Functional Synergy between Cholecystokinin Receptors CCKAR and CCKBR in Mammalian Brain Development

Sayoko Nishimura¹, Kaya Bilgüvar²,³,⁴, Keiko Ishigame¹, Nenad Sestan⁵, Murat Günel¹,²,³,⁵, Angeliki Louvi¹,²,⁵*

¹ Department of Neurosurgery, Yale School of Medicine, New Haven, Connecticut, United States of America, ² Yale Program on Neurogenetics, Yale School of Medicine, New Haven, Connecticut, United States of America, ³ Department of Genetics, Yale School of Medicine, New Haven, Connecticut, United States of America, ⁴ Yale Center for Genome Analysis, Yale School of Medicine, New Haven, Connecticut, United States of America, ⁵ Department of Neurobiology, Yale School of Medicine, New Haven, Connecticut, United States of America

* angeliki.louvi@yale.edu

Abstract

Cholecystokinin (CCK), a peptide hormone and one of the most abundant neuropeptides in vertebrate brain, mediates its actions via two G-protein coupled receptors, CCKAR and CCKBR, respectively active in peripheral organs and the central nervous system. Here, we demonstrate that the CCK receptors have a dynamic and largely reciprocal expression in embryonic and postnatal brain. Using compound homozygous mutant mice lacking the activity of both CCK receptors, we uncover their additive, functionally synergistic effects in brain development and demonstrate that CCK receptor loss leads to abnormalities of corticogenesis, including defects in the formation of the midline and corpus callosum, and cortical interneuron migration. Using comparative transcriptome analysis of embryonic neocortex, we define the molecular mechanisms underlying these defects. Thus we demonstrate a developmental, hitherto unappreciated, role of the two CCK receptors in mammalian neocortical development.

Introduction

Neuropeptides modulate neuronal activity in the mammalian central nervous system (CNS) [1]. Cholecystokinin (CCK), originally discovered in the gastrointestinal tract [2], is one of the most abundant neuropeptides [3] and mediates its actions via two G-protein coupled receptors, CCKAR and CCKBR, which have distinct pharmacology and largely non-overlapping and species-dependent expression in different organs. CCK has emerged as a central regulator of neuronal circuits [4], and has been implicated together with its receptors in the neurobiology of feeding, memory, nociception and exploratory behavior [5, 6] and further associated with neuropsychiatric disorders [4, 7, 8].
CCKAR is the peripheral receptor, having limited expression in the brain [9, 10], whereas CCKBR predominates in the CNS, mostly in neocortical and limbic structures; in the periphery, it is restricted to the stomach, where it serves as a receptor for gastrin, a hormone homologous to CCK [9, 11, 12]. In brain, CCKAR and CCKBR have distinct distribution and selectivity in different rodent species, as suggested by binding assays of radiolabeled CCK peptides [13–18]. CCK is expressed in neocortical pyramidal neurons, including corticocortical projection neurons [19–22] and in a distinct subtype of interneurons [23–29].

Genetic inactivation of Cck [30, 31], Cckar [17] or Cckbr [32, 33] leads to defects in the gastrointestinal system, satiation and control of food intake, memory and exploration, and anxiety-related behaviors [17, 31–42]. CCKAR regulates the migration of gonadotrophin-releasing hormone 1 neurons and olfactory bulb interneurons, as well as female sexual behavior [43–46], suggesting a broader role despite its restricted expression in the brain. On the other hand, Cckbr and Cck mutant mice appear to lack remarkable brain phenotypes [30–33].

In this study, we show that mutant mice lacking both CCK receptors have abnormalities of cortical development, including defects in the formation of the corpus callosum and interneuron migration. Using comparative transcriptome analysis of embryonic neocortex we define the molecular mechanisms underlying these defects. We thus demonstrate a hitherto unappreciated, synergistic role of CCK receptors in mammalian neocortical development.

Materials and Methods

Transgenic mice

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of Yale University (protocol number 2013–10886). All experimental procedures involving animals were performed under deep anesthesia, and all efforts were made to minimize suffering. Approximately two hundred mice were used in this study. Mice were sacrificed by injectable anesthetic overdose followed by cervical dislocation. Mice of either sex were used throughout the study. Details regarding the generation and characterization of Cckar/Cckbr double homozygous mutant mice (JAX 129-Cckar<sup>tm1Kpn</sup>Cckbr<sup>tm1Kpn</sup>/J; stock number 006365) were previously reported [17, 33]. Genotyping of these transgenic mice was performed by PCR (Cckar<sup>tm1Kpn</sup> mutant forward primer: 5’-GAC AAT CGG CTG CTC TGA TG-3’, WT forward primer: 5’-GCT GCA TAG CGT CAC TTG G-3’, WT reverse primer: 5’-GAT GGA GTT AGA CTG CAA CC-3’, Cckbr<sup>tm1Kpn</sup> mutant forward primer: 5’-CTT GGG TGG AGA GGC TAT TC-3’, WT forward primer: 5’-CCA AGC TGC TGG CTA AGA AG-3’, WT reverse primer: 5’-CTT AGC CTG GAC AGA GAA GC-3’; additional information regarding PCR programs can be obtained on request).

Histological analysis

Brains were perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, post-fixed and cryoprotected in 30% sucrose in 4% PFA, then sectioned on a cryomicrotome (Leica Microsystems, Wetzlar, Germany). Nissl staining was performed by standard procedures on serial sections of the forebrain. The area of the SVZ and RMS, distinguishable because of high cell density, was measured using NIH ImageJ on Nissl-stained 36-μm-thick coronal sections.

In situ hybridization

Embryonic and postnatal mouse brains were fixed, respectively, by immersion in or intracardiac perfusion with 4% PFA, post-fixed in 30% sucrose in 4% PFA and sectioned on a
cryomicrotome (Leica Microsystems, Wetzlar, Germany). Human tissue was obtained from several sources, including the Human Fetal Tissue Repository at the Albert Einstein College of Medicine (New York, NY). Serial representative sections along the anterior/posterior axis of the neocortex (generally every third for embryonic and early postnatal or every sixth for adult) were processed for in situ hybridization as described previously [31]. RNA probes complementary to mouse Cckar (IMAGE: 4236240; BC020534), Ccckbr, Cck, claudin 11 (gift from S. Tsukita, Kyoto University, Kyoto, Japan), Ctgf, Tle4, Er81, Rorb (gifts from C. Ragsdale, University of Chicago, Chicago, IL), Tag1 (gift from D. Karagogeos, IMBB, Heraklion, Greece), Gad1, reelin, Lhx6, Cxcl12, Bmp7 (gifts from E. Grove, University of Chicago, Chicago, IL) and Nrp2 or to human CCKAR, CCKBR and CCK, were labeled with digoxigenin-11-UTP following cDNA cloning (S1 Table). Sections were analyzed using a Stemi stereomicroscope or AxioImager (Zeiss, Oberkochen, Germany) fitted with an AxioCam MRc5 digital camera. Images were captured using AxioVision software (Zeiss) and assembled in Adobe Photoshop.

Layer Distribution Analysis
To quantify the distribution of neurons, anatomically matched sections (The Mouse Brain Atlas [47]) were selected. The postnatal neocortex was divided radially into 5 equal-sized bins from the pia to the upper edge of the white matter. The cells in each bin were quantified and reported as the percentage of total cells counted.

Quantitative Analysis
Data were analyzed by two-tailed Student’s t-tests with a significance level of at least P < 0.05 for all statistical comparisons. Numbers of replicates are given in the main text or Fig legends.

Labeling of callosal projections using DiI
Embryonic or postnatal mouse brains were fixed, respectively, by immersion in or intracardial perfusion with 4% PFA. Labeling of callosal projection neurons was achieved by placing DiI crystals (Molecular Probes, Life Technologies) in the cingulate or somatosensory cortex. The brains were returned to 4% PFA, placed in light-tight containers, and incubated at 37°C or at room temperature for 3–5 weeks. The brains were then embedded in 3% agarose and sectioned using a vibratome (Leica Microsystems).

RNA sequencing
Embryonic brains (E17.5) were dissected in ice-cold Hank’s balanced salt solution (HBSS) (without Ca²⁺ or Mg²⁺) supplemented with 0.5% D-glucose and 25mM HEPES, the meninges removed and the neocortex microdissected. RNA was isolated by Trizol and purified with the RNeasy kit (Qiagen, Valencia, CA). The quality of the RNA was evaluated by A260/A280 ratio, and by electrophoresis on an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). RNA was amplified using the Ovation RNA-Seq system (NuGEN, San Carlos, CA) according to the manufacturer’s protocol. Briefly, mRNA was isolated from total RNA using oligo dT on magnetic beads. The mRNA was then fragmented at 94°C and converted into double stranded cDNA. Following polishing of the cDNA ends and addition of adenine bases at the 3’ ends, specific adaptors supplied by Illumina were ligated. The adaptor ligated DNA was amplified by 15 cycles of PCR. The amplified DNA was then purified on the Qiagen PCR purification kit. The insert size and DNA concentration of the sequencing library were determined on an Agilent Bioanalyzer. Following cluster generation via isothermal
solid support bridge amplification, one lane of single-read sequencing was performed for each experiment at a read length of 74 base pairs on the Genome Analyzer IIx (Illumina) according to the manufacturer’s protocol. Image analysis and base calling were performed by the Illumina pipeline version 1.5 with default parameters, installed on the Yale High Performance Computing Cluster. Cluster generation and error rates were also evaluated using the pipeline. We next aligned reads to the mouse genome, Mus musculus MM9, using Bowtie [48] as implemented in TopHat [49]. The splice junctions were mapped by TopHat by using the pre-built splice junction library provided by the authors. We used Cufflinks [50] to assemble transcripts using RefSeq as the reference list.

