Disturbed Prefrontal Cortex Activity in the Absence of Schizophrenia-Like Behavioral Dysfunction in *Arc/Arg3.1* Deficient Mice

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Arc/Arg3.1, an activity regulated immediate early gene, is essential for learning and memory, synaptic plasticity, and maturation of neural networks. It has also been implicated in several neurodevelopmental disorders, including schizophrenia. Here, we used male and female constitutive and conditional *Arc/Arg3.1* knock-out (KO) mice to investigate the causal relationship between *Arc/Arg3.1* deletion and schizophrenia-linked neurophysiological and behavioral phenotypes. Using *in vivo* local field potential recordings, we observed dampened oscillatory activity in the prefrontal cortex (PFC) of the KO and early conditional KO (early-cKO) mice, in which *Arc/Arg3.1* was deleted perinatally. Whole-cell patch-clamp recordings from neurons in PFC slices revealed altered synaptic properties and reduced network gain in the KO mice as possible mechanisms underlying the oscillation deficits. In contrast, we measured normal oscillatory activity in the PFC of late conditional KO (late-cKO) mice, in which *Arc/Arg3.1* was deleted during late postnatal development. Our data show that constitutive *Arc/Arg3.1* KO mice exhibit no deficit in social engagement, working memory, sensorimotor gating, native locomotor activity, and dopaminergic innervation. Moreover, adolescent social isolation, an environmental stressor, failed to induce deficits in sociability or sensorimotor gating in adult KO mice. Thus, genetic removal of *Arc/Arg3.1* *per se* does not cause schizophrenia-like behavior. Prenatal or perinatal deletion of *Arc/Arg3.1* alters cortical network activity, however, without overtly disrupting the balance of excitation and inhibition in the brain and not promoting schizophrenia. Misregulation of *Arc/Arg3.1* rather than deletion could potentially tip this balance and thereby promote emergence of schizophrenia and other neuropsychiatric disorders.

Key words: *Arc/Arg3.1*; excitation/inhibition; knock-out; local field potential; prefrontal cortex; schizophrenia

Significance Statement

The activity-regulated and memory-linked gene *Arc/Arg3.1* has been implicated in the pathogenesis of schizophrenia, but direct evidence and a mechanistic link are still missing. The current study asks whether loss of *Arc/Arg3.1* can affect brain circuitry and cause schizophrenia-like symptoms in mice. The findings demonstrate that genetic deletion of *Arc/Arg3.1* before puberty alters synaptic function and prefrontal cortex activity. Although brain networks are disturbed, genetic deletion of *Arc/Arg3.1* does not cause schizophrenia-like behavior, even when combined with an environmental insult. It remains to be seen whether misregulation of *Arc/Arg3.1* might critically imbalance brain networks and lead to emergence of schizophrenia.

Introduction

Schizophrenia is a debilitating neuropsychiatric disease with a high penetration (1%) of the population and a strong hereditary component. Although heterogeneous, the core behaviors characterizing schizophrenia are divided in positive symptoms, includ-

ing delusions and hallucinations, negative symptoms, including social withdrawal and anhedonia, and cognitive symptoms reflecting deficits in working memory and executive control (van Os and Kapur, 2009; Owen et al., 2016). The neurophysiological

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characteristics of the disease include altered brain activity patterns and uncoordinated inter-areal communication (Lisman, 2012; Uhlhaas and Singer, 2015; Hunt et al., 2017). Although psychosis commonly manifests during puberty and early adulthood, cognitive and neurophysiological phenotypes can be detected earlier, which has led to the prevalent view of schizophrenia as a neurodevelopmental disorder (Rapoport et al., 2012; Birnbaum and Weinberger, 2017). This view holds that the process of normal brain development is interrupted by polygenetic and environmental factors (van Os and Kapur, 2009; Modai and Shomron, 2016; Owen et al., 2016), which ultimately alter neurotransmitter systems, brain connectivity, and cognitive functions.

The activity-regulated gene *Arg3.1* (Link et al., 1995), also known as *Arc* (Lyford et al., 1995), plays an essential role in adult learning and memory and synaptic plasticity (Guzowski et al., 2000; Plath et al., 2006; Messaoudi et al., 2007; Jakkamsetti et al., 2013; El-Boustani et al., 2018; Gao et al., 2018). Additionally, *Arc/Arg3.1* also mediates forms of plasticity that are prominent during development, such as homeostatic synaptic scaling (Chowdhury et al., 2006; Shepherd et al., 2006), metabotropic glutamate receptor-dependent long-term depression (Wang et al., 2008) and synapse elimination (Mikuni et al., 2013). *Arc/Arg3.1* upregulation during early postnatal development shapes hippocampal oscillatory activity and learning capacity (Gao et al., 2018), likely by changing network connectivity and microarchitecture. This may also explain why misregulation of *Arc/Arg3.1* is linked to neurodevelopmental diseases, such as fragile X Syndrome (Niere et al., 2012; Ronesi et al., 2012) and Angelman syndrome (Greer et al., 2010; Cao et al., 2013; Mandel-Brehm et al., 2015). Previous studies on schizophrenia patients implicated *Arc/Arg3.1* as a molecular hub in synaptic gene networks whose abnormalities possibly play a significant role in the pathogenesis of schizophrenia (Kirov et al., 2012; Fromer et al., 2014; Purcell et al., 2014; Fernández et al., 2017). In addition, a recent study (Managò et al., 2016) reported schizophrenia-like behaviors and dysregulated dopaminergic transmission in *Arc/Arg3.1*-deficient mice (Wang et al., 2006). However, the presence of a neomycin cassette and an *EGFP* sequence in the genome of these mice can result in artifactual aberrations in gene expression and confound the conclusions of the study.

