to their home-cage. The percentage of time spent in the open arms (open/open + closed) × 100 was scored as a measure of anxiety-related behaviour.

**Three-chamber.** A three-chamber arena was used to assess the social recognition performance of the mice68. The tested animal (P36) was placed in the middle of the central chamber and allowed to explore all the chambers for 10 min. During this habituation session, small wire cages were present, one in each opposite chamber. After habituation, an unfamiliar conspecific of the same sex and age (Stranger 1) was placed inside a small wire cage whereas the other remained empty. The tested animal was allowed to freely choose between the cage of Stranger 1 for 10 min. At the end of this 10 min, a new unfamiliar mouse of the same sex and the same age (Stranger 2) was placed in the previously unoccupied wire cage and the tested mouse was examined for an additional 10 min to assess preference for social novelty. Stanger 1 and stranger 2 animals originated from different home cages and were in contact with each other, however, each member originated from the same laboratory. Social novelty preference was evaluated by quantifying the time spent by the tested mice in each chamber during the third 10 min session.

**T-maze.** A discrete-two-trials spontaneous alternation paradigm in a T-maze apparatus was used to assess the working memory69. Each test consisted of a two-choice trials separated by a 1 min inter-trial interval (ITI) for 3 consecutive days. At the beginning of each trial, the animals (P38–40) were placed in the starting arm, allowed to freely move until one arm was chosen. The animals were left in the choice arm for 10 s before being removed and placed in the home-cage for 50 s. Then, the individuals were allowed to perform another free-choice trial. If the mice entered in the opposite arm than the first trial, the responses were considered as an alternation.

**Statistical analysis.** Data were expressed as mean ± s.e.m. unless otherwise specified in the legends. Normality tests were performed for all data analysed. Differences between two groups were assessed with the Student’s t-test for normally distributed data or with the Mann–Whitney rank sum test for not-normally distributed data. Differences between multiple groups were assessed with one-way ANOVA with either Dunn’s or Tukey’s post hoc test for non-normally distributed and normally distributed data, respectively. Two-way ANOVA with Sidak’s multiple comparison post hoc test was used for the detection of differences in the novelty social recognition test. Two-way Repeated Measure ANOVA with Bonferroni’s multiple comparison post hoc test was used for analysing light-evoked mIPSCs, considering N of mice as independent replicates. mIPSC frequency and amplitude were represented with Tukey boxplots (median ± 1.5 × interquartile range; box represents 1st–3rd quartile). In the same graph, a cross represented mean mIPSC frequency and amplitude values.
In addition, all individual cell values were reported, using different symbols for different animals. mIPSC frequency and amplitude were analysed using a LMM, modelling animal as a random effect and genotype as fixed effect. We used this statistical analysis because we considered the number of mice as independent replicates and the number of cell in each mouse as repeated measures. For gamma oscillation power analysis, averages from the two genotypes were compared with Wilcoxon rank sum tests since values were not normally distributed. Finally, two-tailed power analysis was performed for all experimental data, using alpha = 0.05 and desired power = 0.8. Statistical analysis was performed using Prism 5.0 (GraphPad Software), SigmaStat 3.5 (Systat) and the function lmer implemented in the R package lme4 specifically for LMM.

Three animals were excluded from the analysis of electrophysiological recordings because the genotype was ambiguous (n = 1 mouse) or tdTomato fluorescence was not detectable after AAV injection (n = 2 mice).

### Data availability

Detailed statistics and data that support the findings of this study are available from the corresponding authors on request.

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Author contributions
M.H.B., B.C., J.-C.L., J.L.M. and G.D.C. designed the experiments. M.H.B., B.C., P.X., I.R., C.B., N.S., J.A.B., M.L., M.A., F.F.H., L.C., J.-C.L. and N.L.V. performed experiments or/and analysed data, M.H.B., B.C., J.-C.L., J.L.M. and G.D.C. wrote the manuscript.

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