Results

Expression of CCK receptors in mouse brain is developmentally regulated

The diverse physiological functions of CCK and its receptors in adult brain, prompted us to investigate whether they also played a role during development. We sought to determine the mRNA expression of the two receptors, as all previous studies of CCK receptor distribution were based on binding assays [13–18]. Contrary to its being considered the peripheral receptor, we discovered robust and widespread expression of Cckar mRNA in the developing brain by in situ hybridization from at least embryonic day (E) 13.5 onward, whereas Cckbr transcripts were not detected until late embryonic stages (Fig 1A and 1B). Confirming these observations, transcriptome analysis of normal mouse neocortex at two different embryonic stages (E13.5 and E17.5) demonstrated opposite trends in expression levels of Cckar (high, early vs. low, late) and Cckbr (low, early vs. high, late) during embryogenesis (our unpublished data). Cck is expressed in select embryonic cell populations (Fig 1C), previously shown to include tangentially migrating interneuron precursors [51]. By the second postnatal week, however, Cckbr is robustly upregulated in the neocortex, as is Cck, whereas Cckar is downregulated in the neocortex and becomes restricted to the hippocampus and select extra-cortical areas (Fig 1F–1H). In contrast, in human fetal brain (20 gestational weeks), we detected expression of CCKBR as well as CCK, but not of CCKAR (Fig 1D and 1E), extending to human previous reports of species-specific differences in receptor expression. CCKBR and CCK (but, again, not CCKAR) were also expressed in human adult frontal and temporal cortices (Fig 1I and 1J). Analyses of Cckar and Cckbr expression trajectories across neocortical layers of postnatal mouse brain over time ([52] and http://hbatlas.org/mouseNCXtranscriptome/) and of CCKAR and CCKBR exon array signal intensity in human brain across development and adulthood ([53–55] and http://hbatlas.org/) were both in agreement with our experimental findings (the corresponding graphs can be retrieved using the web links above and are also shown in S1 Fig).

These observations establish that the two CCK receptors are dynamically expressed, displaying distinct and nearly mutually exclusive patterns in embryonic and postnatal mouse brain, and are in agreement with previous reports that interneuron precursors and some cortical pyramidal neurons express Cck [21, 22, 51]. Considered together with the robust expression of Cck and the relatively mild or absent anatomical defects, respectively, of Cckar and Cckbr mutant brains, these findings also suggested a possible synergy in CCK receptor action in embryonic and early postnatal life, prompting us to analyze brain development of Cckar/Cckbr double mutant mice.
Defects in cortical development and axonal connectivity in mice lacking CCKAR and CCKBR

The Cckar/Cckbr double homozygous mutant mice (JAX 129-Cckar<sup>tm1Kpn</sup>Cckbr<sup>tm1Kpn</sup>/J; henceforth Cckar/br mutants), are viable and fertile, and have no obvious physical abnormalities despite their lacking functional CCK receptors [17, 33]). To examine gross brain cytoarchitecture in Cckar/br mutant mice we analyzed cortical organization at late embryonic stages and up to weaning (P21), by Nissl staining, revealing a spectrum of structural abnormalities, including expansion and thickening of the cingulate/retrosplenial cortex and lateral displacement and compression of the hippocampus (Figs 2A, 2B and S4 Fig). Despite the selective enlargement of the cingulate cortex, the ventral and lateral cortical regions retained normal appearance, without a mediolateral shift of sensory cortical areas. Nissl staining also revealed agenesis of the corpus callosum, the largest commissural tract in the vertebrate brain that coordinates information between the two cerebral hemispheres (Fig 2A and 2B). In situ hybridization with claudin 11, expressed in myelinated oligodendrocytes, including those in white matter tracts [56, 57], confirmed failure of callosal axons to cross (Fig 2C and 2D). However, other major forebrain tracts such as the anterior, ventral hippocampal, and posterior commissures appeared normal, suggesting that commissural tracts were not globally affected (S2 Fig).

The differentiation and establishment of the corpus callosum depends on proper development of the dorsal midline, generation of callosal projection neurons (CPN) and their axons, axonal targeting and growth, and target localization and innervation in the contralateral hemisphere [58]. Agenesis of the corpus callosum is often associated with defects in CPN migration and/or specification [59–61], but can also result from alterations in cortical laminarization. To rule out the latter possibility, we analyzed molecular markers of cortical layers (L) [including Ctgf...
Tle4 (L6, subplate); Er81/Etv1 (L5); Rorb (L4); and Cux2 (L2-4)] by in situ hybridization of representative sections of the entire neocortex, and concluded that layer formation and area patterning were grossly normal in Cckar/br mutants, however a possible reduction of a subset neocortical neuronal subtypes cannot be excluded at this point and warrants further investigation (Fig 3A–3J). In addition, in situ hybridization with Tag1 (marker of commissural neurons) [62–64], Satb2 (marker of CPN general identity) [59, 60] and Lmo4 (expressed in CPN intermediate progenitors) [65, 66] between E14.5 and E17.5 revealed that CPN are generated in Cckar/br mutants (S3 Fig). Therefore, neither cortical layering nor failure of CPN generation appears to account for the agenesis of the corpus callosum in the Cckar/br mutants.

Callosal axons originating in the neocortex represent the bulk of the corpus callosum, however, they depend on pioneering axons from the cingulate cortex, which pave the way by inner-vating the homotypic cingulate cortex in the contralateral hemisphere at E14-E15 [67–69]. Because the cingulate cortex is abnormal in the Cckar/br mutants, we examined medial cortical

![Figure 2. Defects in cortical development in Cckar/br mutant mice.](image-url)

(A and B) Nissl staining of coronal sections of control and Cckar/br mutant brains at postnatal day 14 (P14) reveals thickening of the cingulate cortex, agenesis of the corpus callosum, and lateral displacement of the hippocampus. (C and D) In situ hybridization with claudin 11, which is expressed in myelinated oligodendrocytes thus highlighting white matter tracts, shows that the commissural axons of the corpus callosum do not cross to the contralateral hemisphere in Cckar/br mutants (D) compared to control (C). Of a total of 16 brains from Cckar/br mutants that were fully sectioned and analyzed for callosal development, 13 had complete and 3 had partial agenesis of the corpus callosum. Scale bar, 100 μm. (E and F) Labeling of callosal projections with DiI shows that colossal axons fail to cross the midline in Cckar/br double mutants (F). Green lines (in E,F) and asterisk (in F) indicate the midline and Probst bundles, respectively. Scale bar, 200 μm.

doi:10.1371/journal.pone.0124295.g002
projections with Dil labeling at embryonic (E14.5 and E16.5) and early postnatal stages (P4) to view, respectively, the trajectory of pioneering and callosal axons. Pioneering axons were seen

Fig 3. Normal cortical lamination in Cckar/br mutant mice. (A-J) In situ hybridization with cortical layer specific markers in control (A,C,E,G,I) and Cckar/br (B,D,F,H,J) littermates at P14. Ctgf (A,B): subplate; Tle4 (C,D): L6, subplate; Er81 (E,F): L5; Rorb (G,H): L4; Cux2 (I,J): L2-4. Scale bar, 0.5 mm.

doi:10.1371/journal.pone.0124295.g003
navigating towards the midline in both control and Cckar/br mutant embryos, suggesting that pioneering cingulate neurons were present; furthermore, callosal axons projected towards the midline, however, they failed to cross to the contralateral hemisphere in the mutants following instead aberrant trajectories and forming Probst bundles [70] on either side of the midline (Fig 2E and 2F; see also Figs 4H and 6D). Together, these observations suggest that although CPN neurons are generated, their axons fail to innervate the contralateral hemisphere, prompting us to investigate whether structural defects at the midline, including failure of midline fusion, might explain callosal agenesis in the Cckar/br mutants. We thus examined the differentiation of midline glia (glial wedge, midline zipper glia and induseum griseum), cell populations that have been implicated in callosal axon guidance [58, 71–73], as well as of the subcallosal sling (formerly known as “glial sling”), a transient structure that is comprised of migratory neurons [74, 75] and is necessary to bridge the gap between the two hemispheres providing a substrate onto which callosal axons grow towards the contralateral hemisphere.

Expression of Gfap, a marker of midline glia at perinatal stages, was significantly reduced specifically in the induseum griseum and the midline zipper glia at E17.5 and in neonatal (P0, P1) Cckar/br mutants, compared even with double heterozygous controls; expression at the glial wedge, was also reduced (Fig 4A–4F). These observations suggested abnormalities with midline differentiation. Examination of serial sections from an entire brain stained for Gfap expression indicated that in Cckar/br double mutants, migration of Gfap-expressing cells towards the midline was clearly reduced compared with controls, as were the size and rostrocaudal extent of the induseum griseum and midline zipper glia. Midline defects were also evident in neonatal brains by in situ hybridization with F-spondin [76] and Tag-1 [62] markers of commissural neurons (Fig 4G and 4H). Moreover, the differentiation of the subcallosal sling, visualized by staining with NeuN, and also examined by morphology on Nissl preparations, was abnormal in the Cckar/br double mutants. Indeed, a continuous sling failed to transverse the gap between the two hemispheres, as also indicated by significantly reduced Tag1 expression, marking the sling [Wolfer, 1994 #261], in Cckar/br mutants at P0 (Fig 4G and 4H). These observations suggest that midline differentiation was abnormal in the Cckar/br mutants and that the delayed development/misspecification of the midline zipper glia and the induseum griseum, together with the failure of the subcallosal sling to bridge the gap between the two hemispheres are the likely causes of callosal agenesis.