Here, we used independently generated constitutive and conditional *Arc/Arg3.1* knock-out (KO) mice to ask whether loss of *Arc/Arg3.1* by itself is indeed sufficient to evoke neurophysiological and behavioral dysfunctions of schizophrenia. Alterations in cortical activity, in particular, aberrant gamma oscillations (Lisman, 2012; Uhlhaas and Singer, 2015; Hunt et al., 2017) are considered an endophenotype of schizophrenia and can be observed in prodromal stages of the disease, before manifestations of behavioral symptoms. Hence, we recorded local field potential (LFP) in the prefrontal cortex (PFC) of anesthetized WT and KO mice and compared these to conditional KO mice in which *Arc/Arg3.1* was deleted after late postnatal development. We labeled synaptic proteins in the PFC and measured glutamatergic and GABAergic neurotransmission in PFC neurons to elucidate synaptic mechanisms underlying aberrant network activity and cognitive dysfunction. In addition, we assessed the behavior of adult

Arc/Arg3.1 KO mice in three benchmark tests of schizophrenia in rodents. Moreover, given the longstanding theory about the dysfunction of the dopamine system in schizophrenia (Toda and Abi-Dargham, 2007; Howes and Kapur, 2009; Howes et al., 2015), we examined the sensitivity to dopamine elevation and the dopaminergic innervation of the PFC and striatum. In humans, schizophrenia has a high rate of comorbidity with epilepsy (Mäkiyö et al., 1998; Qin et al., 2005; Clarke et al., 2012), which is recapitulated in several animal models of the disease (Fejgin et al., 2014). To investigate a possible association between *Arc/Arg3.1* deletion, epileptic activity, and schizophrenia, we performed electrocorticogram recordings from chronically implanted WT and KO mice while monitoring their activity over several days. Finally, we tested the interaction between *Arc/Arg3.1* deletion and an environmental stressor on schizophrenia-like behaviors.

Materials and Methods

Mice. Mice aged 3–5 months were kept in a vivarium with an inverted 12 h light/dark cycle (08:00–20:00 dark period) in groups, under standard housing conditions ($23 \pm 1^\circ\text{C}$, 40–50% humidity; food and water *ad libitum*). Animals were housed together in groups of 3–5 mice per cage. Mice subjected to social isolation were housed individually from postnatal day (P)35 until the day of the experiment. Both male and female mice were used in all experiments. All procedures were conducted in accordance with the German and European Community laws on protection of experimental animals and approved by the local authorities of the City of Hamburg. Experimenters were blind to the genotype until the conclusion of experiments and analysis.

Generation of constitutive and conditional *Arc/Arg3.1* KO mice. *Arc/Arg3.1* KO mice were generated as previously described (Plath et al., 2006). Along with the KO mice generation, floxed *Arc/Arg3.1* mutants (*Arc/Arg3.1^{fl/f}*) were generated in parallel and thoroughly validated for use in biochemical and behavioral experiments (Gao et al., 2018). Conditional *Arc/Arg3.1* gene ablation in the brain was accomplished by breeding *Arc/Arg3.1^{fl/f}* with the Cre recombinase transgenic mice (1) Tg(*CaMKIIα-cre*)1Gsc (Casanova et al., 2001) to obtain early conditional KO (early-cKO) mice, and (2) Tg(*CaMKIIα-cre*)T29–1Stl (Tsien et al., 1996) to obtain late conditional KO (late-cKO) mice. Breeding was performed in accordance with recommended procedure for LoxP-Cre recombination (Song and Palmiter, 2018). Briefly, in generation F1 *Arc/Arg3.1^{fl/f}* mice were crossed with *Arc/Arg3.1^{+/+,Cre+}* mice. In generation F2 *Arc/Arg3.1^{fl/+},Cre+* were crossed with *Arc/Arg3.1^{fl/+}* to obtain cKO mice. Mice with genotypes *Arc/Arg3.1^{+/+,Cre+}* and *Arc/Arg3.1^{fl/f},Cre+* were used for experiments as WT controls and cKOs, respectively. Mice were genotyped by PCR for *Arc/Arg3.1* and by PCR and copy number variation for *Cre*. Genotyping was performed between P5 and P7 and again after termination of the experiment.

In vivo LFP recording in the PFC. LFP recording and analysis were performed as previously described (Prox et al., 2013; Fazeli et al., 2017). In short, adult male mice were injected with 0.8 mg/g body weight urethane (10% w/v, in 0.9% sodium chloride; Sigma-Aldrich) and 0.05 μg/g body weight of buprenorphine (Temgesic). Initial anesthesia was induced with 4% isoflurane, which was decreased to 1.0–1.5% isoflurane during surgery. A linear 16-site silicon probe with a 100 μm inter-electrode distance and 177 μm² electrode surface (a1x16-5 mm-100–177; NeuroNexus Technologies) was vertically lowered into the PFC of the right hemisphere (1.5 mm anterior to bregma, 0.3 mm lateral to midline, 3.3 mm deep). Data from the probe was digitally filtered (0.5–9000 Hz bandpass) and digitized as 16-bit integers with a sampling rate of 32 kHz using a Digital Lynx 4SX data acquisition system (NeuraLynx). During the recording, anesthetic depth was monitored from breathing rates, twitching and electrophysiological properties. If required, additional 0.2 mg/g body weight urethane doses were given. The position of the probe, dipped in a DiI-solution before insertion, was verified post-mortem from NeuroTrace fluorescent DAPI-stained (Invitrogen) coro-

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nal slices. Analysis was performed from electrodes located in layer (L)5 of the infralimbic (IL) region of the PFC.

All *in vivo* data were analyzed and visualized in MATLAB (MathWorks) or NeuroScope (Hazan et al., 2006). LFPs were downsampled to 1.28 kHz from raw traces. Multitaper time-frequency spectra were computed using Chronux (<http://chronux.org>). One power spectrum per mouse was calculated from concatenated epochs (nFFT = 4096, nw = 1). Theta (3.8–6 Hz) and gamma (20–50 Hz) power was calculated for selected rapid eye movement (REM)-like epochs by integrating the area below the theta and gamma ranges.

