Circular RNAs (circRNAs) are abundant in mammalian brain and some show age-dependent expression patterns. Here, we report that circGRIA1, a conserved circRNA isoform derived from the genomic loci of α-mmino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit Gria1, shows an age-related and male-specific increase in expression in the rhesus macaque prefrontal cortex and hippocampus. We show circGRIA1 is predominantly localized to the nucleus, and find an age-related increase in its association with the promoter region of Gria1 gene, suggesting it has a regulatory role in Gria1 transcription. In vitro and in vivo manipulation of circGRIA1 negatively regulates Gria1 mRNA and protein levels. Knockdown of circGRIA1 results in an age-related improvement of synaptogenesis, and GluR1 activity-dependent synaptic plasticity in the hippocampal neurons in males. Our findings underscore the importance of circRNA regulation and offer an insight into the biology of brain aging.
BRAIN AGING IS CHARACTERIZED BY MULTIPLE CHANGES IN CELL STRUCTURE; MOLECULAR, PHYSIOLOGICAL, AND BIOPHYSICAL PROPERTIES OF ITS RESIDENT NEURONS. AT A SYSTEM LEVEL, THERE DEGENERATIVE CHANGES ARE ACCOMPANIED BY A DECREASED SYNAPTIC PLASTICITY AND OCCASIONALLY BY NEURONAL LOSS. WHILE DESCRIPTIVE STUDIES OF BRAIN AGING ARE NUMEROUS, MOLECULAR MARKERS THAT OFFER INSIGHT INTO THE MECHANISMS UNDERLYING THE BIOLOGY OF BRAIN AGING REMAIN ELUSIVE AND UNDEFINED.

AGE-RELATED ALTERATIONS IN NEURONAL FUNCTION ARE PARITIONALLY RESULTED FROM CHANGES IN HOMEOSTATIC SYNAPTIC PLASTICITY AND CALCIUM HOMEOSTASIS DUE TO SUCH FACTORS AS DECREASED EXPRESSION OF NEUROTRANSMITTER RECEPTORS AND SELECTIVE ION CHANNEL MESSANGER RNA (mRNAs) AND AMPA RECEPOTRS ARE THE PREDOMINANT EXCITATORY NEUROTRANSMITTER RECEPTORS IN THE MAMMALIAN BRAIN. COTMAN ET AL. NOTED A 45% LOSS IN GLUTAMATE RECEPTORS THAT OCCUR IN AGED RATS WHEN COMPARED WITH YOUNG ANIMALS. YET STUDIES SUCH AS THESE ARE SILENT ON THE MECHANISMS BEHIND THIS AGE-RELATED DECREASE.

CIRCFAQNS ARE A NOVEL CLASS OF TRANSCRIPTS THAT ARE SYNTHESIZED BY HEAD-TO-TAIL SPLICING OF LINEAR RNA MOLECULES. THEIR EXISTENCE HAS BEEN KNOWN FOR MANY YEARS, BUT THE CONSEQUENCES OF THEIR COMPLEX TISSUE- AND SPATIOTEMPORAL-EXPRESSION PATTERNS ARE NOT YET FULLY UNDERSTOOD. ONE MAJOR ROLE OF CIRCFAQNS IS TO SERVE AS miRNA “SPONGES” THAT BUFFER THE ACTION OF miRNAs. SLIGHTLY DEVICED FROM THAT, FOR INSTANCE, MUSCLE BLIND PROTEIN, INDEPENDENTLY MODULATES HOST mRNA PRODUCTION. BREAKING WITH IT FOR SPlicing AND OTHER MATURATION FACTORS. IN ADDITION, CIRCFAQNS ALSO REGULATE THE INTRACELLULAR TRANSPORT OF RNA THROUGH THEIR ABILITIES TO SEQUESTR RNA-BINDING PROTEINS.

Adding to the potential regulatory complexity, studies have shown that circRNAs can be translated into peptides in a cap-independent way. Taken together, circRNAs represent a heterogeneous and dynamic class of noncoding transcripts that potentially regulates brain function through a series of diverse mechanisms.

CIRCFAQNS ARE ABUNDANTLY PRODUCED IN THE BRAIN AND ARE NATURALLY PRESENT IN NERVE CELL TYPES, WHERE THEY POTENTIALLY SERVE AS A NEW AND RELATIVELY UNEXPLORATORY NETWORK THAT MAY BE ACTIVE IN BRAIN DEVELOPMENT MATURATION AND AGING. Indeed, our recent work has described dynamic changes in circRNA expression in rhesus macaque brain during aging, and indicated that the complicate correlation between circRNA and host mRNA expression may be involved in the biology of brain aging.

In this study, we focus specifically on the AMPA receptor gene-derived circGRIA1. Utilizing postmortem brain tissues of macaque together with in vivo and in vitro manipulation of circGRIA1 expression, we disclose an age-related and sex-related increase in circGRIA1 expression in the male macaque brain that likely explains the loss of synaptic dysfunction over the aging states.

RESULTS

Age- and sex-related changes in circGRIA1 expression in the macaque brain. Previously, using deep RNA profiling, we described a comprehensive map of changes in circRNA expression in rhesus macaque (macaca mulatta) brain during aging. The study explored the variable age-related correlations between circRNA and host mRNA expression. We identified 11 age-related circRNAs and host mRNAs whose functions make them prime candidates for serving to regulate brain aging. Of these eleven circRNAs, nine are negatively correlated with the aging processing, while two are positively correlated.