Different guidance mechanisms are in place in the environment of the callosal trajectory [58] and several chemoattractant and chemorepellant cues are crucial for proper development of the corpus callosum [77–83]. We thus examined the expression of various guidance molecules to establish whether callosal axons were prevented from crossing owing to cell non-autonomous defects in the Cckar/br mutants. We did not detect differences in expression of the chemorepellent Slit2 in glial wedge or the induseum griseum [78], or of multiple Eph receptors (EphB1, EphB3, EphA4 or EphA5) that regulate midline crossing of callosal fibers [84, 85], or Sema3f [82] (not shown). Taken together these observations suggest that abnormal patterning or differentiation of the midline may be the underlying cause of corpus callosum agenesis in the Cckar/br mutants.

Defects in corpus callosum development in mice lacking CCKAR and CCKBR

To assess the relative contributions of each receptor to the formation of the corpus callosum, we examined Cckar and Cckbr single mutants, which we generated by crossing the Cckar/br mutants (maintained in the 129/J background) to 129/SvJ wild type mice in order to maintain the mutations in a uniform genetic background, thus eliminating genetic variability known to
influence corpus callosum development as well as to control for sporadic occurrence of callosal agenesis in some strains [86, 87]. We analyzed brain sections through the entire anterior/posterior extent of the corpus callosum between birth and P21 by Nissl staining, and for each genotype, we scored brains displaying complete (failure of all callosal axons to cross the midline) or partial (at least part of the tract could be identified) agenesis. The Cckar/br mutants (n = 16) had either complete (n = 13) or partial (n = 3; in these, at least some callosal axons could be

Fig 4. Abnormal differentiation of the midline in Cckar/br mutant embryos. (A-F) In situ hybridization with Gfap, a marker of midline glia at perinatal stages, reveals that two midline cell populations, the induseum griseum (IG) and midline zipper glia (mzg), are reduced in Cckar/br double mutants (D-F) compared even with Cckar<sup>+/+</sup>;Cckbr<sup>+/+</sup> double heterozygous embryos (A-C) at E17.5. Expression of Gfap at the glial wedge (gw) does not appear to be affected to the same extent, suggesting defective differentiation of the midline. Scale bar, 200 μm. (G and H) In situ hybridization with Tag1, a marker of commissural neurons, which also marks the subcallosal sling (scs), demonstrates that a continuous sling is present in control Cckbr<sup>+/+</sup> pups (G) at P0, but it fails to transverse the gap between the two hemispheres in Cckar/br mutants (H). Scale bar, 200 μm. (I-N) In situ hybridization demonstrates a modest increase in Cckbr transcripts in the ventral hypothalamus of Cckar single mutants (J,L,N) compared with wild type (I,K,M) at P14. The sections shown are at three different rostro-caudal levels. Scale bar, 0.5 mm.

doi:10.1371/journal.pone.0124295.g004
seen crossing anteriorly, but none did so at posterior levels) agenesis of the corpus callosum. On the other hand, almost all (9 out of 10) Cckar homozygous single mutants had normal corpus callosum with the remaining showing complete agenesis, whereas Cckbr homozygous single mutants (n = 9), as well as wild type 129/SvJ mice (n = 7), all had normal corpus callosum. The cytoarchitecture of the cingulate cortex and hippocampus was normal in both single mutants. These observations suggest functional synergy of CCKAR and CCKBR in cingulate cortex and corpus callosum development, possibly with a more important contribution of CCKAR, and, likely, functional compensation between the two receptors in the formation of the major forebrain commissural track. In support of this notion, we detected a modest but reproducible increase of Cckbr expression by in situ hybridization in the ventromedial hypothalamus of Cckar single mutants; any increase in the neocortex would be masked by the already robust Cckbr expression in this region (Fig 4I–4N; see also Fig 1G).

Neuronal migration defects in Cckar/br mutants

In light of the synergistic action of the receptors and the migration defects of neuroendocrine neurons and of olfactory bulb interneurons previously reported in Cckar single mutants [43, 44, 46, 88], we reasoned that the Cckar/br double mutants would also have neuronal migration abnormalities. We noticed that the rostral migratory stream (RMS), observed on Nissl stained coronal and sagittal brain sections, had abnormal morphology and was severely thickened in the Cckar/br mutants (S4 Fig), as was the subventricular zone (SVZ) (S4 Fig). Quantitative analyses revealed that the volume of the RMS (P14: control, 0.356 +/- 0.024; Cckar/br, 1.41575 +/- 0.19876; n = 3; p = 0.02379 and P21: control, 0.37083 +/- 0.040819; Cckar/br, 0.63075 +/- 0.11605; n = 3; p = 0.03807) was significantly increased in the Cckar/br mice compared with controls, suggesting that the rostral migration of neuroblasts was affected, consistent with previous findings of migration defects in adult olfactory bulb interneurons in Cckar single mutants.

As shown in Fig 3, we did not detect any obvious defects in cortical lamination suggesting that migration of cortical pyramidal neurons was grossly normal. In contrast, we observed a noticeable reduction of cortical interneurons, which are generated in the medial and caudal ganglionic eminences (respectively, MGE and CGE) of the embryonic ventral forebrain and migrate tangentially into the cortex, where they assume their final laminar positions by radial migration [89–91]. Analysis of Gad1 (a.k.a. Gad67, a general interneron marker encoding glutamate decarboxylase 1, an enzyme involved in GABA biosynthesis), Lhx6 (a marker of MGE-derived interneuronal lineages [92, 93]) and reelin (a marker of CGE- and pre-optic area [POA]- derived interneurons [91, 94]) at P7 indicated a significant overall reduction in Lhx6+ and reelin+ cortical interneurons in the Cckar/br mutants (Fig 5A–5F). Quantification of Lhx6-expressing cells at P7 (n = 3) indicated a 26% reduction (control: 179.3±7.839; Cckar/br: 133.3±8.373; p = 0.0160) in MGE-derived cortical interneurons in the mutants compared with controls (Fig 5G). However, their laminar allocation was not grossly affected (Fig 5H), indicating that those interneurons reaching the neocortex, were indeed able to respond to local guiding cues controlling their final position. Furthermore, quantification of reelin-expressing cells at P7 (n = 4) indicated a 15% reduction (control: 98.75±4.289; Cckar/br: 84.75±1.652; p = 0.0226) in CGE- and POA-derived interneurons in the Cckar/br mutants, compared to controls (Fig 5I). On the other hand, striatal cholinergic interneurons, which are born in the basal forebrain but do not migrate into the cortex, were generated in Cckar/br mutants in numbers comparable to wild type, as indicated by quantification of Lhx8-expressing cholinergic interneurons [95, 96] in a defined area of the striatum at P0 (control: 159.3±7.78; Cckar/br: 168.1 ±12.51, p = 0.55971) and at P7 (control: 93.9±5.55; Cckar/br: 105.2±9.66, p = 0.3238) (S5 Fig).
A reduction in cortical interneuron number could be due to cellular malfunctions at the site of origin (e.g. fewer interneuron progenitors in the MGE/CGE), abnormal tangential migration, or failure to survive within the cortex. We examined the $Cckar/br$ mutants at E14.5 (a day

**Fig 5. Neuronal migration defects in Cckar/br mutants.** (A-F) In situ hybridization with markers of cortical interneurons at P7: *Gad1* (A,B) (a general interneuron marker); *Lhx6* (C,D) (a marker of MGE-derived interneurons); and *reelin* (E,F) (a marker of CGE- and pre-optic area derived interneurons) indicates a significant overall reduction of interneurons in $Cckar/br$ mutants, irrespective of their origin. Scale bar, 200 μm. (G) Quantification of *Lhx6*-expressing interneurons. $Cckar/br$ mutants have a 26% reduction in MGE-derived cortical interneurons at P7 (p = 0.0160, n = 3). (H) Distribution of *Lhx6*-expressing interneurons. Despite the reduction in their number, cortical interneurons are distributed in a similar fashion in control and $Cckar/br$ mutant neocortex, suggesting that interneurons that successfully completed tangential migration are able to respond to local cues in the neocortex and settle into their final position. The fraction of *Lhx6*-positive interneurons per bin is similar in control and $Cckar/br$ mutant neocortices (p is at least > 0.15 for all comparisons, n = 3). (I) Quantification of *reelin*-expressing interneurons. $Cckar/br$ mutants have a 15% reduction in CGE- and POA-derived cortical interneurons at P7 (p = 0.0226, n = 4). Error bars represent s.e.m.

doi:10.1371/journal.pone.0124295.g005
after the peak of interneuron progenitor generation in the MGE [97]), when tangential migration into the cortex is underway [98, 99]. In situ hybridization with Gad1 (A,B) and Lhx6 (E,F), expressed at this stage by MGE-derived progenitors and post-mitotic migrating interneurons [89, 100], suggested that fewer interneurons were engaged in tangential migration (Fig 6A,6B,6E and 6F).

Tangentially migrating interneurons employ two routes into the cortex, one superficial within the marginal zone and one deep at the subplate/SVZ interphase [101]. Lhx6 and Gad1

Figure 6. Defects in tangentially migrating interneurons in Cckar/br mutants. (A,B,E,F) Intereuron progenitors are generated normally in Cckar/br mutant embryos compared to controls, as demonstrated by in situ hybridization with Gad1 (A,B) and Lhx6 (E,F). However their tangential migration appears delayed in the mutants. Arrows in (A,B) indicate the onset of tangential routes employed by migrating interneurons. Note a delay/reduction in tangential migration in Cckar/br mutants. Red marks in (E,F) indicate the extent of tangential migration (deep route) in the neocortex. Scale bar (B,D), 200 μm. (C,D,G,H) In situ hybridization with Gad1 and Lhx6 at birth (P0, C,D) or late embryogenesis (E17.5, G,H) demonstrates that despite the delay in migration, a majority of interneurons settle into the neocortex. Scale bar (F,H), 200 μm.

doi:10.1371/journal.pone.0124295.g006
expression indicated that both routes were disrupted; the deep route appeared not as focused, and did not extend as far dorsally as in control littermates, and fewer migrating interneurons employed the superficial route into the cortex at this stage. Despite this migration delay, interneurons did reach the neocortex at later stages (Fig 6C, 6D, 6G and 6H), albeit in lower than normal numbers (Fig 5G and 5I).