In vitro recordings in PFC slices. Mice, 3–4 months old, were anesthetized with isoflurane and decapitated. Brains were rapidly removed and immersed in ice-cold slicing artificial CSF (slicing-ACSF), continuously gassed with a mixture of 95% O₂, 5% CO₂. Coronal slices (350 μ m) were cut (Microme 650H vibratome, ThermoFisher Scientific), immediately transferred into a holding chamber containing oxygenated warmed recording-ACSF and allowed to recover for 1–4 h at 36°C. Slicing-ACSF contained the following (in mM): 212 sucrose, 2.6 KCl, 1 CaCl₂, 3 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose. Recording-ACSF contained the following (in mM): 119 NaCl, 2.5 KCl, 2.5 CaCl₂, 1.3 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose, and was maintained at 37°C and pH 7.4.

Recordings were made in a submerged chamber continuously perfused with oxygenated recording-ACSF (3 ml/min) maintained at 35–37°C. Slices containing the PFC (bregma coordinates 1.4–2.2) were visualized with an inverted Olympus BX51WI microscope. A 4 $\times$ objective was used to localize L5 in the IL or prelimbic (PrL) regions of the PFC and to place a stimulating electrode (concentric stainless steel, 0.1 M Ω impedance) in L1 of the same region. Neurons in L5 were visualized and targeted for patching with infrared differential interference contrast microscopy at 63 $\times$ magnification. Pipettes (5–8 M Ω) were pulled from borosilicate glass and filled with a recording solution containing the following (in mM): 115 potassium gluconate, 4 KCl, 10 HEPES, 10 phosphocreatine-Na₄ ATP-Mg, 0.3 guanosine triphosphate, and 0.5% biocytin. Osmolarity was adjusted to 280–290 mOsm with KOH. This solution was chosen to increase the driving force for Cl[−] currents (calculated as −82 mV) and facilitate detection of spontaneous IPSCs (sIPSCs). Somatic whole-cell patch-clamp recordings were made in current- and voltage-clamp modes with a MultiClamp 700b amplifier (Molecular Devices). Signals were sampled at 10 kHz and filtered (3 kHz) using Digidata 1422 (Molecular Devices). Series resistance (range 12–40 M Ω) was monitored continuously but compensated only in the current clamp mode. Experiments where *R_s* changed >30% were excluded from analysis. Data were collected and visualized using the pCLAMP 10 software (Molecular Devices), exported and analyzed off-line, using Clampfit 10 and Igor Pro 6.3 (WaveMetrics). A typical experiment consisted of a series of hyperpolarizing and depolarizing currents steps in current-clamp, to determine the input resistance (*R_{in}*) of the cell and its action potential firing pattern and frequency. Subsequently, neurons were voltage clamped at membrane potential of −70 and −50 mV to measure spontaneous EPSCs (sEPSCs) and sIPSCs, respectively. sIPSCs recorded at −50 mV were consistently abolished following application of the GABA_A antagonist gabazine (10 μ M; Abcam). To measure evoked PSCs (ePSCs), neurons were clamped to −70 mV and a series of incremental current steps (0.1 ms, 10–50 μ A) was applied by a stimulus isolator (Iso-Flex, AMPI) to the extracellular electrode in L1. Finally, gabazine (10 μ M) was added to the ACSF and recordings of the sEPSC, sIPSCs, and ePSCs were repeated. At the end of experiments, slices were fixed in 4% paraformaldehyde (PFA) and processed, as previously published (Ohana et al., 2012), to reveal the biocytin in the recorded neurons. Stained neurons were carefully examined at the light microscope and their dendritic morphology and laminar and regional locations were noted. Only neurons with typical pyramidal morphology in L5 were selected for analysis (van Aerde and Feldmeyer, 2015). Few neurons were 3-dimensionally reconstructed at a 40 $\times$ magnification with the NeuroLucida system (MBF Bioscience).

Data analysis: for sEPSC and sIPSC analysis, current traces were filtered off-line at 1 kHz and sEPSCs and sIPSCs were detected automatically in Clampfit at a threshold of −5 and 5 pA, respectively. All events

were visually inspected by the experimenter. Traces of PSCs evoked by repeated stimuli (3–6 per stimulus intensity) were baseline subtracted and averaged. At each stimulus intensity, the averaged eEPSC trace evoked under gabazine was subtracted from the ePSC trace recorded under control ACSF to obtain a hypothetical evoked IPSC trace (eIPSC). The charge was calculated from the averaged ePSC, eEPSC, and eIPSC traces by integrating the current over an identical time window beginning 3 ms after stimulus and ending when the longer trace returned to baseline. The excitation/inhibition (E/I) ratio of the network was calculated by dividing the eEPSC charge carried by eIPSC. Experiments were included in the analysis, which contained a complete series of ePSC recordings at all current steps between 15 and 50 μ A (see Fig. 3*D, E*) in both ACSF and gabazine.

Adolescent social isolation. WT and *Arc/Arg3.1* KO mice were socially isolated by individual housing from P35 and onward. Behavioral tests were conducted when the mice were 3 months old (Niwa et al., 2013; Li et al., 2018).

Sociability and social novelty tests. This test was done in a three-chambered arena made of transparent Plexiglas (20 $\times$ 38.5 cm for the left and right side chambers, 12 $\times$ 38.5 cm for the middle chamber). Plexiglas walls separated the three chambers, which were connected by open doors in the walls. Two transparent plastic cylinders with holes were placed in the left and right chamber. During the habituation phase, each mouse was released in the middle chamber and allowed to explore the arena for 10 min every day for 2 d. In the sociability test, a Dummy Lego mouse and Novel Mouse 1 were placed underneath a plastic cylinder separately. A weighted object was placed on top to prevent the mice from climbing to the top or toppling the cylinder. The test mice were placed into the middle chamber for 10 min of free exploration. Two zones, centered on either cylinder with a radius 1.5 cm larger than the cylinders were defined as the close interaction zones. The duration the test mouse spent within these zones and in each chamber was recorded. The location of the Dummy Lego mouse or Novel Mouse 1 was pseudorandomized. Subsequently, the same test mouse was subjected to the social novelty test. In this test, the Dummy Lego mouse was replaced by Novel Mouse 2, and Novel Mouse 1 was kept in the same cylinder as in the sociability test. The test mouse was again released into the middle chamber and allowed to explore for another 10 min. The duration the test mouse spent within the close interaction zones and duration spent in exploring the three chambers was recorded. All the novel mice used in the experiments are wild types with gender and genetic background the same as the test mice. Videos were recorded, and mice exploration behaviors were tracked with EthoVision XT (Noldus Information Technology). Ethanol (35%) was used for cleaning the arena between subjects.