CircGRIA1 negatively correlates to Grial mRNA expression. Next, we decided to investigate whether circGRIA1 expression was associated with the biological process of brain aging. First, using BASEscope in situ hybridization (ISH) for detection of circRNA with the junction site probes, we verified the age-related increase of circGRIA1 expression in prefrontal cortex (PFC), hippocampal CA1, and dentate gyrus (DG) of 20-year-old male macaque (Fig. 2a, b). Using RNAseq ISH for detection of linear RNA with the specific probe sets against host Gria1 mRNA, we examined the levels of Grial expression, and found an age-related decrease in PFC and hippocampus of 20-year-old male macaque brain (Fig. 2c, d). Interestingly, in 20-year-old female PFC and hippocampus, where there was no detectable age-related increase in circGRIA1 expression, we nonetheless found an age-related decrease in Grial expression was found (Supplementary Fig. 2a, b). Data from immunohistochemistry (IHC) and western blot analyses further validated that the GluR1 decreased in PFC and hippocampus of 20-year-old both sexes (Supplementary Fig. 3). This suggests a different molecular basis to the regulation of Grial expression and function in the brains of male and female macaques.
To investigate whether in vitro hippocampal cultures of fetal *macaque* might reproduce these in vivo age-related changes, we used BASEscope ISH and RT-qPCR to examine the levels of circGRIA1 expression in hippocampal cultures of male fetal *macaque* at 14 days in vitro (DIV14) and DIV28 (Supplementary Fig. 4). As with aging brain, we found increased levels of circGRIA1 expression in neurons at DIV28 compared with that of the DIV14. By contrast, data from RNAscope ISH and RT-qPCR showed decreases in *Gria1* expression in hippocampal cultures of male fetal *macaque* at DIV28 compared with that of the DIV14 (Supplementary Fig. 4).
Association of circGRIA1 with the promoter region of its parental gene. Recent study reveals that the gene regulation functions of a circRNA are accomplished by its ability to compete with the factors needed to splice the linear native mRNA. In this way they serve as an important regulator of mRNA maturation\(^\text{19,30}\). To explore whether circGRIA1 negatively correlates to its host mRNA expression in the cis-acting manner, we investigated potential interactions between circGRIA1 and chromatin. Using chromatin isolation by RNA purification (ChIRP) we examined genomic DNA coprecipitated with circGRIA1 through biotin-labeled oligonucleotides complementary to circGRIA1 junction sites. We focused our analysis on the 5′-UTR and 3′-UTR regions of the Gria1 gene elements that were coprecipitated with circGRIA1 from both in vivo and in vitro preparations. In our samples, ChIRP with circGRIA1 pulled down endogenous circGRIA1 itself, but failed to precipitate the normal, linear mRNA transcript. As negative controls we used 5.8S RNA and circGRIN2A (Fig. 3a–c). Importantly, when ChIRP with circGRIA1 was performed on lysates from hippocampal tissues of 20-year-old macaques, a substantial amount of PCR product complementary to the 5′-UTR of the Gria1 gene was recovered, especially compared with comparable assay using brain samples from 10-year-old animal (Fig. 3d, e). No detectable 3-UTR genomic DNA was found. Sanger sequencing of RT-PCR products verified the identity of the Gria1 5′-UTR (Fig. 3f).

To determine whether association circGRIA1 with the promoter region of its parental gene affects its transcription, SH-SY5Y cells, which express no detectable circGRIA1, were transfected with the 5′-UTR sequence (~330 bp) of macaque Gria1 in pGL4.11 vector together with either circGRIA1 or Tet-on circRNA vector or pLCDH-cir vector. Twenty-four hours later, we assayed for luciferase activity. We found that the relative levels of luciferase activity were consistent with our hypothesis that circGRIA1 strongly downregulates transcription from the Gria1 gene by its competitive association with the promoter region (Supplementary Fig. 5c, d). Next, using RNA–DNA dual fluorescent in situ hybridization (FISH) we further validated co-localization of nuclear circGRIA1 with the genomic loci of Gria1 in hippocampal cultures of male macaque at DIV28 compared with that of the DIV14 (Supplementary Fig. 6).

CircGRIA1 negatively regulates Gria1 mRNA expression. Next, using BASEscope and RNAscope ISH we examined correlation between the levels of circGRIA1 and Gria1 expression in hippocampal cultures of male macaque at DIV14 and DIV28. We introduced the junction site-targeting siRNAs against circGRIA1 as well as the mismatched junction site-targeting siRNA-control at DIV5. Analysis of the average intensities of BASEscope ISH signals revealed a significant reduction of circGRIA1 expression at DIV28 infected with viral particles of siRNA (Fig. 4a–c). By contrast, analysis of the average intensities of RNAscope ISH signals revealed that knockdown of circGRIA1 led to a significant increase in Gria1 expression at DIV28 (Fig. 4a–c). Notably, no substantial response was seen at DIV14 with introduction of siRNA consistent with the barely detectable levels of endogenous circGRIA1. Since circRNAs are quite stable, we sought to exclude the effect of age-related accumulation of circGRIA1 on the negative correlation between circGRIA1 and its host mRNA expression, nascent RNA was purified by use of the Click-IT Nascent RNA Capture Kit followed by qPCR. Indeed, both knockdown and overexpression of circGRIA1 could reverse the changes in Gria1 mRNA expression (Fig. 4c).

CircGRIA1 contributes to synaptogenesis. Brain aging entails many chemical, biological, and structural changes including synaptogenesis\(^\text{31-33}\). NMDA and AMPA receptor activities are required for both long-term potentiation and neural activity-dependent synaptogenesis\(^\text{34}\). To investigate whether the negative correlation between circGRIA1 and Gria1 expression is involved in regulation of pre- and post-synaptogenesis over the aging states, we first microinjected AAV viral particles of siRNAs against circGRIA1 into hippocampus of 10- and 20-year-old male and female macaques under the guidance of magnetic resonance imaging (MRI) (Supplementary Fig. 7). Six weeks later, the levels of circGRIA1 and Gria1 expression were examined using BASEscope ISH (Fig. 5a, b and Supplementary Fig. 8a, b), RNAscope ISH (Fig. 5d, e and Supplementary Fig. 8d, e), and RT-qPCR (Fig. 5c, f and Supplementary Fig. 8c, f). Knockdown of circGRIA1 led to significant decreases in circGRIA1 expression, and increases in Gria1 mRNA expression in the hippocampal neurons of male but not female macaque. Next, we examined age-related changes in the levels of several synaptic components in the brains of rhesus macaques. IHC showed significant decreases in the levels of synapsin-1 and PSD95 in PFC and hippocampus (CA1 and DG) of 20-year-old male and female macaques compared with that of the 10 years (Fig. 6a, c and Supplementary Fig. 9a, c). In addition, despite little change in VAMP2, both pre- and postsynaptic components including synapsin-1, synaptotagmin-I, syntaxin-2, PSD95, and neuroligin-1 all showed...
age-related decreases in the brain tissues of 20-year-old male *macaques* (Fig. 6d, e and Supplementary Fig. 10a, b).

To determine whether knockdown of circGRIA1 prevents the decreases in the presynaptic vesicle protein, synapsin-I and the postsynaptic protein, PSD95, cryostat sections of postmortem hippocampus from 10- and 20-year-old male and female *macaques* in which circGRIA1 has been knocked down were examined. Knockdown of circGRIA1 robustly increased the levels of synapsin-I, in the hippocampal neurons of 20-year-old male *macaque* but not that of 20-year-old female *macaque* (Fig. 6b, f and Supplementary Fig. 9b, d). Curiously despite these findings, in vivo knockdown of circGRIA1 led to few changes in the levels of PSD95 in either male and female *macaques* at 20 years of age. Indeed, similar changes were also found in hippocampal cultures of male fetal *macaque* with introduction of siRNA (Supplementary Fig. 10c–f). Despite significant circGRIA1-induced decreases in the densities of both of synapsin-I and PSD95 found at DIV28, the cultures infected with siRNAs at DIV5 showed significantly increases in the densities of synapsin-I at DIV28 with little effect on PSD95.