Our findings suggest that tangential migration of interneurons into the cortex is partially disrupted in the Cckar/br mutants. For tangential migration to occur, different classes of interneurons respond to a variety of molecular cues via guidance receptors, the expression of which is under region-specific transcriptional control [89, 99]. Neuregulin-1, a short- and long-range chemoattractant, at E17.5 is expressed in the cortex and migrating interneurons express its receptor ErbB4 [102, 103]. Migrating MGE-derived interneurons expressed ErbB4 in both Cckar/br and controls at E15.5 and E17.5, and we failed to detect any differences in Nrg1 expression in the cortex between control and Cckar/br double mutants at E15.5 or E17.5 (S6 Fig). These observations suggest the abnormal tangential migration we observed cannot be explained by disruption in ErbB4 expression. The molecular factors controlling the tangential migration of interneurons originating in the CGE and pre-optic area remain to be defined, preventing detailed examination.

### Comparative transcriptional profiling offers insight into the brain abnormalities of Cckar/br mutant mice

To gain mechanistic insight into CCK receptor signaling and determine gene targets associated with CCK action, we performed comparative transcriptome analysis of Cckar/br vs. wild type control neocortical tissue at E17.5. We reasoned that RNA-Seq at this stage would be potentially informative for both developmental processes that are disrupted in the mutants, as at E17.5, the formation of the corpus callosum as well as tangential migration of interneurons are still underway, the latter nearing completion. The analysis was performed on two sets of biological triplicates (RNA Integrity Number [RIN] for control samples: 9.7, 9.6, 9.7 and for Cckar/br samples: 9.8, 9.8, 9.7). After mapping the RNA-seq data, we used pathway analysis (http://www.ingenuity.com/) to determine which biological pathways were differentially modulated in normal and Cckar/br mutant neocortex. As would be expected in the absence of functional CCK receptors, CCK-mediated signaling was significantly downregulated [-log(p-value): 6.64E-01; ratio: 2.83E-02], as were the ERK/MAPK signaling pathway [4.74E-01, 1.96E-02], which is known to control cell proliferation and migration induced by CCKBR and the transcriptional regulation of gastrin-sensitive genes, but also protein translation induced by CCKAR in several cellular contexts [104], the CXCR4 signaling pathway [6.45–01, 2.37E-02], and the BMP signaling pathway [4.98E-01, 2.5E-02] (Table 1). Two additional pathways were strongly affected: glutamate receptor signaling [2.68E00, 7.25E-02] and axonal guidance signaling [2.39E00, 3.24E-02] (Table 1). Within these pathways, we identified a number of candidate genes whose expression was modulated in Cckar/br mutants compared with controls, that could, on the basis of their known roles in brain development, provide a potential mechanistic explanation for the phenotypes we observed (Table 2). We selected for further study and validation three transcripts, two of which were significantly downregulated in the Cckar/br mutants, Cxcl12 (fold change: -2.104, p = 6.14E-08) and Bmp7 (fold change: -2.092, p = 2.13E-03), and one, Nrp2, that was upregulated, (fold change: 1.510, p = 3.29E-04). We note that Grin3a and Grin2b, encoding subunits of the NMDA receptor were also found to be upregulated (respectively, 2.033, p = 5.97E-04 and 1.707, 8.71E-04), but were not experimentally validated (Table 2; see Discussion).
Cxcl12 (previously called Sdf1) encodes a chemokine that is a known attractant for interneurons facilitating their migration through both the superficial and deep routes and their final laminar distribution [105–112]. CXCL12 is expressed by the meninges adjacent to the marginal zone (the superficial route), and by intermediate progenitor cells in the SVZ (the deep route). Indeed, in situ hybridization demonstrated that Cxcl12 was expressed at lower levels in the SVZ of the Cckar/br mutants compared with double heterozygous animals; expression in the meninges was not affected (Fig 7A–7D).

Bmp7, the second transcript we investigated, encodes a secreted protein recently implicated in modulating callosal axon outgrowth via its participation in a signaling cascade from the meninges [113] as well as in the timely differentiation of midline “guidepost” cells involved in corpus callosum formation [114]. In Cckar/br mutants at E15.5 and E17.5, Bmp7 was downregulated in the induseum griseum and the glial wedge (Fig 7E and 7F), providing a plausible explanation for the midline defects in the Cckar/br mutant mice.

Our comparative transcriptome analysis further indicated that Nrp2 (Neuropilin 2), encoding a receptor for class III semaphorins, was more abundant in the Cckar/br mutants compared with controls. The neuropilin receptors, and their ligands, semaphorins, regulate repulsive axon guidance as well as neuronal cell migration [115]. Indeed, Nrp2 was upregulated in the midline of Cckar/br mutant embryos at E14.5-E17.5, especially in the induseum griseum, whose orientation was further distorted (Fig 7G–7L). Nrp2 is also expressed by tangentially migrating interneurons [99], and not surprisingly, we detected somewhat lower levels of expression of Nrp2 in these cells, which are migrating in smaller than normal number in the Cckar/br mutants, again indicating defects at the palial-subpallial boundary and in the deep migratory route along the subplate/SVZ interphase (S7 Fig).

Thus, we were able to experimentally validate findings of RNA-Seq and gain insights into the potential mechanisms underlying the structural abnormalities documented in the Cckar/br mutant brains. These observations highlight the power of comparative transcriptome analysis of mutant vs. control tissues in understanding biological function.

---

Table 1. Pathway analysis (Ingenuity.com) of RNA-Seq data.

| Pathway                  | -log(p-value) | ratio   |
|--------------------------|---------------|---------|
| Glutamate Receptor Signaling | 2.68E+00     | 7.25E-02|
| Axonal Guidance Signaling | 2.39E00       | 3.24E-02|
| CCK/gastrin Signaling     | 6.64E-01      | 2.83E-02|
| CXCR4 Signaling           | 6.45E-01      | 2.37E-02|
| BMP Signaling Pathway     | 4.98E-01      | 2.5E-02  |
| ERK/MAPK Signaling        | 4.74E-01      | 1.96E-02|

doi:10.1371/journal.pone.0124295.t001

Table 2. Select transcripts identified by comparative transcriptional profiling.

| Transcript | Fold change Down-regulated in Cckar/br | p-value  |
|------------|----------------------------------------|---------|
| Cxcl12     | -2.104                                 | 6.14E-08|
| Bmp7       | -2.092                                 | 2.13E-03|
| Nrp2       | Up-regulated in Cckar/br               |         |
| Grin3a      | 1.510                                  | 3.29E-04|
| Grin2b      | 2.033                                  | 5.97E-04|
|            | 1.707                                  | 8.71E-04|

doi:10.1371/journal.pone.0124295.t002
Discussion

Here we demonstrate that the CCK receptors are critical regulators of mammalian brain development. Constitutive loss of functional CCKAR and CCKBR, but not of either receptor alone, leads to midline structural anomalies in the developing brain resulting in agenesis of the corpus callosum and further impacts on tangential migration of cortical interneurons. These findings assign a new and previously unappreciated role to the CCK system in the development of the mammalian brain and suggest that the two receptors, whose expression is developmentally regulated and nearly complementary in embryonic and postnatal brain, display functional synergy and can compensate for one another.

The analysis of Cckar/br double mutant mice was prompted by our intriguing observations that one hand, CCKAR, long thought to be the peripheral receptor for CCK, is widely expressed in the embryonic mouse brain, as demonstrated by in situ hybridization and transcriptome analyses and on the other, that the expression of the two receptors is developmentally regulated with opposite trends, considered together with the relatively mild to even absent defects of each single mutant. The two receptors, which may be transiently co-expressed in some cells during late embryonic and early postnatal development, appear to act synergistically and to
compensate for one another in certain contexts, e.g. in corpus callosum formation (this study) and in satiety control, as reported recently [116]. Contrary to the Cckar/br mutants, the Cck mutants apparently lack remarkable structural brain phenotypes (but do exhibit behavioral abnormalities related to satiation and control of food intake) [Lacourse, 1999 #41; Lo, 2008 #44; Hannibal, 2010 #235; Lo, 2012 #233]. Cck mutant mice do not upregulate gastrin (the peripheral ligand of the CCK receptors) in the brain, and Cck/gastrin double mutants have only been examined for (normal) pancreatic morphology [Lacourse, 1999 #41]. Despite these reports, however, whether brain development has been analyzed in any detail in the Cck mutants remains unclear. Should it be normal, however, the intriguing possibility of an additional ligand for these receptors in the brain should be investigated.

Our findings support and extend previous observations that correct establishment and patterning of the midline are essential for the formation of the corpus callosum [58, 61]. The lack of structural integrity of the midline, the distorted orientation and smaller size of the induseum griseum, and the reduction in size of other midline "guidepost" cell populations, including the subcallosal sling, considered together with the downregulation of Bmp7 in the glial wedge and induseum griseum, suggest that abnormal differentiation of the midline is the most likely cause of callosal agenesis. BMP7 has indeed been implicated in callosal development [Sanchez-Camacho, 2011 #164] [Choe, 2012 #258]: genetic manipulation of BMP7 levels specifically in the meninges, normally expressing and secreting this molecule, either prevents callosal axons from crossing (when in excess), or leads to a larger corpus callosum (when reduced). BMP7 is also required for the proper differentiation of midline glia; in Bmp7 null mutants these are defective, and the corpus callosum does not form. Considered together with our findings, these observations suggest a fundamental role of BMP7 in the formation of the corpus callosum. Our finding that other forebrain commissures are not disrupted in Cckar/br mice also suggests that cell non-autonomous mechanisms (such as the delayed development of the midline), rather than cell intrinsic defects of commissural neurons, are responsible for callosal agenesis. Thus, the Cckar/br mutants must now be added to the list of several mouse models with midline defects that have callosal phenotypes of varying penetrance [61]. The CCK receptors should also be evaluated as candidates for human syndromes of callosal agenesis for which a causative gene has not been identified.