Y-maze continuous spontaneous alternation. The Y-maze apparatus consisted of three identical arms (length $\times$ width $\times$ height: 37.5 $\times$ 7.5 $\times$ 16 cm) made of opaque beige Plexiglas and interconnected at 120° from a central triangle. It was located in a room with diffuse lighting (40 lux in each arm). For each trial, mice were placed in one arm of the maze and allowed to explore freely for 5 min during which the number and sequence of arm entries were noted down. A total number of arm entries was used as a measure for activity. The number of alternations, defined as successive entries into each of the three arms as an overlapping triplet set was calculated. Percentage alternation scores were then computed as follows: spontaneous alternation (%) = [(Number of alternations)/(Total number of arm entries − 2)] $\times$ 100.

Prepulse inhibition of the acoustic startle reflex. Prepulse inhibition (PPI) of the acoustic startle reflex was examined in two startle response systems (SR-LAB, San Diego Instruments). The test platform consisted of a transparent cylindrical mouse enclosure mounted on a Plexiglas base and an accelerometer sensor connected at the base. This was housed inside a sound-attenuating chamber containing a light source and a sound generation system that emitted continuous background white noise (65 dB) and the various acoustic stimuli used during the test session. Before testing, startle calibration, and chamber standardization was conducted by mounting an SR-LAB Standardization Unit (San Diego Instruments) onto the animal enclosures to attain a reasonable waveform response (700 $\pm$ 15 mV) and to ensure that a given baseline response was similar for both test chambers.

The test session began by introducing the mice in the animal enclosures. Following a 5 min acclimation period, mice were first presented with six pulse-alone trials to habituate and stabilize their startle response. The animals were subsequently presented with 12 main blocks of trials, each comprising a mixture of 12 discrete trial types: pulse-alone trials, prepulse-plus-pulse trials, prepulse-alone trials, and trials in which no discrete stimulus other than constant background noise was presented. The discrete trial types within each block were presented in pseudorandom order with variable intertrial intervals ranging from 10 to 20 s. All stimuli were in the form of white noise with a rise time of ~ 1 ms presented against a constant background noise level of 65 dB. Pulse stimuli were 40 ms in duration and 120 dB in intensity. Prepulse stimuli were 20 ms in duration and were 69, 73, 77, 81, or 85 dB in intensity. This corresponded to +4, +8, +12, +16, or +20 dB units above background noise. There were, therefore, five possible combinations of prepulse-plus-pulse trials, for which a stimulus onset asynchrony of 100 ms between the two stimuli was used. The test session concluded with the presentation of six pulse-alone trials and lasted ~ 48 min.

Whole-body responses were transduced by the accelerometer sensor, digitized, and transmitted to a computer running the SR-LAB software. Startle reaction was analyzed by measuring the maximum ($V_{\max}$) of the waveform response (from 0 to 65 ms) to the stimulus for every trial. Percentage of PPI for each prepulse trial (69, 73, 77, 81, or 85 dB) was calculated according to the following formula: percentage PPI = [(mean startle response to pulse alone trials) – (mean startle response for prepulse-plus-pulse trials)] / (mean startle response to pulse alone trials) $\times 100$.

Locomotor activity and amphetamine sensitivity test in the open field. Spontaneous exploration and locomotor activity were evaluated in an open-field arena ($50 \times 50 \times 50$ cm) constructed from opaque white forex plates. In this test, mice are allowed to freely explore the arena for 1 h. Locomotor activity was video-recorded under dim indirect illumination (40 lux) by using EthoVision XT video-tracking program (Noldus Information Technology). To test the dopamine sensitivity, an independent group of mice was injected with D-amphetamine intraperitoneally (1.5–2.5 mg/kg body weight, Fagron). Locomotor activity was recorded for 10 min before injection and for 1 h after the injection. For all tests, the path length and velocity were analyzed. The arena was cleaned with 70% ethanol between subjects.

Immunofluorescence staining and confocal imaging. Adult mice were deeply anesthetized and transcardially perfused with 0.1 M PBS followed by 4% PFA. Brains were removed, postfixed with 4% PFA for 2–6 d, cryoprotected in 30% sucrose for 2 d, embedded in Tissue-Tek OCT (Sakura, Finetek) and sectioned ($20 \mu\text{m}$) on a cryostat (Hyrax C60, Microm). Free-floating sections were blocked with 10% horse serum, 0.2% bovine serum albumin (BSA), and 0.3% TritonX in PBS for 1 h and then incubated for 12 h (TH) or 48 h (synaptic proteins) in primary antibody solution containing 1% horse serum, 0.2% BSA, and 0.3% Triton X-100 in PBS. For synaptic clusters analysis, antibodies against the presynaptic marker synaptophysin were coapplied with antibodies against the post-synaptic scaffolding proteins PSD-95 (excitatory synapse marker) and gephyrin (inhibitory synapse marker). Primary antibodies used were as follows: mouse anti-TH monoclonal antibody (1:5000; IMMUNOSTAR, 22941), mouse anti-gephyrin (1:300; Synaptic Systems, 147 011), rabbit anti-PSD-95 (1:500; Invitrogen, 51-6900), guinea pig anti-synaptophysin 1 (1:1000; Synaptic Systems, 101004). Sections were subsequently incubated with fluorophore-conjugated secondary antibodies at room temperature for 2 h. Secondary antibodies used were as follows: goat anti-mouse AlexaFluor 555 (1:1000; ThermoFisher, A-21422), goat anti-mouse DyLight 633 (1:200; ThermoFisher, 35513), goat anti-rabbit AlexaFluor 555 (1:200; ThermoFisher, A-21428), and goat anti-guinea pig AlexaFluor 488 (1:200; ThermoFisher, A-11073). Sections were then rinsed and mounted with ProLong Gold Antifade Mountant with DAPI (Invitrogen, P36931) and stored in the dark.