**CircGRIA1 regulates synaptic plasticity and Ca$^{2+}$ homeostasis.** Homeostatic synaptic plasticity is associated with AMPA and
NMethyld-aspartate receptors-mediated neuronal activity (mEPSCs) are used to model this activity as their levels are dependent on the number of synapses formed and/or the presynaptic rate of vesicle release. At equilibrium, mEPSCs amplitude is dependent on either postsynaptic glutamate responsiveness or a postsynaptic glutamate content of synaptic vesicles or both. Since circGRIA1 not only affects GluR1 expression, but also regulates the densities of presynaptic component synapsin-1, we were curious whether circGRIA1 contributed to homeostatic synaptic plasticity. Hippocampal cultures of fetal macaques were prepared, then infected on DIV5 with the junction site-targeting siRNAs against circGRIA1 or the mismatched junction site-targeting siRNA-control. We then tracked the effects of this manipulation on the mEPSCs at different times after infection. Both the amplitude and frequency of spontaneous mEPSCs showed the normal decreases in control cultures (siRNA-control) at DIV28 neurons compared with DIV14. By contrast, siRNA against circGRIA1, when introduced into male neurons significantly attenuated this decreases (Fig. 7a–c). We next asked whether circGRIA1 also contributed to GABA receptor blockade-induced change by manipulating neuronal activity with bicuculline (a GABA receptor antagonist) treatment. At DIV28, bicuculline enhanced excitatory neural activity,
leading to a homeostatic decrease in the mEPSCs amplitude but not frequency. Interestingly, similar to the observed patterns of spontaneous mEPSCs, the changes in mEPSC amplitude but not frequency induced by bicuculline were unaffected by reducing circGRIA1 expression (Fig. 7a–c). To further confirm this finding, we induced chemical LTP (cLTP) at DIV14 and DIV28, and found that circGRIA1 levels in male neurons affect cLTP as well as mEPSCs (Fig. 7d).

Age-related neural plasticity deficits are tightly associated with age-induced alterations in calcium homeostasis36. This has important consequences for the nerve system as both AMPA and NMDA receptors require calcium influx for their functioning35,37. A large body of evidence suggests that glutamate receptor signaling-induced disturbances of calcium homeostasis may be a causative factor in the biology of brain aging as well as Alzheimer’s disease7,9. We therefore sought to determine whether increased levels of circGRIA1 expression could have a potential role in disturbances of AMPA receptor activity-dependent calcium homeostasis. We used a microscope-based fluorimeter to examine enriched populations of Fura-2-loaded female and

**Fig. 4** CircGRIA1 negatively regulates Gria1 mRNA expression. a Representative images of BASEscope and RNAscope ISH showing circGRIA1 and Gria1 expression in hippocampal cultures of male fetal macaque at DIV14 and DIV28. The images are representative of three independent experiment of each sample from 2 to 3 fetal cultures per group. Red boxes indicate areas magnified. Scale bar, 100 μm. b Average intensities of circGRIA1 and Gria1 signals per neuron illustrated in (a) were quantified by use of Image J. Data are present as mean ± S.D. (n = 30–39 per group; *p < 0.01), p values were calculated using two-tailed Student’s t test or one-way ANOVA with Sidak’s correction for multiple comparisons. c Negative correlation between nascent circGRIA1 and Gria1 expression. Data are present as mean ± S.D. Each bar represents the average of three independent experiment; error bars denote S.D.; *p < 0.05, p values were calculated using two-tailed Student’s t test or one-way ANOVA with Sidak’s correction for multiple comparisons. d Protein extracts from DIV14, DIV28 male fetal macaque hippocampal neurons infected at DIV5 with viral circGRIA1, or siRNAs against circGRIA1 or control, were immunoblotted with GluR1 antibody. α-Tubulin was loading control. The blots represent three independent experiment of each hippocampal culture from 2 to 3 male fetal macaques. e Relative intensities of immunoblotted signals of GluR1 illustrated in (d) were quantified by use of Image J. Data are present as mean ± S.D. Each bar represents the average of three independent experiment; error bars denote S.D.; n.s. no significance; *p < 0.05, p values were calculated using two-tailed Student’s t test or one-way ANOVA with Turkey’s correction for multiple comparisons. Source data are provided as a Source Data file.
male fetal macaque hippocampal neurons. We tracked Fura-2 fluorescence in groups of six to eight neurons after stimulation with glutamate followed by 100 μM cyclothiazide (CTZ, an AMPA receptor modulator, which binds to and desensitizes the AMPA receptor) treatment. The intracellular neuronal calcium concentrations, normally 38.6 ± 12.5 nM, increased and maintained at 150.9 ± 33.8 nM (~1.5-fold from the original baseline) after stimulation with Glutamate followed by CTZ treatment at DIV28 neurons (Fig. 7e). On the contrary, circGRIA1 knockdown in hippocampal cultures of male but female fetal macaques led to...
a significant increase in the intracellular calcium concentrations after stimulation with Glutamate followed by CTZ treatment at DIV28, which is nearly recovered to its level in DIV14 neurons. The intracellular calcium concentrations were calculated to range from 49.8 ± 12.5 nM and increased to and maintained around 206.8 ± 28.6 (≈4.1-fold from the original baseline) after stimulation with Glutamate followed by CTZ treatment at DIV28 neurons.

**Discussion**

During the normal course of eukaryotic transcript, the original RNA transcript is a precursor mRNA that is spliced to remove noncoding regions and form a mature mRNA. With circRNA this process is subverted and one or more noncanonical splicing events occurs [Salzman et al.38 #72][Zhang et al.39 #78]. We have previously reported that there are a huge number of brain-specific circRNAs, many of which show age-related changes in their
expression as compared to their linear mRNA during brain aging. While the molecular mechanism and physiological function of changes in circRNA expression in the biology of brain aging remains unclear, we report here an example of the molecular consequences of such a change. Based on our data from circGRIA1, we propose that age-related increases in circRNA expression are the result of increased noncanonical splicing of newly generated transcripts in postmitotic neurons, and play an important role in the biology of brain aging.

CircGRIA1, a conserved but previously unannotated circRNA isoform derived from AMPA receptor GluR1 locus, increases in the brain of rhesus macaque in an age- and gender-related fashion. The increase has a substantial impact on glutamate receptor levels and therefore on synaptic plasticity. Combining in vivo and in vitro systems, we have investigated the regulatory role of circGRIA1 in the aging process of brain. We found that circGRIA1 expression is negatively associated with its host gene Gria1 mRNA expression. Unexpectedly, the most robust correlation between circGRIA1 and its host mRNA expression in both macaque brain and fetal hippocampal cultures occurs in males. The effect is cell autonomous as well find it to be true both in whole brains as well as in dissociated cells in culture.

Our finding that circGRIA1 negatively regulates the transcriptional activity of its host Gria1 gene via its association with the promoter region of the parental genomic locus offers potential insight into the mechanism by which gene expression is tuned by circRNA-chromatin association. We note that circGRIA1 theoretically has no direct DNA-binding ability. This suggests that its increased association with its parental gene 5′-UTR may involve other regulatory binding partners. This suggestion receives support from our finding of a strong gender bias to the action of circGRIA1 coupled with previous studies showing that sex-biased expression of protein-coding genes in the brain can be the result of multiple factor such as hormones and other epigenetic regulations. Thus of nuclear receptors for sex hormones may play an important role in the biology of brain aging. Our in depth characterization of these molecular events will show the evolution of these effects with age in the nonhuman primate brains, independent of genetic influences. We believe that our in depth characterization of these molecular events will enhance future explorations of circRNA-mediated regulation, and function underlying the biology of brain aging with its associated age-related mental disorders.