Our findings also demonstrate neuronal migration abnormalities in the Cckar/br mutant mice. Previous analyses of Cckar single mutants indicated defects in two cell populations with opposing migratory routes: migration of neuroendocrine neurons that secrete gonadotrophin-releasing hormone (GnRH) [43] and fewer neuroblasts giving rise to adult olfactory bulb interneurons [44, 46]. GnRH-secreting neurons originate in the nasal compartment and migrate in association with the olfactory nerve to enter into the forebrain and reach their final destinations in the hypothalamus [117, 118]. Conversely, olfactory bulb interneurons originate in the adult SVZ and migrate via the RMS to the olfactory bulb, where they integrate into its circuitry [119]. The increase in thickness of the RMS suggests that olfactory bulb interneuron migration is affected in the Cckar/br mutants. Previous findings of concomitant impairment of corpus callosum formation and neuroblast migration from the SVZ to the olfactory bulb in Cdk5 conditional mutant mice led to the suggestion that agenesis of the corpus callosum may interfere with the environment of the SVZ, thus contributing to the neuroblast migration defects [120], a hypothesis that also seems plausible for the Cckar/br mutants analyzed in this study.

We further observed that tangential migration of interneurons from their birthplace in subcortical areas towards the neocortex was delayed in the Cckar/br double mutants, and, consequently the number of neocortical interneurons was significantly reduced. The consequences of CCK receptor loss appear to be more pronounced in the subpopulation of interneurons migrating along the superficial route. MGE- and CGE- and POA-derived interneurons
(respectively characterized by Lhx6 and reelin expression) appear to be affected, suggesting that the defect is not specific to CCK-expressing neocortical interneurons, which are thought to derive from the CGE [28, 121, 122]. Although the molecular mechanisms guiding tangential migration of CGE-derived interneurons remain to be elucidated [91], we propose that the lower levels of Cxcl12 expression that we detected in SVZ progenitors contribute to rendering the Cckar/br neocortex less attractive to tangentially migrating interneurons, regardless of their origin. Interestingly has been reported that the migration of GnRH neurons to the ventral forebrain, which is modulated by CCK and defective in Cckar single mutants [43], requires CXCL12/CXCR4 signaling [123]. Therefore, the CXCL12/CXCR4 signaling perturbations suggested by our study may underlie defects in several migrations over a distance in the Cckar/br mutants, including that of GnRH neurons [43] and cortical interneurons (this study).

Considered together, these defects not only highlight the important role of CCK-mediated signaling in the brain, but also have intriguing parallels. In the absence of functional CCK receptors, commissural axons fail to cross the midline to form the corpus callosum (this study); fewer cortical interneurons that have to travel across the pallial-subpallial boundary, enter the neocortex (this study) and fewer olfactory bulb interneurons reach their targets [44, 46, 88]; yet, more peripherally generated neuroendocrine neurons cross into the forebrain [43]. It appears therefore that CCK signaling is involved in regulating midline crossing by commissural axons and migratory travel across boundaries, two processes that have indeed been proposed to be analogous [91].

Comparative transcriptome analysis led to the identification of two significantly downregulated transcripts in the Cckar/br mutant neocortex, encoding the chemokine CXCL12, a known attractant for tangentially migrating interneurons [106, 108–111], and the secreted factor BMP7, which was recently implicated in corpus callosum formation [113, 114]. Both were validated in vivo, and their modulation helps explain two of the phenotypes we detected in the Cckar/br mutant mice. A third transcript, Nrp2, which we also validated in vivo, is upregulated in the midline of the mutants, where it could be acting as a repellant to guide cingulate pioneer axons [82]. Interestingly, Nrp2, acting as an attractant, has also been implicated in the migration of GnRH neurons [124], and therefore, Nrp2 upregulation may explain the increase in GnRH neurons in the Cckar mutants [43]. Finally, Grin3a and Grin2b, encoding subunits of the NMDA receptor, were upregulated in the Cckar/br mutants (this observations was not validated experimentally). Both are “juvenile” NMDA receptor subtypes that are developmentally replaced with “mature” subtypes during postnatal periods of activity-dependent rearrangement of synaptic connectivity, a developmental switch of functional importance [125–129]. An interplay of CCK signaling and NMDA receptor has been reported in several contexts, including food intake and anxiety behavior [130–132]; furthermore, CGE-derived CCK interneurons have a distinct NMDA subunit composition [133]. For all these reasons, the modulation of expression levels of juvenile NMDA receptor subtypes suggested by comparative transcriptomics, warrants further investigation. Our findings thus underscore the power of deep transcriptional profiling as a novel and fertile genomic approach in the identification of downstream targets of CCK signals and the elucidation of the mechanisms underlying CCK receptor function.

In conclusion, we document an important and additive (synergistic?) role of the two CCK receptors in the formation of the corpus callosum and tangential migration of cortical interneurons, highlighting a developmental role for CCK receptor signaling in the mammalian brain.

**Supporting Information**

S1 Fig. Developmental expression trajectories of Cckar and Cckbr in mouse and human brain (data retrieved from Brain Atlas; [http://hbatlas.org/mouseNCXtranscriptome/](http://hbatlas.org/mouseNCXtranscriptome/) and
http://hbatlas.org/). Abbreviations: SgL (subgranular layer); L4 (layer 4); IgL (infragranular layer); NCX (neocortex); HIP (Hippocampus); AMY (amygdala); STR (striatum); MD (mediodorsal nucleus of the thalamus); CBC (cerebellar cortex).

S2 Fig. Formation of anterior (ac), and posterior (pc) commissures.

S3 Fig. In situ hybridization with Satb2 and Lmo4.

S4 Fig. Morphology of the rostral migratory stream (RMS) and subventricular zone (SVZ). (A,B) are sagittal sections (anterior to the left); (C-I) are coronal sections.

S5 Fig. In situ hybridization with Lhx8.

S6 Fig. In situ hybridization with Nrg1 and ErbB4.

S7 Fig. In situ hybridization with Nrp2.

S1 Table. Primers for in situ hybridization.

Author Contributions

Conceived and designed the experiments: AL. Performed the experiments: SN KI AL. Analyzed the data: SN KB MG AL. Contributed reagents/materials/analysis tools: NS. Wrote the paper: AL.

References

1. van den Pol AN. Neuropeptide transmission in brain circuits. Neuron. 2012; 76(1):98–115. doi: 10.1016/j.neuron.2012.09.014 PMID: 23040809

2. Ivy AC, Oldberg E. A hormone mechanism for gallbladder contraction and evacuation. Am J Physiol. 1928; 86:599–613.

3. Vanderhaeghen JJ, Signeau JC, Gepts W. New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature. 1975; 257(5527):604–5. PMID: 1165787

4. Lee SY, Soltesz I. Cholecystokinin: a multi-functional molecular switch of neuronal circuits. Dev Neurobiol. 2011; 71(1):83–91. doi: 10.1002/dneu.20815 PMID: 21154912

5. Rehfeld JF. The endoproteolytic maturation of progastrin and procholecystokinin. J Mol Med. 2006; 84(7):544–50. PMID: 16680481

6. Rehfeld JF, Friis-Hansen L, Goetze JP, Hansen TV. The biology of cholecystokinin and gastrin peptides. Curr Top Med Chem. 2007; 7(12):1154–65. PMID: 17584137

7. Noble F, Roques BP. Cholecystokinin peptides in brain function. In: Lajtha A, Lim R, editors. Hand- book of neurochemistry and molecular neurobiology. New York: Springer; 2006. p. 545–71.

8. Zwanzger P, Domschke K, Bradwejn J. Neuronal network of panic disorder: the role of the neuropeptide cholecystokinin. Depression and anxiety. 2012; 29(9):762–74. doi: 10.1002/da.21919 PMID: 22653078

9. Moran TH, Robinson PH, Goldrich MS, McHugh PR. Two brain cholecystokinin receptors: implications for behavioral actions. Brain Res. 1986; 362(1):175–9. PMID: 3002550

10. Hill DR, Campbell NJ, Shaw TM, Woodruff GN. Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J Neurosci. 1987; 7(8):2967–76. PMID: 3625281
11. Noble F, Roques BP. Phenotypes of mice with invalidation of cholecystokinin (CCK(1) or CCK(2)) receptors. Neuropeptides. 2002; 36(2–3):157–70. PMID: 12507432

12. Gaudreau P, Quirion R, St-Pierre S, Pert CB. Characterization and visualization of cholecystokinin receptors in rat brain using [3H]pentagastrin. Peptides. 1983; 4(5):755–62. PMID: 6318206

13. Williams JA, Gryson KA, McChesney DJ. Brain CCK receptors: species differences in regional distribution and selectivity. Peptides. 1986; 7(2):293–6. PMID: 3016683

14. Sekiguchi R, Moroji T. A comparative study on characterization and distribution of cholecystokinin binding sites among the rat, mouse and guinea pig brain. Brain Res. 1986; 399(2):271–81. PMID: 3828764

15. Hill DR, Shaw TM, Woodruff GN. Species differences in the localization of ‘peripheral’ type cholecystokinin receptors in rodent brain. Neurosci Lett. 1987; 79(3):286–9. PMID: 3658220