Brain sections were imaged with a confocal microscope (TCS SP8, Leica). For quantification of TH immunoreactivity, stacks of 15 images at $1 \mu\text{m}$ increment steps were acquired at $20\times$ magnification and a 1 AU pinhole. Images were analyzed with FIJI blind to the genotypes. Images were background subtracted, and Z-projected to generate a summed-

intensity image. The mean TH immunofluorescence was measured from regions-of-interest (ROIs) positioned over the striatum or PFC and normalized to the ROI area.

Synaptic clusters were quantified in the PrL and IL regions of the PFC from three WT and three KO mice. Three brain sections per mouse were taken at bregma coordinates 1.7–1.9. Non-overlapping image stacks (12 per mouse) were captured in the cell-sparse and dendrites-enriched L1, using a 63×1.4 NA oil-immersion objective and a $2.4\times$ digital zoom with the pinhole set to 1 AU. Stacks of four consequent images were captured at 1024×1024 pixels and $0.25 \mu\text{m}$ z-steps increment, resulting in imaged region size of $76.88 \times 76.88 \times 1 \mu\text{m}$ and a voxel size of $0.075 \times 0.075 \times 0.25 \mu\text{m}$. Laser intensity, detector sensitivity and line averaging were optimized against sections stained with secondary antibodies only. Identical image acquisition parameters were applied to the sections of all mice. Image stacks were analyzed in Imaris 9.3 (Bitplane) using the Spot function and the MATLAB R2017 (MathWorks) extension for Spot colocalization. Images were background subtracted. Spots were automatically detected above a threshold of $0.2 \mu\text{m}$ diameter for PSD-95 and gephyrin and $0.3 \mu\text{m}$ for synaptophysin. Colocalization between synaptophysin and either gephyrin or PSD-95 spots was set at a maximal center-to-center distance of $0.7 \mu\text{m}$.

Kainic acid-induced seizures. Mice were taken from their cages and received an intraperitoneal injection of $20 \mu\text{g}$ kainic acid per gram body weight. From there on animals were monitored for seizure symptoms. Seizures were defined as having occurred when the animals showed typical symptoms of rearing with forelimb clonus, falling, and rotating. If animals did not show any of these symptoms within 30 min, they were administered additional equal or lower dosages of kainic acid until seizures occurred. The seizure strength was scored by the following criteria: (1) Strong seizure activity like clonus of forelimbs and head, rearing but no falling; (2) as in 1, but additionally falling; (3) as in 2, but more severe with additional jumping, uncontrolled circling or repetitive falling. The final dose of kainic acid injected ($\mu\text{g/g}$ body weight) was calculated from the sum of all injection volumes.

Electrocorticogram recording and analysis. Data were collected as previously described (Marguet et al., 2015). Telemetric electrocorticogram analyses were performed using implantable radio transmitters (models TA11EA-F20 or TA11ETA-F20, DataSciences International). Adult (at least 3 months old) male mice received 0.05 mg/kg buprenorphine (subcutaneously) for analgesia and were anesthetized with 1.5–2% isoflurane in 100% oxygen. Midline skin incisions were made on the top of the skull and in the neck. The transmitter body was implanted subcutaneously. The tips of the leads were placed 2 mm posterior to bregma and 1.8 mm right to the midline for the recording electrode, and 1 mm posterior to lambda and 1–2 mm left to the midline for the reference electrode. The electrodes were fixed with dental cement. Radio transmitters allowed simultaneous monitoring of electrocorticogram and motor activity in undisturbed, freely moving mice that were housed in their home cages. Telemetry data and corresponding video data were recorded 1 week after surgery at the earliest and were stored digitally using Ponemah software v5.1 (Data Sciences International). Electrocorticogram recordings lasted over 50 h, sampled at 500 Hz, with continuous video recording. Electrocorticograms were analyzed off-line in MATLAB R2014b (MathWorks).

To find any ictal activity, we first tried to automatically detect the largest nonpathological electrocorticogram pattern available, namely K-complexes. Detection settings for putative K-complexes were defined as having a positive and negative peak of at least $2\times$ the amplitude of baseline activity, at least 15 ms in length each and where the end of a positive peak necessarily needed to be followed by the start of a negative peak within 15 ms. An additional criterion was to restrict detection during slow-wave sleep. For this, the electrocorticogram was Butterworth filtered in the delta range ($0.5\text{--}3 \text{ Hz}$, third order). The instantaneous delta amplitude (moving average filter, width 2 s) at any putative K-complex needed to be $>95\%$ of the mean instantaneous delta amplitude of the entire recording. Then, any event that exceeded twice the median peak-to-peak amplitude of these detected putative K-complexes was investigated for possible signs of ictal activity.

For automatic REM-like sleep detection, the electrocorticogram was Butterworth filtered in the following frequency ranges: delta ($0.5\text{--}3 \text{ Hz}$,

Figure 1. Genetic deletion of *Arc/Arg3.1* prenatally or perinatally results in hypoactive PFC networks. **A**, Illustration of LFP recording in the PFC (top) and histological verification of Dil-coated probes (red) with DAPI staining shown in blue (bottom). **B**, Spectrograms of exemplary excerpts of PFC activity for WT, KO, and E-cKO mice. **C, D**, Reduced theta power in the PFC of KO mice. **E, F**, Reduced gamma power in the PFC of KO mice. **G, H**, E-cKO mice exhibit significantly reduced theta power in the PFC compared with their WT littermates. **I, J**, Significantly reduced gamma power in the PFC of E-cKO mice. Power spectra were calculated from the LFP during REM-like states. Means are plotted as continuous lines, and shades represent $\pm$ SEM. In corresponding boxplots, hourglass markers represent the median, 25th and 75th percentiles. Crosses mark outliers. WT: $n = 11$, KO: $n = 10$; WT: $n = 7$, E-cKO: $n = 10$. Mann–Whitney–Wilcoxon test was applied in **D, F, H**, and **J** with $*p < 0.05$, $**p < 0.01$.