Methods

Antibodies and chemical reagents. Antibodies against GluR1 (ab31232), GluR2 (ab133477), GFP (ab1213, ab183734), PSD95 (ab2723, ab13552), synaptin-1 (ab64581), syntaxin-2 (ab233275), syntaxotagmin (ab13299), VAMP2 (ab181869), neurologin-1 (ab153821), α-tubulin (ab7291), and MAP2 (ab5392) were purchased from Abcam. Secondary antibodies used for immunocytochemistry were: Alexa 488-labeled chicken anti-mouse or anti-rabbit; Alexa 594-labeled donkey anti-mouse or anti-rabbit (Invitrogen, Eugene, OR); all used at a dilution of 1:500. 4′,6-Diamidino-2-phenylindole (DAPI) (4′, 6-diamidino-2-phenylindol) (Invitrogen) was used as a nuclear counterstain at 1 μg/ml. CTZ was purchased from Tocris Bioscience, and Furo-2, AM (F1201) was ordered from ThermoFisher. Bicuculline (S2694) and 5-Fluoro-2′-deoxyuridine (FDU) (F5303) were purchased from Sigma Aldrich.

Constructs and plasmids. All plasmids were constructed with restriction-enzyme digestion and ligation or alternative with recombinant methods. Oligos for all plasmid construction, probe preparation, siRNAs, and biotin-oligonucleotides are listed in Supplementary Tables 2 and 3. The AAV-shRNA vectors against circGRIA1 were purchased from Vigen Biosciences with scrambled shRNA as negative control. Viral stocks were stored at −80 °C until use. All plasmids were
Fig. 7 CircGRIA1 regulates synaptic plasticity and calcium homeostasis. a Representative of mEPSC from hippocampal neurons. For bicuculline treatment, the neurons were incubated with bicuculline for 48 h followed by recording. Notes: fDIV14 and mDIV14, fDIV28 and mDIV28, hippocampal cultures of female and male fetal macaques at DIV14 and DIV28, respectively. b, c The average mEPSC wave forms and quantification of mEPSC amplitudes and frequency. The histogram showing a significant decrease in mEPSC amplitudes and frequency at DIV28 (*p = 0.00236 for amplitude, and *p = 0.00237 for frequency). Knockdown of circGRIA1 caused a significant increase in mEPSC amplitudes and frequency at DIV28 (*p = 0.00362 for amplitude, and *p = 0.00437 for frequency). Bicuculline treatment led to a significant decrease in mEPSC amplitudes. ANOVA F2,79 = 13.594 *p = 0.0002, Tukey’s multiple comparisons DIV14 control vs. bicuculline, *p = 0.026; siRNA-control DIV28 control vs. bicuculline, *p = 0.026 (n.s. no significance, n = 6-8 recordings of each culture from 2 to 3 fetal macaques per sex group). d Involvement of circGRIA1 in essential properties of cLTP. Data are presented as mean ± S.E.M.; *p < 0.05 vs. basal (ANOVA, Tukey post hoc test, n = 8-10 neurons of each culture from 2 to 3 fetal macaques per sex group). e CircGRIA1 regulates calcium flux. Relative ratios of fluorescence at an emission frequency of 510 nm and excitation frequencies of 340 and 380 nm was collected and analyzed. Data are present as mean ± S.E.M. (n = 8-10 cells of each culture from 2 to 3 fetal macaque per sex group). *p < 0.05, unpaired t-test. p values were calculated using two-tailed Student’s t test or one-way ANOVA with Sidak’s correction and two-way ANOVA with Turkey’s correction for multiple comparisons. Source data are provided as a Source Data file.

sequenced for confirmation. For the overexpression of circGRIA1, lentiviral plasmid with inserts coding three exons of circGRIA1 (with 5′-AG and 3′-GT included in the insertion) was used. The 5′UTR sequence (~330 bp) of macaque GRIA1 containing circGRIA1 binding site was amplified and cloned into pGL4.11 vector. To construct the overexpressing circGRIA1, the cDNA of circGRIA1 was cloned and inserted into the pLCDH-ciR, Tet-on circRNA, and AAV-hSyn1 expression vectors with cyclization sequence.[85,86]

Animals and samples collection. Frozen postmortem tissue samples from rhesus macaque were obtained from Kunming Primate Research Center of the Chinese Academy of Sciences (KPRC). Brain regions were systematically collected from well-characterized rhesus monkeys born and raised at the KPRC in outdoor, 6-acre enclosures that provide a naturalistic setting and normal social environment. Extensive health, family lineage, and dominance information were maintained on all animals. For circRNA-seq analysis, two male and two female specimens at each of stages representing adult (10-year old) and old (20-year old) were profiled (GEO, GSE94027).[26] According to a widely used macaque brain atlas and brainmaps (http://www.brainmaps.org), tissues spanning eight anatomically distinct regions were selected and collected from each specimen. The detailed information was described as below: the PFC was sampled at the main sulci, the posterior cingulate cortex was sampled at the Brodmann’s area 23, the temporal cortex at the superior temporal gyrus, the parietal cortex at the middle sylvian fissure, the occipital cortex at the V1, and the cerebellar cortex was sampled at the cauda cerebellum. The hippocampus (including CA1 and DG) was also sampled. All the collected samples were washed with RNA later solution (AM7021, Ambion, USA) and put in freezing tubes to store at liquid nitrogen temperature.

All animal procedures were in strict accordance with the guidelines for the National Care and Use of Animals approved by the National Animal Research Authority (China) and the Institutional Animal Care and Use Committee of the Kunming Institute of Zoology of Chinese Academy of Sciences. The nonhuman primate cares and experimental protocols were approved by the Ethics Committee.
of Kunming Institute of Zoology and the KPRC, Chinese Academy of Sciences (AAALAC accredited), and the methods were carried out in accordance with the approved guidelines (Approval No: SYDW20160311).

Conservation analysis for macaque circRNAs. To analyze the homologous feature between macaque circRNAs and other mammalian species, we downloaded the human and mouse circRNAs sequence from the circBase (http://www.circbase.org/) [Glazar et al. 47 #56]. Then we aligned the circRNA sequence to the human and mouse circRNAs by blastn with E value 1e–3. Meanwhile, we also adopted the conservation calculation method in Rybak-Wolf et al. 23 [Rybak-Wolf et al. 23 #2], and performed this analysis among three species: macaque, mouse, and human.