16. Miceli MO, Steiner M. Novel localizations of central- and peripheral-type cholecystokinin binding sites in Syrian hamster brain as determined by autoradiography. J. Eur J Pharmacol. 1989; 169(2–3):215–24. PMID: 2572437

17. Kopin AS, Mathes WF, McBride EW, Schmitz F, et al. The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. J Clin Invest. 1999; 103(3):881–91. PMID: 997499

18. Ji B, Kopin AS, Logsdon CD. Species differences between rat and mouse CCKA receptors determine the divergent acinar cell response to the cholecystokinin analog JMV-180. J Biol Chem. 2000; 275(25):19115–20. PMID: 10749842

19. Ingram SM, Krause RG 2nd, Skenes J, Lewis ME. Neuronal localization of cholecystokinin mRNA in the rat brain by using in situ hybridization histochemistry. J Comp Neurol. 1989; 287(2):260–72. PMID: 2794128

20. Burgunder J.M., Young WS 3rd. Cortical neurons expressing the cholecystokinin gene in the rat: distribution in the adult brain, ontogeny, and some of their projections. J Comp Neurol. 1990; 300(1):26–46. PMID: 2229486

21. Gallopin T, Geoffroy H, Rossier J, Lambolez B. Cortical sources of CRF, NKB, and CCK and their effects on pyramidal cells in the neocortex. Cereb Cortex. 2006; 16(10):1440–52. PMID: 16339088

22. Watakabe A, Hirokawa J, Ichinohe N, Ohsawa S, Kaneko T, Rockland KS, et al. Area-specific sub-stratification of deep layer neurons in the rat cortex. J Comp Neurol. 2012; 520(16):3553–73. doi: 10.1002/cne.23160 PMID: 22678985

23. Nunzi MG, Gorio A, Milan F, Freund TF, Smith AD. Cholecystokinin-Immunoreactive Cells Form Symmetrical Synaptic Contacts with Pyramidal and Nonpyramidal Neurons in the Hippocampus. Journal of Comparative Neurology. 1985; 237(4):485–496. PMID: 2044896

24. Kubota Y, Kawauchi Y. Two distinct subgroups of cholecystokinin-immunoreactive cortical interneurons. Brain Research. 1997; 752(1–2):175–83. PMID: 9106474

25. Fereczou I, Cau I, Hill EL, Rossier J, Hamel E, Lambolez B. 5-HT3 receptors mediate serotonergic fast synaptic excitation of neocortical vasoactive intestinal peptide/cholecystokinin interneurons. J Neurosci. 2002; 22(17):7389–97. PMID: 12196560

26. Wang Y, Gupta A, Toledo-Rodriguez M, Wu CZ, Markram H. Anatomical, physiological, molecular and circuit properties of nest basket cells in the developing somatosensory cortex. Cerebral Cortex. 2002; 12(4):395–410. PMID: 11884355

27. Gonchar Y, Wang Q, Burkhalter A. Multiple distinct subtypes of GABAergic neurons in mouse visual cortex identified by triple immunostaining. Front Neuroanat. 2008; 1(3):1–11.

28. Morozov YM, Torilli M, Rakic P. Origin, Early Commitment, Migratory Routes, and Destination of Can- cerbord/bhp028 PMID: 19346272

29. Xu XM, Roby KD, Callaway EM. Immunochemical Characterization of Inhibitory Mouse Cortical Neurons: Three Chemically Distinct Classes of Inhibitory Cells. Journal of Comparative Neurology. 2010; 518(3):389–404. doi: 10.1002/cne.22229 PMID: 19950390

30. Lacourse KA, Swanberg LJ, Gillespie PJ, Rehel JF, Saunders TL, Samuelson LC. Pancreatic function in CCK-deficient mice: adaptation to dietary protein does not require CCK. Am J Physiol. 1999; 276(5 Pt 1):G1302–9. PMID: 10530022

31. Tamrider G, Boylan AJ, Diluna ML, Pricola KL, Louvi A, Gunel M. PDCD10, the gene mutated in cerebro- cavernous malformation 3, is expressed in the neurovascular unit. Neurosurgery. 2008; 62(4):930–8; discussion 8. doi: 10.1227/01.neu.0000318179.02912.ca PMID: 18496189

32. Nagata A, Ito M, Iwata N, Kuno J, Takano H, Minowa O, et al. G protein-coupled cholecystokinin-B/gastrin receptors are responsible for physiological cell growth of the stomach mucosa in vivo. Proc Natl Acad Sci U S A. 1996; 93(21):11825–30. PMID: 8876222
33. Langhans N, Rindi G, Chiu M, Rehfeld JF, Ardman B, Beinborn M, et al. Abnormal gastric histology and decreased acid production in cholecystokinin-B/gastrin receptor-deficient mice. Gastroenterology. 1997; 112(1):280–6. PMID: 9873869

34. Rindi G, Langhans N, Rehfeld JF, Beinborn M, Kopin AS. Abnormal gastric morphology and function in CCK-B/gastrin receptor-deficient mice. Yale J Biol Med. 1998; 71(3–4):347–54. PMID: 10604785

35. Miyasaka K, Shinozaki H, Suzuki S, Sato Y, Kanai S, Masuda M, et al. Disruption of cholecystokinin (CCK)-B receptor gene did not modify bile or pancreatic secretion or pancreatic growth: a study in CCK-B receptor gene knockout mice. Pancreas. 1999; 19(2):114–8. PMID: 10438156

36. Miyasaka K, Ichikawa M, Ohta M, Kanai S, Yoshida Y, Masuda M, et al. Energy metabolism and turnover are increased in mice lacking the cholecystokinin-B receptor. J Nutr. 2002; 132(4):739–41. PMID: 11925470

37. Horinouchi Y, Akiyoshi J, Nagata A, Matsushita H, Tsutsumi T, Isogawa K, et al. Reduced anxious behavior in mice lacking the CCK2 receptor gene. Eur Neuropsychopharmacol. 2004; 14(2):157–61. PMID: 15013032

38. Miyasaka K, Ohta M, Kanai S, Yoshida Y, Sato N, Nagata A, et al. Enhanced gastric emptying of a liquid gastric load in mice lacking cholecystokinin-B receptor: a study of CCK-A, B, and AB receptor gene knockout mice. J Gastroenterol. 2004; 39(4):319–23. PMID: 15168241

39. Chen Q, Nakajima A, Meacham C, Tang YP. Elevated cholecystokininergic tone constitutes an important molecular/neuronal mechanism for the expression of anxiety in the mouse. Proc Natl Acad Sci U S A. 2006; 103(10):3881–6. PMID: 16537459

40. Jin G, Ramanathan V, Quante M, Baik GH, Yang X, Wang SS, et al. Inactivating cholecystokinin-2 receptor inhibits progastrin-dependent colonic crypt fission, proliferation, and colorectal cancer in mice. J Clin Invest. 2009; 119(9):2691–701. doi: 10.1172/JCI38918 PMID: 19652364

41. Chen Q, Tang M, Mamiya T, Im Hl, Xiong X, Joseph A, et al. Bi-directional effect of cholecystokinin receptor-2 overexpression on stress-triggered fear memory and anxiety in the mouse. PLoS One. 2010; 5(12):e15999. doi: 10.1371/journal.pone.0015999 PMID: 21209861

42. Lo CC, Langhans W, Georgievsky M, Arnold M, Caldwell JL, Cheng S, et al. Apolipoprotein AIV requires cholecystokinin and vagal nerves to suppress food intake. Endocrinology. 2012; 153(12):5865–6. doi: 10.1210/en.2012-1427 PMID: 23027805

43. Giacobini P, Kopin AS, Beart PM, Mercer LD, Fasolo A, Wray S. Cholecystokinin modulates migration of gonadotropin-releasing hormone-1 neurons. J Neurosci. 2004; 24(20):4737–48. PMID: 15152034

44. Stanic D, Paratcha G, Ledda F, Herzog H, Kopin AS, Hokfelt T. Peptidergic influences on proliferation, migration, and placement of neural progenitors in the adult mouse forebrain. Proc Natl Acad Sci U S A. 2008; 105(9):3610–5. doi: 10.1073/pnas.0712303105 PMID: 18305161

45. Xu X, Coats JK, Yang CF, Wang A, Ahmed OM, Alvarado M, et al. Modular genetic control of sexually dimorphic behaviors. Cell. 2012; 148(3):596–607. doi: 10.1016/j.cell.2011.12.018 PMID: 22304924

46. Sui Y, Vermeulen R, Hokfelt T, Horne MK, Stanic D. Female mice lacking cholecystokinin 1 receptors have compromised neurogenesis, and fewer dopaminergic cells in the olfactory bulb. Frontiers in cellular neuroscience. 2013; 7:13. doi: 10.3389/fncel.2013.00013 PMID: 23459364

47. Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. Second edition ed. San Diego, CA: Academic Press; 2001.

48. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009; 10(3):R25. doi: 10.1186/gb-2009-10-3-r25 PMID: 19261174

49. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009; 25(9):1105–11. doi: 10.1093/bioinformatics/btp120 PMID: 19289445

50. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010; 28(5):511–5. doi: 10.1038/nbt.1621 PMID: 20434646

51. Batista-Brito R, Machold R, Klein C, Fishell G. Gene expression in cortical interneuron precursors is prescient of their mature function. Cerebral cortex. 2008; 18(10):2306–17. doi: 10.1093/cercor/bhn258 PMID: 18250082

52. Furtuzinhos S, Li M, Kawasawa YI, Iavic V, Franjic D, Singh D, et al. Laminar and temporal expression dynamics of coding and noncoding RNAs in the mouse neocortex. Cell Rep. 2014; 6(5):938–50. doi: 10.1016/j.celrep.2014.01.036 PMID: 24561256

53. Kang HJ, Kawasawa YI, Cheng F, Zhu Y, Xu X, Li M, et al. Spatio-temporal transcriptome of the human brain. Nature. 2011; 478(7370):483–9. doi: 10.1038/nature10523 PMID: 22031440
54. Johnson MB, Kawasawa YI, Mason CE, Krnik Z, Coppola G, Bogdanovic D, et al. Functional and evolutionary insights into human brain development through global transcriptome analysis. Neuron. 2009; 62(4):494–509. doi: 10.1016/j.neuron.2009.03.027 PMID: 19477152

55. Pletikos M, Sousa AM, Sedmak G, Meyer KA, Zhu Y, Cheng F, et al. Temporal specification and bilaterality of human neocortical topographic gene expression. Neuron. 2014; 81(2):321–32. doi: 10.1016/j.neuron.2013.11.018 PMID: 24373884

56. Morita K, Sasaki H, Fujimoto K, Furuse M, Tsukita S. Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. J Cell Biol. 1999; 145(3):579–88. PMID:10225958

57. Gow A, Southwood CM, Li JS, Pariali M, Riordan GP, Brodie SE, et al. CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell. 1999; 99(6):649–59. PMID:19612400

58. Donahoo AL, Richards LJ. Understanding the mechanisms of callosal development through the use of transgenic mouse models. Semin Pediatr Neurol. 2009; 16(3):127–42. doi: 10.1016/j.spen.2009.07.003 PMID: 19778710

59. Alcamo EA, Chirivella L, Dautzenberg M, Dobreva G, Farinas I, Grosschedl R, et al. Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. Neuron. 2008; 57(3):364–77. doi: 10.1016/j.neuron.2007.12.012 PMID: 18255030

60. Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, et al. Satb2 is a postmitotic determinant for upper-layer cell specification in the neocortex. Neuron. 2008; 57(3):378–92. doi: 10.1016/j.neuron.2007.12.028 PMID: 18255031

61. Edwards TJ, Sherr EH, Barkovich AJ, Richards LJ. Clinical, genetic and imaging findings identify new causes for corpus callosum development syndromes. Brain: a journal of neurology. 2014.

62. Wolfer DP, Henehan-Beatty A, Stoeckli ET, Sonderegger P, Lipp HP. Distribution of TAG-1/axonin-1 in fibre tracts and migratory streams of the developing mouse nervous system. J Comp Neurol. 1994; 345(1):1–32. PMID:8089271

63. Yamamoto M, Boyer AM, Crandall JE, Edwards M, Tanaka H. Distribution of Stage-Specific Neurite-Associated Proteins in the Developing Murine Nervous-System Recognized by a Monoclonal-Antibody. Journal of Neuroscience. 1986; 6(12):3576–94. PMID:3794790

64. Furley AJ, Morton SB, Manalo D, Karagogeos D, Dodd J, Jessell TM. The Axonal Glycoprotein Tag-1 Is an Immunoglobulin Superfamily Member with Neurite Outgrowth Promoting Activity. Cell. 1990; 61(1):157–70. PMID:2317872

65. Arlotta P, Molyneaux BJ, Chen J, Inoue J, Kominami R, Macklis JD. Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuroen. 2005; 45(2):207–21. PMID: 15664173

66. Bulchand S, Subramanian L, Tole S. Dynamic spatiotemporal expression of LIM genes and cofactors in the embryonic and postnatal cerebral cortex. Developmental dynamics: an official publication of the American Association of Anatomists. 2003; 226(3):460–9. PMID: 12619132

67. Koester SE, O’Leary DD. Connectional distinction between callosal and subcortically projecting cortical neurons is determined prior to axon extension. Dev Biol. 1993; 160(1):1–14. PMID: 8224528

68. Koester SE, O’Leary DD. Axons of early generated neurons in cingulate cortex pioneer the corpus callosum. J Neurosci. 1994; 14(11 Pt 1):6608–20. PMID: 7965064

69. Rash BG, Richards LJ. A role for cingulate pioneering axons in the development of the corpus callosum. J Comp Neurol. 2001; 434(2):147–57. PMID: 11331522

70. Ozaki HS, Wahlsten D. Cortical axon trajectories and growth cone morphologies in fetuses of acallosal mouse strains. J Comp Neurol. 1993; 336(4):595–604. PMID: 7504000

71. Silver J. Glia-neuron interactions at the midline of the developing mammalian brain and spinal cord. Perspect Dev Neurobiol. 1993; 1(4):227–36. PMID: 8087547

72. Shu T, Puche AC, Richards LJ. Development of midline glial populations at the corticoseptal boundary. J Neurosci. 2003; 137(1):81–94. PMID: 12973830

73. Silver J, Edwards MA, Levitt P. Immunocytochemical demonstration of early appearing astroglial structures that form boundaries and pathways along axon tracts in the fetal brain. J Comp Neurol. 1993; 328(3):415–36. PMID: 8440789

74. Silver J, Lorenzo SE, Wahlsten D, Coughlin J. Axonal guidance during development of the great cerebral commissures: descriptive and experimental studies, in vivo, on the role of preformed glial pathways. J Comp Neurol. 1982; 210(1):10–29. PMID: 7130467

75. Shu T, Li Y, Keller A, Richards LJ. The glial sling is a migratory population of developing neurons. Development. 2003; 130(13):2929–37. PMID: 12756176
76. Burstyn-Cohen T, Tzavarlty F, Frumkin A, Feinstein Y, Stoeckli E, Klar A. F-spondin is required for accurate pathfinding of commissural axons at the floor plate. Neuron. 1999; 23(2):233–46. PMID: 10399931

77. Barallobre MJ, Del Rio JA, Alcantara S, Borrell V, Aguado F, Ruiz M, et al. Aberrant development of hippocampal circuits and altered neural activity in netrin 1-deficient mice. Development. 2000; 127(22):4797–810. PMID: 11044395

78. Shu T, Richards L.J. Cortical axon guidance by the glial wedge during the development of the corpus callosum. J Neurosci. 2001; 21(8):2749–58. PMID: 11306627

79. Bagi A, Marin O, Plump AS, Mak J, Pleasure SJ, Rubenstein JL, et al. Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. Neuron. 2002; 33(2):233–48. PMID: 11804571

80. Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, Richards LJ, et al. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Developmental cell. 2003; 5(1):45–57. PMID: 12852851

81. Niquille M, Garel S, Mann F, Hornung JP, Otstmane B, Chevalley S, et al. Transient Neuronal Populations Are Required to Guide Callosal Axons: A Role for Semaphorin 3C. Plos Biol. 2009;7(10: ).

82. Piper M, Plachez C, Zalucki O, Fothergill T, Goudreau G, Erzurumlu R, et al. Neuropilin 1-Sema signaling regulates crossing of cingulate pioneering axons during development of the corpus callosum. Cereb Cortex. 2009; 19 Suppl 1:11–21. doi: 10.1093/cercor/bhp027 PMID: 19357391

83. Hand R, Polleux F. Neurogenin2 regulates the initial axon guidance of cortical pyramidal neurons projecting medially to the corpus callosum. Neural development. 2011; 6:30. doi: 10.1186/1749-8104-6-30 PMID: 21864333

84. Hu ZL, Yue X, Shi GF, Yue Y, Crockett DP, Blair-Flynn J, et al. Corpus callosum deficiency in transgenic mice expressing a truncated ephrin-A receptor. Journal of Neuroscience. 2003; 23(34):10963–70. PMID: 14645492

85. Mendes SW, Henkemeyer M, Liebl DJ. Multiple Eph receptors and B-class ephrins regulate midline crossing of corpus callosum fibers in the developing mouse forebrain. Journal of Neuroscience. 2006; 26(3):882–92. PMID: 16421308

86. Wahlsten D. Deficiency of corpus callosum varies with strain and supplier of the mice. Brain Res. 1982; 239(2):329–47. PMID: 7093694

87. Wahlsten D, Bishop KM, Ozaki HS. Recombinant inbreeding in mice reveals thresholds in embryonic corpus callosum development. Genes, brain, and behavior. 2006; 5(2):170–88. PMID: 16507008

88. Sui Y, Horne MK, Stanic D. Reduced proliferation in the adult mouse subventricular zone increases survival of olfactory bulb interneurons. PLoS One. 2012; 7(2):e31549. doi: 10.1371/journal.pone.0031549 PMID: 22363671

89. Batista-Brito R, Fishell G. The developmental integration of cortical interneurons into a functional network. Curr Top Dev Biol. 2009; 87:81–118. doi: 10.1016/S0070-2153(09)01203-4 PMID: 19427517

90. Bartolini G, Ciceri G, Marin O. Integration of GABAergic interneurons into cortical cell assemblies: lessons from embryos and adults. Neuron. 2013; 79(5):849–64. doi: 10.1016/j.neuron.2013.08.014 PMID: 24012001

91. Marin O. Cellular and molecular mechanisms controlling the migration of neocortical interneurons. Eur J Neurosci. 2013; 38(1):2019–29. doi: 10.1111/ejn.12225 PMID: 23651101

92. Cobos I, Long JE, Thwin MT, Rubenstein JL. Cellular patterns of transcription factor expression in developing cortical interneurons. Cereb Cortex. 2006; 16 Suppl 1:i82–8. PMID: 16766712

93. Fogarty M, Grist M, Gelman D, Marin O, Pachnis V, Kessaris N. Spatial genetic patterning of the emperipolesis system requires Lhx8. Development. 2006; 202(2):381–41. doi: 10.1242/dev.038083 PMID: 17928435

94. Alcantara S, Ruiz M, D’Arcangelo G, Ezan F, de Lecea L, Curran T, et al. Regional and cellular patterns of reelin mRNA expression in the forebrain of the developing and adult mouse. J Neurosci. 1998; 18(19):7779–99. PMID: 9742148