third order), theta (6–11 Hz, third order), gamma (50–90 Hz, sixth order), and high frequency (150 high-pass, 14th order). The instantaneous amplitudes were further filtered with a moving average filter (width: 2 s), from which a mean was calculated for each frequency band. Putative REM-like epochs needed to meet the following criteria: (1) exceed 65 and 70% of the mean theta and gamma amplitude, respectively; (2) be <95 and 120% of the mean delta and high-frequency amplitude, respectively; (3) show an absence of any detected movement activity; (4) exceed 7.5 s in length; and (5) be $<120\%$ of the mean high-frequency amplitude 2 min before the putative REM-like epoch. Gaps between detected REM-like epochs <5 s in length were still determined to be part of a REM epoch. Putative REM-like epochs that were still detected in between bouts of activity were deemed false positives and excluded. One power spectrum per mouse was calculated from concatenated epochs (nFFT = 4096, $nw = 1$) and normalized for the full range of 0–250 Hz.

Radioactive in situ hybridization. Sagittal brain slices (14 μ m) were prepared using a cryostat and mounted on slides (Superfrost plus, ThermoFisher Scientific). *In situ* hybridization was performed as described before (Hermeijer et al., 2013). A fragment corresponding to nucleotides 2342–2923 of the *Arc/Arg3.1* three prime untranslated region (3' UTR) was used as a probe and specificity of signals was verified by comparing with KO. Probes labeled with [α - 35 S]-UTP were generated according to the manufacturer's instructions (Promega). Transcribed probes were cleaned using G-50 mini columns (GE Healthcare) and diluted in hybridization buffer (4 $\times$ SSC, 50% formamide, 1 M Denhardt's solution, 10% dextran sulfate, 0.5 mg/ml salmon sperm DNA, 0.25 mg/ml yeast t-RNA) to a concentration of 5000 cpm/ μ l. Mounted cryosections of brains were fixed in 4% PFA, acetylated, dehydrated, and hybridized at 55°C overnight. Ribonuclease A treatment was performed for 30 min at 37°C. Following a high stringency wash in 0.1 $\times$ saline sodium citrate buffer at 55°C, slides were exposed to x-ray films (Kodak Biomax MR; GE Healthcare Bioscience).

Immunoblotting. Cortical tissues were dissected and quick-frozen on dry ice and stored at -80°C until used. Dissected tissue was homogenized in lysis buffer containing 5 mM EDTA, 5 mM EGTA, 50 mM NaF, 1

mm Na_2VO_4 , 1% Triton X-100, 1 mM PMSF, 4 μ g/ml aprotinin, 1 μ g/ml leupeptin, and 200 ng/ml pepstatin A in PBS. Protein concentrations were determined using BCA assay (ThermoFisher, Pierce). Equal amounts of protein were electrophoresed on 12% SDS polyacrylamide gels and transferred to PVDF membranes. Western blots were blocked in 5% nonfat milk in PBS with 0.01% Tween and incubated overnight with a polyclonal anti-*Arc/Arg3.1* antibody (1:300,000; Synaptic Systems) or monoclonal anti-GAPDH antibody (1:500,000; Millipore). After secondary antibody incubation, bands were visualized using SuperSignal chemiluminescence reagent (ThermoFisher, Pierce). Immunopositive bands were detected by ImageQuant LAS 4000 (Fujifilm) and analyzed using ImageJ (NIH).

Experimental design and statistical analysis. Statistical tests used were as follows: two-tailed two-sample Student's *t* test, Mann–Whitney–Wilcoxon test, Kolmogorov–Smirnov test, two-way ANOVA with repeated measures and Bonferroni *post hoc* test. Type of test is indicated in the main text. Statistics were done with SPSS Statistics 19 (IBM) and OriginPro 2017 (OriginLab); $p < 0.05$ was considered as significant. All graphs were generated with OriginPro 2017, Igor Pro 6.3 (WaveMetrics), Adobe Illustrator CS5.5, and MATLAB R2014b/R2017b (MathWorks). Values presented in figures are mean $\pm$ SEM or median with 25th and 75th percentile, as indicated.

Results

Deletion of *Arc/Arg3.1* before but not during puberty results in reduced prefrontal network activity

Schizophrenia is widely considered a neurodevelopmental disease, caused by combined genetic deficits and environmental assaults inflicted during brain development (Rapoport et al., 2012; Birnbaum and Weinberger, 2017). Disrupted brain development often results in aberrant brain activity preceding disease onset and psychosis (Lisman, 2012; Uhlhaas and Singer, 2015; Hunt et al., 2017). This aberrant activity is particularly evident in the prefrontal cortex, a region implicated in schizophrenia pathology.

To examine whether genetic deletion of *Arc/Arg3.1* might affect network activity, we performed LFP recordings from the PFC of urethane-anesthetized WT and KO mice (Fig. 1A). LFP recordings during REM-like episodes revealed a significant loss of power in the theta (Mann–Whitney–Wilcoxon test, $U = 20$, $**p = 0.0076$, WT: $n = 11$, KO: $n = 10$; Fig. 1B–D) and gamma ($U = 20$, $*p = 0.022$; Fig. 1B,E,F) bands in KO mice.

To investigate whether the reduced network activity reflects a developmental effect of *Arc/Arg3.1*, we first bred the *Arc/Arg3.1*^{fl/fl} mice with *CaMKII α -Cre* mice (Casanova et al., 2001). The progeny of this breeding are cKO mice in which *Arc/Arg3.1* is irreversibly removed between P7 and 14 (Gao et al., 2018). We named these mice early-cKO. Remarkably, like KO mice, adult early-cKO mice also displayed significantly reduced theta ($U = 68$, $**p = 0.00758$, WT: $n = 7$, E-cKO: $n = 10$; Fig. 1B,G,H) and gamma ($U = 64$, $**p = 0.0027$; Fig. 1B,I,J) power in the PFC compared with their WT littermates.