Analysis of the relationships between circRNAs and host mRNAs. To explore the expression relationship of circRNAs and their host mRNAs, we used the circRNA-seq data (GEO, GSE94027) [Xu et al. 18 #26] and the poly(A) selected RNA-seq data (GEO, GSE85377) [Liu et al. 48 #48]. The biological samples of the two studies were the same and total RNAs were extracted from the brain tissue of PFC and hippocampus of Kunming Institute of Zoology and the KPRC, Chinese Academy of Sciences (AAALAC accredited), and the methods were carried out in accordance with the approved guidelines. The meninges were removed, and cortical tissue was mechanically dissociated using steel scalp blades and washed in sterile phosphate-buffered saline (PBS). Cells were pelleted by centrifugation at 1200 × g for 10 min, and the pellet was subsequently enzymatically dissociated with 0.25% trypsin in the presence of DNase (50 μg/ml) at 37 °C for 30 min. Cells were further mechanically dissociated in a 0.1% trypsin solution and were washed in cold sterile PBS by centrifugation at 2200 × g at 4 °C for 10 min. The collected cell pellet was mechanically dissociated by trituration and further enzymatically dissociated with DNase (50 μg/ml) at RT for 10 min. After several further washes with cold sterile PBS, cells were seeded onto poly-D-lysine-coated glass coverslips or T75 flasks at a density of 1 × 10^6 cells/ml in DMEM/10% FCS, FDU (10 μM), a uridine analogue that is toxic to dividing astrocytes, was added to cultures to inhibit overproliferation of glial cells on days 5, 8, and 12 in vitro. Neurons were collected and prepared for biochemistry, immunocytochemistry, electrophysiology, and calcium imaging assays at indicated time.

RNA extraction and reverse transcription PCR. RNA was using PureLink micro-to-midi Total RNA isolation system (Invitrogen). RNA quantity and quality were measured by Nano Drop ND-1000 spectrophotometer (Nano Drop Thermo, Wilmington, DE) and RNA integrity was assessed by agarose gel electrophoresis. Specific divergent primers were designed to amplify the circular and linear Rhesus macaque transcripts. Semi-quantitative RT-PCR was performed with Superscript III one-step RT-PCR system with platinum Taq High Fidelity (Invitrogen). For quantitative real-time PCR, cDNAs were prepared by using oligonucleotide (dT), random primers, and a Thermos Reverse transcription kit (Signal way Bio-technology). qPCR was performed with SYBR Green 1 Master Roche (4707516001) on Light Cycler 480 II. qPCR was performed in 10–20 μl of reaction volume, including 2 μl of cDNA, 5 μl 2x Master Mix, 0.5 μl of Forward Primer (10 μM), 0.5 μl of reverse primer (10 μM), and 2 μl of double distilled water. The reaction was set at 95 °C for 10 min for pre-denaturation, then at 95 °C for 10 s, and at 60 °C for 60 s repeating 40 cycles. Gapdh was used as a reference. Both target and reference were amplification in triplicate wells. The level of each circular and linear transcript was calculated using the 2−ΔΔCt method. All PCR primer sequences can be found in Supplementary Data 3.

Subcellular fractionation. Cytoplasmic and nuclear fractional RNAs were extracted and prepared from postmortem fresh hippocampal tissues of 20-year-old male macaques according to the instruction of the Cytoplasmic and Nuclear RNA Purification Kit (Norgen). Extracted RNAs were subjected to RT-qPCR analysis to verify the subcellular localization of circGRIA1 with β-actin as the cytoplasm control and U6 as the nuclear control.

BASExcope assay for circRNA detection. BASExcope assays were performed using BaseScope Detection Reagent Kit-RED (#322900-USM, Advanced Cell Diagnostics (ACD) Hayward, CA) and circGRIA1 junction site-targeting or non-targeting labeled probes conjugated to HRP from ACD (#700001 and #700002). Briefly, 10-μm cryostat macaque brain sections were pretreated with hydrogen peroxide followed by performing target retrieval using an Oster™ Steamer. Dried slides were placed on the slide rack, and incubated with RNAscope® Protease III at 40 °C for 30 min in the HybEZ™ system (ACD). Next, the slides were incubated at 40 °C in order to hybridize probes (circGRIA1) for 2 h in HybEZ™ system. The slides were then performed signal amplification with the following steps: AMP 6 for 30 min; AMP 1 for 15 min; AMP 2 for 30 min; AMP 3 for 30 min; AMP 4 for 15 min; AMP 5 for 30 min; AMP 6 for 15 min. After each step, slides were washed with wash buffer three times at room temperature (RT). Chromogenic detection was performed using BASEscope® Fast RED followed by counterstaining with haematoxylin (American CytoChem Scientific). Two slides were collected using a Zeiss Olympus IX-81 microscope with either a 40× or 100× objective running Metamorph. For image analysis, regions of interest (50 × 50 μm) were manually drawn, and after background subtraction, BASExcope intensity was normalized to the control. Images were thresholded in ImageJ and, using the Image Calculator tool, a third image was generated showing those pixels which were positive in all input channels. Using the Particle Analysis tool, the size and number of the thresholded clusters were analyzed. Microsoft Excel was used to calculate the fraction of positive clusters. GraphPad Prism was used to perform ANOVAs and t-tests and to visualize bar charts. Error bars represent SD. The target genes, probed regions, and sequences of target probes are listed in Supplementary Table S1.

RNAscope assay for Gria1 mRNA detection. Detection of macaque Gria1 was performed on cryostat brain sections of PFC and hippocampus of rhesus macaques using RNAscope® Probe- Hs-GRIA1 (Cat No. 472441, ACD) and RNAscope® 2.5 LS Multiplex Fluorescent Reagent Kit v2 assays (323100-USM, ACD). Positive [RNAscope® 3-plex LS Multiplex Control Positive Probe—Min pol2A, PPBP, abc; ACD] and negative [Rn – Hs LS Multiplex Negative Control Probe DAPB; ACD] controls were performed in parallel. Slides were thawed at RT for 10 min before baking at 40 °C for 45 min. The sections were then post-fixed in pre-chilled 4% PFA for 15 min at 4 °C, washed in 3 changes of PBS for 5 min each before dehydration through 50%, 70% and 100% ethanol for 5 min each. The slides were air-dried for 5 min before loading onto a Bond Rx instrument (Leica Biosystems). Slides were prepared using the frozen slide delay prior to pretreatments using Eptiope Retrieval Solution 2 (Leica Biosystems) at 95 °C for 5 min, and ACD enzyme from the Multiplex Reagent kit at 40 °C for 10 min. Probe hybridization and signal amplification was performed according to manufacturer’s instructions. The following TSA plus fluorophores were used to detect corresponding RNAscope probes using a Bond Rx platform. After hybridization and signal amplification, the slides were post-fixed on the Bond Rx and mounted using Prolong Diamond (ThermoFisher Scientific). Slides were imaged on a CellDiscoverer 7 microscope (Zeiss). Gria1 positive cells were detected using the HALO FISH v2.1.6 analysis module based on intensity thresholds set using negative controls for both the fluorescein and Cy5 channels. Neurons detected as positive for Gria1 were checked by eye, and were only included in final analysis if there were three or more spots corresponding to Gria1 mRNA. The target genes, probed regions, and sequences of target probes are listed in the product information of RNAscope® Probe- Hs-GRIA1 (Cat No. 472441, ACD).