95. Zhao Y, Marin O, Hermesz E, Powell A, Flames N, Palkovits M, et al. The LIM-homeobox gene Lhx8 is required for the development of many cholinergic neurons in the mouse forebrain. Proc Natl Acad Sci U S A. 2003; 100(15):9005–10. PMID: 12855770

96. Fragkouli A, van Wijk NV, Lopes R, Kessaris N, Pachnis V. LIM homeodomain transcription factor-dependent specification of bipotential MGE progenitors into cholinergic and GABAergic striatal interneurons. Development. 2009; 136(22):3841–51. doi: 10.1242/dev.038083 PMID: 19855026

97. Miyoshi G, Butt SJ, Takebayashi H, Fishell G. Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. J Neurosci. 2007; 27(29):7786–98. PMID: 17634372
98. Corbin JG, Nery S, Fishell G. Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. Nature neuroscience. 2001; 4 Suppl:1177–82.
99. Marin O, Yaron A, Bagri A, Tessier-Lavigne M, Rubenstein JL. Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. Science. 2001; 293(5531):872–5. PMID: 11486090
100. Grigoriou M, Tucker AS, Sharpe PT, Pachnis V. Expression and regulation of Lhx6 and Lhx7, a novel subfamily of LIM homeodomain encoding genes, suggests a role in mammalian head development. Development. 1998; 125(11):2063–74. PMID: 9570771
101. Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci. 1999; 19(18):7881–8. PMID: 10479690
102. Yau HJ, Wang HF, Lai CR, Liu FC. Neural development of the neuregulin receptor ErbB4 in the cerebral cortex and the hippocampus: Preferential expression by interneurons tangentially migrating from the ganglionic eminences. Cerebral Cortex. 2003; 13(3):252–64. PMID: 12571115
103. Flames N, Long JE, Garratt AN, Gassmann M, Birchmeier C, et al. Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. Neuron. 2004; 44(2):251–61. PMID: 15473965
104. Dufesné M, Seva C, Fourmy D. Cholecystokinin and gastrin receptors. Physiol Rev. 2006; 86(3):805–47. PMID: 16816139
105. Stumm RK, Zhou C, Ara T, Lazarini F, Dubois-Dalcq M, Nagasawa T, et al. CXCR4 regulates interneuron migration in the developing neocortex. The Journal of Neuroscience: the official journal of the Society for Neuroscience. 2003; 23(12):5123–30. PMID: 12832536
106. Tiveron MC, Rossel M, Moepps B, Zhang YL, Seidenfaden R, Favor J, et al. Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1-CXCL12/CXCR4 signaling in the cortical subventricular zone/intermediate zone. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2006; 26(51):13273–8. PMID: 17182777
107. Stumm R, Kolodziej A, Schulz S, Kohtz JD, Holtt V. Patterns of SDF-1alpha and SDF-1gamma mRNAs, migration pathways, and phenotypes of CXCR4-expressing neurons in the developing rat telencephalon. The Journal of comparative neurology. 2007; 502(3):382–99. PMID: 17366607
108. Li G, Adesnik H, Li J, Long J, Nicoll RA, Rubenstein JL, et al. Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2008; 28(5):1085–98. doi: 10.1523/JNEUROSCI.4602-07.2008 PMID: 18234887
109. Liapi A, Pritchett J, Jones O, Fuji N, Parnavelas JG, Nadarajah B. Stromal-derived factor 1 signalling regulates radial and tangential migration in the developing cerebral cortex. Dev Neurosci. 2008; 30(1–3):117–31. doi: 10.1159/000191212 PMID: 19127062
110. Lopez-Bendito G, Sanchez-Alcaniz JA, Ortega JA, Ocana I, Alcantara S, Bovolenta P. Appropriate Bmp7 levels are required for the differentiation of midline guidepost cells involved in corpus callosum formation. J Neurobiol. 2011; 71(5):1739–45. doi: 10.1002/jneu.2011.11.036 PMID: 22365545
111. Caronia-Brown G, Grove EA. Timing of cortical interneuron migration is influenced by the cortical hem. Cerebral cortex. 2011; 21(4):748–55. doi: 10.1093/cercor/bhq142 PMID: 20713502
112. Lysko DE, Putt M, Golden JA. SDF1 regulates leading process branching and speed of migrating interneurons. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2011; 31(5):1739–45. doi: 10.1523/JNEUROSCI.3118-10.2011 PMID: 21289183
113. Choe Y, Siegenthaler JA, Pleasure SJ. A cascade of morphogenetic signaling initiated by the meninges controls corpus callosum formation. Neuron. 2012; 73(4):689–712. doi: 10.1016/j.neuron.2011.11.036 PMID: 22365545
114. Sanchez-Camacho C, Ortega JA, Ocana I, Alcantara S, Bovolenta P. Appropriate Bmp7 levels are required for the differentiation of midline guidepost cells involved in corpus callosum formation. Dev Neurobiol. 2011; 71(5):337–50. doi: 10.1002/dneu.20865 PMID: 21485009
115. Bagri A, Tessier-Lavigne M. Neuropilins as Semaphorin receptors: in vivo functions in neuronal cell migration and axon guidance. Advances in experimental medicine and biology. 2002; 515:13–31. PMID: 12613540
116. Mohammad S, Ozaki T, Takeuchi K, Unno K, Yamoto K, Morioka E, et al. Functional compensation between cholecystokinin-1 and -2 receptors in murine paraventricular nucleus neurons. J Biol Chem. 2012; 287(47):39391–401. doi: 10.1074/jbc.M112.416214 PMID: 23038256
117. Schwanzel-Fukuda M, Pfaff DW. Origin of luteinizing hormone-releasing hormone neurons. Nature. 1989; 338(6211):161–4. PMID: 2645530
118. Wray S, Grant P, Gainer H. Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. Proc Natl Acad Sci U S A. 1989; 86(20):8132–6. PMID: 2682637

119. Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo PM. Becoming a new neuron in the adult olfactory bulb. Nature neuroscience. 2003; 6(5):507–18. PMID: 12704391

120. Hirota Y, Otshimi T, Kaneko N, Ikeda M, Iwasato T, Kulkarni AB, et al. Cyclin-dependent kinase 5 is required for control of neuroblast migration in the postnatal subventricular zone. Journal of Neuroscience. 2007; 27(47):12829–38. PMID: 18032654

121. Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. J Neurosci. 2010; 30(50):16796–808. doi:10.1523/JNEUROSCI.1869-10.2010 PMID: 21159951

122. Vucurovic K, Gallopin T, Ferezou I, Rancilliac A, Chameau P, van Hooft JA, et al. Serotonin 3A receptor subtype as an early and protracted marker of cortical interneuron subpopulations. Cereb Cortex. 2010; 20(10):2333–47. doi: 10.1093/cercor/bhp310 PMID: 20083553

123. Schwarting GA, Henion TR, Nugent JD, Caplan B, Tobet S. Stromal cell-derived factor-1 (chemokine C-X-C motif ligand 12) and chemokine C-X-C motif receptor 4 are required for migration of gonadotropin-releasing hormone neurons to the forebrain. J Neurosci. 2006; 26(25):6834–40. PMID: 16793890

124. Cariboni A, Hickok J, Rakic S, Andrews W, Maggi R, Tischkau S, et al. Neuropilins and their ligands are important in the migration of gonadotropin-releasing hormone neurons. J Neurosci. 2007; 27(9):2387–95. PMID: 17329436

125. Philpot BD, Sekhar AK, Shouval HZ, Bear MF. Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. Neuron. 2001; 29(1):157–69. PMID: 11182088

126. Barria A, Malinow R. Subunit-specific NMDA receptor trafficking to synapses. Neuron. 2002; 35(2):345–53. PMID: 12160751

127. Bellone C, Nicoll RA. Rapid bidirectional switching of synaptic NMDA receptors. Neuron. 2007; 55(5):779–85. PMID: 17785184

128. Roberts AC, Diez-Garcia J, Rodriguez RM, Lopez IP, Lujan R, Martinez-Turrillas R, et al. Downregulation of NR3A-Containing NMDARs Is Required for Synapse Maturation and Memory Consolidation. Neuron. 2009; 63(3):342–56. doi: 10.1016/j.neuron.2009.06.016 PMID: 19679074

129. Wang CC, Held RG, Chang SC, Yang LL, Delpire E, Ghosh A, et al. A Critical Role for GluN2B-Containing NMDA Receptors in Cortical Development and Function. Neuron. 2011; 72(5):789–805. doi: 10.1016/j.neuron.2011.09.023 PMID: 22153375

130. Campos CA, Wright JS, Czaja K, Ritter RC. CCK-Induced Reduction of Food Intake and Hindbrain MAPK Signaling Are Mediated by NMDA Receptor Activation. Endocrinology. 2012; 153(6):2633–46. doi: 10.1210/en.2012-1025 PMID: 22508518

131. Campos CA, Shina H, Silvas M, Page S, Ritter RC. Vagal Afferent NMDA Receptors Modulate CCK-Induced Reduction of Food Intake Through Synapsin I Phosphorylation in Adult Male Rats. Endocrinology. 2013; 154(8):2633–U87. doi: 10.1210/en.2013-1062 PMID: 23715865

132. Xiao ZY, Jaiswal MK, Deng PY, Matsui T, Shin HS, Porter JE, et al. Requirement of phospholipase C and protein kinase C in cholecystokinin-mediated facilitation of NMDA channel function and anxiety-like behavior. Hippocampus. 2012; 22(6):1438–50. doi: 10.1002/hipo.20984 PMID: 22072552

133. Matala JA, Pelkey KA, Craig MT, Chittajallu R, Jeffries BW, McBain CJ. Developmental origin dictates interneuron AMPA and NMDA receptor subunit composition and plasticity. Nature neuroscience. 2013; 16(8):1032–U87. doi: 10.1038/nn.3459 PMID: 23852113