We further assessed the effects of *Arc/Arg3.1* ablation during puberty, on network activity by using a second line of conditional *Arc/Arg3.1* KO mice in which *Arc/Arg3.1* was genetically deleted through *CaMKII α -Cre*-mediated recombination starting at P21

and completing ~P36 (Fig. 2*A–C*; Gao et al., 2018). The progeny of this breeding were named late-cKO mice. In contrast to the constitutive KO and early-cKO, LFP recordings in the PFC of late-cKO mice exhibited normal theta and gamma power, indistinguishable from their WT littermates (theta: $U = 64$, $p = 0.154$, gamma: $U = 63$, $p = 0.172$, WT: $n = 10$, L-cKO: $n = 10$; Fig. 2*D–H*). Compared with all WT and WT-controls pooled together, KO mice exhibited significantly reduced theta and gamma power ($p < 0.05$), whereas late-cKO exhibited unchanged gamma and theta power ($p > 0.05$).

Collectively, these findings show that expression of *Arc/Arg3.1* during early brain development is crucial for establishing normal oscillatory network activity in the PFC. Later deletion of *Arc/Arg3.1* had no overt effect on network activity in the PFC, similar to our findings in the hippocampus (Gao et al., 2018).

Altered excitatory and inhibitory synaptic transmission in the PFC of KO mice

Alterations in the function, size, or numbers of excitatory and inhibitory synapses affect network oscillations (Buzsáki and Wang, 2012) and are linked to schizophrenia and other neuropsychiatric disorders (Uhlhaas and Singer, 2015). To elucidate synaptic mechanisms underlying altered network oscillations in the KO mice we recorded spontaneous and evoked synaptic currents from L5 pyramidal neurons in PFC slices obtained from WT and KO mice (Fig. 3*A, B*).

To measure the strength and temporal overlap of excitation and inhibition, we recorded ePSCs in ACSF and the presence of the type A GABA ($GABA_A$) receptor antagonist gabazine. Example current traces recorded in WT and KO neurons are shown in Figure 3*C*. In the presence of gabazine, eEPSCs increased in amplitude and duration and displayed additional delayed peaks, indicative of recurrent excitation. Accordingly, the differential trace (eEPSC subtracted from ePSC; Fig. 3*C*) reflects predominantly the recurrent inhibitory current (eIPSC). As exemplified in Figure 3*C*, eEPSCs and eIPSCs in WT neurons were often much larger compared with KO neurons, although ePSCs recorded in ACSF were comparable. The mean net charge transferred by coactive excitatory and inhibitory synapses (Fig. 3*D*) increased gradually in both WT and KO neurons and was not significantly different between them (two-way ANOVA with repeated measures, genotype: $F_{(1,10)} = 0.53$, $p = 0.48$; intensity: $F_{(4,40)} = 1.47$, $p = 0.23$; interaction: $F_{(4,40)} = 0.83$, $p = 0.52$). In

Figure 2. Conditional deletion of *Arc/Arg3.1* late in development does not alter PFC network activity. **A**, *Arc/Arg3.1^{fl/f}* mice were bred with *Cre* recombinase carrying mice to generate L-cKO mice. **B**, Radioactive *in situ* hybridization with *Arc/Arg3.1* antisense probe, performed on sagittal sections of WT and L-cKO brains. Ablation of *Arc/Arg3.1* takes place between P21 and P36 in cKO mice. **C**, Representative Western blots of *Arc/Arg3.1* protein from the dissected cerebral cortex of adult WT, KO, and L-cKO mice. **D**, Spectrograms of exemplary excerpts of PFC activity for WT and L-cKO mice. **E**, **F**, Comparable theta power in WT and L-cKO mice. **G**, **H**, Indistinguishable gamma power in WT and L-cKO mice. Power spectra were calculated from the LFP during REM-like states. Means are plotted as continuous lines and shades represent $\pm$ SEM. In corresponding boxplots, hourglass markers represent the median, 25th and 75th percentiles. Crosses mark outliers. WT: $n = 10$, L-cKO: $n = 10$. Mann–Whitney–Wilcoxon test was applied in **F** and **H**; n.s., not significant.

Figure 3. Reduced recurrent excitatory and inhibitory synaptic currents in PFC slices from KO mice. **A**, Image of a patch-clamp recording from L5 neurons in a PFC slice with an extracellular stimulation electrode placed in L1. **B**, Examples of biocytin-filled and reconstructed WT and KO neurons with similar dendritic morphology and burst firing patterns. **C**, Examples of evoked PSC recorded in ACSF and gabazine and the subtracted eIPSC trace. Stimulus intensity was 50 μ A. Stimulus artifacts were replaced by dashed lines. **D**, The mean net charge during the ePSC (ACSF) was similar in WT and KO neurons. **E**, The mean excitatory and inhibitory charges were significantly larger in WT neurons compared with KO. **F**, The E/I charge ratio was not different between WT and KO neurons. Two-way ANOVA with repeated measures was applied in **D–F**. Symbols show mean $\pm$ SEM; $n = 6$ neurons from 3 mice per group; main genotype effect, $\#p < 0.05$; Bonferroni *post hoc*, $*p < 0.05$.

contrast, the mean excitatory and inhibitory charges increased abruptly at stimuli >20 μ A (WT) and 40 μ A (KO; Fig. 3*E*), and were significantly larger in the WT neurons (excitatory charge: two-way ANOVA with repeated measures, genotype: $F_{(1,10)} = 5.93$, $\#p = 0.035$; intensity: $F_{(4,40)} = 6.79$, $p < 0.001$; interaction: $F_{(4,40)} = 4.96$, $p = 0.002$; Bonferroni *post hoc*, $*p = 0.038$, 0.022 ,