Dual DNA-RNA fluorescence in situ hybridization (FISH). circGRIA1 RNA probe was transcribed by the TranscriptAid T7 High Yield Fission (Thermo Scientific), with the corresponding insertion of circGRIA1 junction site in the T vector as a template for transcription, and were labeled with Alexa Fluor647 with the ULYSIS Nucleic Acid Labeling Kit (Invitrogen), which added a Fluor on every G of the probe to amplify the fluorescence intensity. Fixed neurons were washed in PBS, treated with RNase A at 37 °C for 30 min, and then neurons and circGRIA1 RNA probe were denatured at 80 °C for 15 min and then incubated at 42 °C for 24 h with human Cot-1 DNA (Life Technologies, final concentration 30 ng/μl). Slides were washed with 2x SSC at 45 °C for 10 min. FISH signal for circGRIA1 was detected with junction probe if not specified. For DNA-RNA dual-fluorescent RNA probes were hybridized with ISH DNA with the ACD protocol: Fluorescin (Akoya Biosciences), and Cy3 (Akoya Biosciences) were slides that were then removed from the Bond Rx and mounted using Prolong Diamond (ThermoFisher Scientific). Slides were imaged on a CellDiscoverer 7 microscope (Zeiss). Gria1 positive cells were detected using the HALO FISH v2.1.6 analysis module based on intensity thresholds set using negative controls for both the fluorescein and Cy5 channels. Neurons detected as positive for Gria1 were checked by eye, and were only included in final analysis if there were three or more spots corresponding to Gria1 mRNA. The target genes, probed regions, and sequences of target probes are listed in the product information of RNAscope® Probe- Hs-GRIA1 (Cat No. 472441, ACD).

Immunohistochemistry (IHC). For DAR/bright field staining, ten-micron cryostat sections of macaque brain were pretreated in 0.3% hydrogen peroxide in methanol for 10 min to remove endogenous peroxidase activity, rinsed in Tris-buffered saline (TBS), and then treated with 0.1-M citrate buffer in a microwave at sufficient power to keep the solution at 100 °C for 20 min. Sections were cooled in the same buffer at RT for 30 min and rinsed in TBS. Slides were incubated in 10% goat serum in PBS blocking solution for 1 h at RT, after which primary antibodies Gria1 and GAD were applied in sections that were blocked overnight. The sections were washed three times in TBS before applying the secondary antibody (Vector Laboratories). Secondary antibody was applied for 1 h at
promoter sequence was added to the reverse primer to obtain an antisense probe.

buffer for 30 min at RT followed by three rinses with PBS. Primary antibody GluR1 fraction of positive clusters. GraphPad Prism was used to perform

images were collected using a Zeiss Olympus IX-81 microscope with either a 40× or

circRNA speci

primers were designed using standard primer designing tools (Primer Premier 5.0)

chloroform extraction and subsequent precipitation. Probes were used at 50 ng/ml

in vitro transcription reaction. In vitro transcription was performed using the

DIG-RNA labeling mix (Roche) according to manufacturer

Protein extraction and western blot

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-17435-7 | www.nature.com/naturecommunications

Chromatin isolation by RNA purification (CHIRP). CHIRP or biotin-oligonucleotides pulldown was carried out according to the established protocol with modifications50. Briefly, fresh macaque brain tissues digested by 0.25% trypsin or fresh macaque hippocampal neurons were crosslinked with 1% formaldehyde in PBS for 10 min at RT, and cross-linking was then quenched with 0.125-M glycine in PBS for 5 min. The brains or neurons were pelleted and resuspended in buffer (0.1 M Tris, pH 7.0, 10 mM KOAc, and 15 mM MgOAc, with freshly added 1% NP-40, 1 mM DTT, complete protease inhibitor, and 0.1 μl RNase inhibitor) for 10 min on ice. Cell suspensions were then homogenized and pelleted at 2500 g for 5 min. Nuclei were further lysed in nuclear lysis buffer (50 mM Tris, pH 7.0, 10 mM EDTA, and 1% SDS) with freshly added 1 mM DTT, complete protease inhibitor, and 0.1 μl RNase inhibitor on ice for 10 min and were sonicated until most chromatin had solubilized and DNA was in the size range of 100–500 bp. Chromatin was diluted in two times volume with hybridization buffer (750 mM NaCl, 1% SDS, 50 mM Tris, pH 7.0, 1 mM EDTA, 15% formamide, 1 mM DTT, and 0.1% sodium dodecyl sulfate (SDS)). Biotinylated RNA probes were added to the buffer (100 pmol) were prepared with the ULYSIS Nucleic Acid Labeling Kit (MP 21650, Invitrogen) and added to 3 ml of diluted chromatin, which was mixed by end-to-end rotation at 37 °C for 4 h. M-280 Streptavidin Dynabeads (Life Technologies) were added after three times in nuclear lysis buffer which was blocked with 500 ng/ml RNA and 1 mg/ml BSA for 1 h at RT. Then washed three times in nuclear lysis buffer before being resuspended. 100 μl Dynabeads were added per 100 pmol of biotin-antisense oligos, and the whole mix was then rotated for 30 min at 37 °C. Beads were captured by magnets (Life Technologies) and washed five times with 40 μl of the volume of Dynabeads with wash buffer (2× SSC, 0.5% SDS, and 0.1 mM DTT). PMSE (fresl). Beads were then subjected to RNA elution and DNA elution.

Preparation of nascent RNAs. Nascent transcripts were isolated by fluorescent labeling of nascent RNA by use of the Click-IT Nascent RNA Capture Kit (ThermoFisher Scientific Inc.). Briefly, fresh macaque hippocampal neurons at indicated DIV were incubated for 24 h in medium (see above) containing 0.5 mM 5-ethyluridine (EU) at 37 °C, 5% CO2, 100% humidity. After incubation, neurons were once washed in PBS followed by proceeding to RNA purification using TRIzol. Next, biotinylation of RNA by the Click-IT reaction was performed. The biotinylation of EU-labeled RNA was precipitated and proceeded to bind biotinylated RNA to Dynabeads® MyOne™ Streptavidin T1 magnetic beads. The cDNA synthesis using the RNA captured on the beads as a template was immediately performed. The cDNA was stored at −20 °C until further use.

MRI-guided AAV viral particles microinjection in vivo. Three-paired 10- and 20-year-old male and female rhesus macaque weighing 6–10 kg were used in microinjection of the viral particles. The monkeys were housed in adjoining individual primate cages under typical conditions of humidity, temperature, and light and fed with standard monkey chow and daily supplements of fruit and vegetables to ensure their health and welfare. Animals were fasted overnight prior to the MRI sessions and surgery. 3.0 T MRI (uMR770, United Imaging) imaging was conducted to determine the microinjected location of hippocampus with a circular 12-channel coil. Before the scan, each macaque was sedated with xetamid (10 mg/kg, i.m.) and atropine (0.05 mg/kg, i.m.), anesthetized with pentobarbital (25 mg/kg, i.m.) and then placed into the scanner in the prone position. The whole-brain images were acquired with B0-image by use of the high-resolution sequence (TR = 16.6 ms, TE = 3.6 ms, Ti = 0 ms, NEX = 2, slice thickness = 0.5 mm, matrix size = 256 × 256, FOV = 124 × 124 mm) and a 3D T2-weighted sequence (TR = 2300 ms, TE = 3800.88 ms, Ti = 0 ms, NEX = 2, slice thickness = 0.5 mm, matrix size = 256 × 256, FOV = 124 × 124 mm). In order to design and optimize the target trajectory, all the coronal and sagittal brain images were analyzed by use of the software BrainSight (Rogue Research). One hemisphere received five intrahippocampal injections (Bregma −9.5/−12.6/−21.4/−14/−18 mm, ML +12.45/+9.5/+13.5/+/14.8/+15 mm, DV 34/34/33.5/30.50 mm) of 20 μl (total 100 μl) regulative AAV vectors (serotype AAV9 and titer ~ 3 × 1014 vg/ml), and the contralateral hemisphere (Bregma −9.5/−12.6/−21.4/−14/−18 mm, ML −12.5/−9.5/−13.5/−14.8/−15 mm, DV 34/34/33.5/30.50 mm) received control vectors (serotype AAV9 and titer ~ 3 × 1014 vg/ml). All surgical procedures were conducted under strict aseptic conditions. After the skull was exposed, holes were punched in the corresponding positions and the vectors were then infused through a 31-gauge Hamilton syringe with a 10-Gaussian sterile needle attached by use of a 27-Gaussian sterile instrument. A map of the microinjection sites is shown in Supplementary Fig. 7 according to the Macaque Scalable Brain Atlas with modifications (Author copy:...
and RNAscope ISH, and IHC was tested using either an unpaired excitation frequencies of 380 nm and are representative of three separate experiments single factor ANOVAs since the normality of the distribution was pretested using the Lilliefors test. No statistical methods were used to predetermine comparisons. The statistical significance was calculated by employing a template-matching algorithm. Recordings were started 5 min after patching and the recording duration usually ranged from 5 to 10 min. Only recording epochs in which series and input resistances varied <10% were averaged. At least ten neurons were subjected to recording for each group. Statistical comparisons were made using unpaired student’s t test; for multiple comparisons single factor ANOVAs followed by nonparametric Mann–Whitney U test were performed.

Chemically induced LTD (cLTP). Whole-cell voltage-clamp recordings were obtained from hippocampal cultures of fetal macaques as described below. Xenopus laevis tadpole oocytes were used for data acquisition and analysis. Neurons were transfected with plasmids to express HCN1 and HCN2 channels and were held at −70 mV. In whole-cell configuration, the excitation frequencies of 380 nm and are representative of three separate experiments single factor ANOVAs since the normality of the distribution was pretested using the Lilliefors test. No statistical methods were used to predetermine comparisons. The statistical significance was calculated by employing a template-matching algorithm. Recordings were started 5 min after patching and the recording duration usually ranged from 5 to 10 min. Only recording epochs in which series and input resistances varied <10% were averaged. At least ten neurons were subjected to recording for each group. Statistical comparisons were made using unpaired student’s t test; for multiple comparisons single factor ANOVAs followed by nonparametric Mann–Whitney U test were performed.

Calcium flux analysis. For calcium flux analysis, hippocampal cultures from fetal macaque were prepared as described above, seeded onto 110-mm-thick coverslips, and cultured as above. Cells were washed with PBS and loaded with 5-μM Fura-2 (Molecular Probes, Eugene, OR) for 1 h in a dark chamber at 37 °C. Cells were then washed with PBS, and DMEM/1% FCS was added to the cultures. Neurons were kept at 37 °C until analyzed for calcium flux responses (up to 1 h). For calcium flux analysis, coverslips were placed into a 37 °C warming chamber and examined under an inverted microscope connected to a spectrophotometer. Fetal macaque neurons with infection of the junction site-targeting siRNAs against circGRIA1 or the mismatched junction site-targeting siRNA control, respectively, were tested for response to CTZ. Neurons were stimulated with 25 μM glutamate before exposure to 100 μM CTZ. Calcium flux tracings were analyzed for the maximum increase in intracellular calcium according to the formula [Ca]₀ = [R − Rmin]/(Rmax − R), assuming a Kᵢ of 224 nM, and R is the ratio of fluorescence at 340 and 380 nm. Calcium concentrations are expressed as the mean ± S.D., and all data were presented as relative ratio of fluorescence at an emission frequency of 510 nm and excitation frequencies of 380 nm and are representative of three separate experiments performed under different conditions for each neuronal preparation.

Statistical analysis. Two-sided paired t test was performed to calculate the differentially expressed circRNAs. The statistical significance of the data of BASiScope and RNAscope ISH, and IHC was tested using either an unpaired t test or multiple comparisons single factor ANOVAs since the normality of the distribution was pretested using the Lilliefors test. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those generally employed in the field. Data collection and analysis were not performed blind to the conditions of the experiment and no randomization of data was performed.

Received: 6 May 2019; Accepted: 1 July 2020; Published online: 17 July 2020

References
1. Finch, C. E. & Morgan, D. G. RNA and protein metabolism in the aging brain. *Ann. Rev. Neurosci.*, 13, 73–88 (1990).
2. Shrivara Shetty, M. & Sajikumar, S. ‘Tagging’ along memories in aging: synaptic tagging and capture mechanisms in the aged hippocampus. *Aging Res. Rev.*, 35, 22–35 (2015).
3. Uchida, S. et al. Learning-induced and stathmin-dependent changes in microtubule stability are critical for memory and disrupted in aging. *Nat. Commun.*, 5, 4389 (2014).
4. Duda, P., Wojcicka, O., Winnie, J. R. & Rakus, D. Global quantitative TPA-based proteomics of mouse brain structures reveals significant alterations in expression of proteins involved in neuronal plasticity during aging. *Aging* 10, 1682–1697 (2018).
5. Coleman, P. D. & Flood, D. G. Neuron numbers and dendritic extent in normal aging and Alzheimer’s disease. *Neurobiol. Aging* 8, 521–545 (1987).
6. Shetty, M. S., Sharma, M. & Sajikumar, S. Chelation of hippocampal zinc enhances long-term potentiation and synaptic tagging/capture in CA1 pyramidal neurons of aged rats: implications to aging and memory. *Aging Cell* 16, 136–148 (2017).
7. Khachaturian, Z. S. Calcium, membranes, aging, and Alzheimer’s disease. *Introduction and overview. Ann. N. Y. Acad. Sci.* 568, 1–4 (1989).
8. Khachaturian, Z. S. The role of calcium regulation in brain aging: reexamination of a hypothesis. *Aging* 1, 17–34 (1989).
9. Gareri, P., Mattace, R., Nava, F. & De Sarro, G. Role of calcium in brain aging. *Gen. Pharmacol.* 26, 1651–1657 (1995).
10. Satriustegi, D., Bogonez, E., Vitorka, Z., Blanko, P. & Martinez-Serrano, A. [Changes in the calcium transport systems of rat brain synaptosomes and their possible role in the pathophysiology of aging]. *Fiziol. Zh.* 36, 42–50 (1990).
11. Foster, T. C., Kyritsopoulos, C. & Kumar, A. Central role for NMDA receptors in redox mediated impairment of synaptic function during aging and Alzheimer’s disease. *Behav. Brain Res.* 322, 223–232 (2017).
12. Deisseroth, K. & Dody, L. A. Alzheimer’s Association California Hypothesis Workinggroup. Calcium hypothesis of Alzheimer’s disease and brain aging: A framework for integrating new evidence into a comprehensive theory of pathogenesis. *Alzheimers Dement.* 13, 178–182.e117 (2017).
13. Cotman, C. W., Geddes, J. W., Bridges, R. J. & Monaghan, D. T. N-methyl-D-aspartate receptors and Alzheimer’s disease. *Neurobiol. Aging* 10, 603–605 (1989). discussion 618-620.
14. Nigro, I. M. et al. Scrambled exons. *Cell* 64, 607–613 (1991).
15. Hansen, T. B., Kjems, J. & Damgaard, C. K. Circular RNA and miR-7 in cancer. *Cancer Res.* 73, 5609–5612 (2013).
16. Jek, W. R. & Sharpless, N. E. Detecting and characterizing circular RNAs. *Nat. Biotechnol.* 32, 453–461 (2014).
17. Jek, W. R. et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 19, 141–157 (2013).
18. Memczak, S. et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495, 333–338 (2013).
19. Ashwal-Fluss, R. et al. circular RNA biogenesis competes with pre-mRNA splicing. *Nature* 570, 166–173 (2019).
20. Hansen, T. B. et al. miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. *EMBO J.* 30, 4414–4422 (2011).
21. Pamudurti, N. R. et al. Translation of Circular RNAs. *Mol. Cell* 66, 9–21. e7 (2017).
22. Legnini, I. et al. Circ-ZNF609 Is a Circular RNA that Can Be Translated and Mediated Cleavage of a Circular Antisense RNA. *Cell* 165, 607–616 (1991).
23. Rybak-Wolf, A. et al. Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. *Mol. Cell* 58, 870–885 (2015).
24. You, X. et al. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat. Neurosci.* 18, 603–610 (2015).
25. Gruner, H., Cortes-Lopez, M., Cooper, D. A., Bauer, M. & Miura, P. Circular RNA accumulation in the aging mouse brain. *Sci. Rep.* 6, 38907 (2016).
26. Xu, K. et al. Annotation and functional clustering of circRNA expression in rhesus macaque brain during aging. Cell Discov. 4, 48 (2018).
27. Gao, Y., Zhang, J. & Zhao, F. Circular RNA identification based on multiple seed matching. Brief. Bioinform. 19, 803–810 (2017).
28. Ji, P. et al. Expanded expression landscape and prioritization of circular RNAs in mammals. Cell Rep. 26, 3444–3460.e5 (2019).
29. Zhang, J. et al. Circular RNA profiling provides insights into their subcellular distribution and molecular characteristics in HepG2 cells. RNA Biol. 16, 220–232 (2019).
30. Li, Z. et al. Exon-intron circular RNAs regulate transcription in the nucleus. Nat. Struct. Mol. Biol. 22, 256–264 (2015).
31. Mostany, R. et al. Altered synaptic dynamics during normal brain aging. J. Neurosci.: Off. J. Soc. Neurosci. 33, 4094–4104 (2013).
32. Hof, P. R. & Morrison, J. H. The aging brain: morphomolecular senescence of cortical circuits. Trends Neurosci. 27, 607–613 (2004).
33. Kolb, B. & Whishaw, I. Q. Brain plasticity and behavior. Annu. Rev. Psychol. 49, 43–64 (1998).
34. Constantine-Paton, M. & Cline, H. T. LTP and activity-dependent synaptogenesis: the more alike they are, the more different they become. Curr. Opin. Neurobiol. 8, 139–148 (1998).
35. Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C. & Nelson, S. B. Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391, 892–896 (1998).
36. Burke, S. N. & Barnes, C. A. Neural plasticity in the ageing brain. Nat. Rev. Neurosci. 7, 30–40 (2006).
37. Lalanne, T., Oyryr, J., Farrant, M. & Sjostrom, P. I. Synapse type-dependent expression of calcium-permeable AMPA receptors. Front. Synaptic Neurosci. 10, 34 (2018).
38. Salzman, J., Gawad, C., Wang, P. L., Lacayo, N. & Brown, P. O. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PloS One 7, e30733 (2012).
39. Zhang, Y. et al. Circular intronic long noncoding RNAs. Mol. Cell 51, 792–806 (2013).
40. Kang, H. J. et al. Spatio-temporal transcriptome of the human brain. Nature 478, 483–489 (2011).
41. Weickert, C. S. et al. Transcriptome analysis of male-female differences in prefrontal cortical development. Mol. Psychiatry 14, 558–561 (2009).
42. Guo, J. U., Agarwal, V., Guo, H. & Bartel, D. P. Expanded identification and characterization of mammalian circular RNAs. Genome Biol. 15, 409 (2014).
43. Catterall W. A. Voltage-gated calcium channels. Cold Spring Harb. Perspect. Biol. 3, a003947 (2011).
44. Buonarati, O. R., Hammes, E. A., Watson, J. F., Greger, I. H. & Helf, J. W. Mechanisms of postsynaptic localization of AMPA-type glutamate receptors and their regulation during long-term potentiation. Sci. Signal. 12, eaar6889 (2019).
45. Zhang, Y. et al. The Biogenesis of Nascent Circular RNAs. Cell Rep. 15, 611–624 (2016).
46. Liang, D. & Wilusz, J. E. Short intronic repeat sequences facilitate circular RNA production. Genes Dev. 28, 2233–2247 (2014).
47. Glazer, P., Papavasileiou, P. & Rajewsky, N. circBase: a database for circular RNAs. RNA 20, 1666–1670 (2014).
48. Liu, S. et al. Annotation and cluster analysis of spatiotemporal- and sex-related IncRNA expression in Rhesus macaque brain. Genome Res. 27, 1608–1620 (2017).
49. Negishi, T., Ishii, Y., Kyuwa, S., Kuroda, Y. & Yoshikawa, Y. Primary culture of rhesus macaque monkey (Macaca fascicularis) fetuses. J. Neurosci. Methods 131, 133–140 (2003).
50. Chu, C., Qu, K., Zhong, F. L., Artandi, S. E. & Chang, H. Y. Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. Mol. Cell 44, 667–678 (2011).