Figure 4. Altered synaptic properties in the PFC of KO mice. **A**, Immunolabeling of gephyrin, PSD-95, and synaptophysin in the PFC of WT and KO mice exhibit reduced gephyrin clusters in the KO. **B**, **C**, Quantification of gephyrin and PSD-95 clusters colocalized (**B**) or non-colocalized (**C**) with synaptophysin. Gephyrin but not PSD-95 clusters were significantly reduced in the KO mice (unpaired two-tailed *t* test, $*p < 0.05$). **D**, **E**, Example traces of sEPSCs and sIPSCs recorded at -70 and -50 mV, respectively, from a WT (**D**) and KO (**E**) neuron. Boxed regions in the left traces are shown magnified on the right. **F–H**, Cumulative probability histograms of the sEPSC and sIPSC amplitudes (**F**, **I**), IEI (**G**, **J**), and decay τ (**H**, **K**). Significant changes in the shape of the distributions were detected for all parameters (Kolmogorov–Smirnov test, $***p < 0.001$). Insets, Averaged median values of the corresponding biophysical property. Only the sIPSC decay τ showed a significant reduction in the median values, with faster decay τ in the KO (unpaired two-tailed *t* test, $*p < 0.05$).

0.019; inhibitory charge: two-way ANOVA with repeated measures, genotype: $F_{(1,10)} = 6.48$, $\#p = 0.029$; intensity: $F_{(4,40)} = 6.69$, $p < 0.001$; interaction: $F_{(4,40)} = 5.34$, $p = 0.002$; Bonferroni *post hoc*, $*p = 0.033$, 0.018 , 0.019). Between 25 and 40 μ A, the mean excitatory and inhibitory charges in WT were ~ 10 - and 7-fold larger than in KO neuron, respectively. However, the ratio between the excitatory and inhibitory charges (E/I ratio; Fig. 3F) was similar in WT and KO neurons (two-way ANOVA with repeated measures, genotype: $F_{(1,10)} = 0.68$, $p = 0.43$; intensity: $F_{(4,40)} = 0.18$, $p = 0.95$; interaction: $F_{(4,40)} = 0.77$, $p = 0.55$). These data indicate that the excitatory recurrent network gain is reduced in PFC slices of KO mice, but the E/I balance is preserved. Reduced network gain in the KO slices can be the consequence of decreased synapse numbers, altered synaptic properties, or reduced rates of specific recurrent connections within the network.

We, therefore, first examined the number of synapses in the PFC by immunolabeling of the presynaptic marker synaptophysin, the excitatory postsynaptic marker PSD-95 and the inhibitory postsynaptic protein gephyrin (Fig. 4A). Labeling revealed a significant reduction in colocalized (Fig. 4B) and non-colocalized (Fig. 4C) gephyrin clusters in the KO PFC sections (unpaired two-tailed *t* test, colocalized: $t_{(70)} = 2.06$, $*p = 0.043$, $n = 36$ ROIs, 3 mice per group; non-colocalized: $t_{(70)} = 2.01$, $*p = 0.047$, $n = 36$ ROIs, 3 mice per group) without a change in the number of PSD-95 clusters (unpaired two-tailed *t* test, colocalized: $t_{(70)} = 0.18$, $p = 0.86$, $n = 36$ ROIs, 3 mice per group; non-colocalized: $t_{(70)} = 0.013$, $p = 0.99$, $n = 36$ ROIs, 3 mice per group; Fig. 4A–C).

Next, we analyzed sEPSCs recorded at -70 mV (Fig. 4D,E, top traces) and sIPSCs at -50 mV (Fig. 4D,E, bottom traces). Example traces show a high-frequency of sEPSCs and a lower frequency of sIPSC in both WT and KO neurons (Fig. 4D,E). Cumulative histograms show differences between WT and KO sEPSCs only at the right tail of the distributions, indicating the presence of more events with larger amplitudes (Kolmogorov–Smirnov test, $***p < 0.001$; Fig. 4F), shorter inter-event intervals (IEIs; Kolmogorov–Smirnov test, $***p < 0.001$; Fig. 4G) and faster decay time constants (Kolmogorov–Smirnov test, $***p < 0.001$; Fig. 4H) in the KO neurons. The median values per neuron were not different between WT and KO for any of the measured properties (unpaired two-tailed *t* test, amplitude: $t_{(21)} = -0.99$, $p = 0.33$, IEI: $t_{(21)} = 1.30$, $p = 0.21$, decay τ : $t_{(21)} = 1.60$, $p = 0.13$; WT: $n = 11$, KO: $n = 12$; Fig. 4F–H, insets). Comparisons of the sIPSCs amplitude and IEIs between WT and KO neurons indicated fewer events with large amplitudes (Kolmogorov–Smirnov test, $***p < 0.001$; Fig. 4I) and longer IEIs (Kolmogorov–Smirnov test, $***p < 0.001$; Fig. 4J) in the KO neurons but equal median values in the WT and KO neurons (unpaired two-tailed *t* test, amplitude: $t_{(21)} = 0.86$, $p = 0.39$, IEI: $t_{(21)} = -0.36$, $p = 0.72$; WT: $n = 12$, KO: $n = 11$; Fig. 4I,J, insets). In contrast, the decay time was significantly shorter over the entire distribution of sIPSCs in the KO neurons (Kolmogorov–Smirnov test, $***p < 0.001$; Fig. 4K). Accordingly, the median values of the sIPSC decay τ were significantly reduced compared with the WT (unpaired two-tailed *t* test, decay τ : $t_{(21)} = 2.39$, $*p = 0.026$; Fig. 4K, inset). Together, these data suggest that the majority of excitatory synapses onto PFC L5 neurons have similar properties in WT and KO mice. In contrast, KO inhibitory synapses have distinctly faster kinetics.

The reduced PFC activity in the KO mice might result in schizophrenia-like behavior. To test this possibility, we subjected both WT and KO mice to a battery of behavioral benchmark tests of schizophrenia.

Prenatal ablation of *Arc/Arg3.1* does not affect social behaviors in adult mice

Patients with schizophrenia frequently display abnormal social behaviors, typically expressed by reduced social engagement (Owen et al., 2016). We, therefore, tested *Arc/Arg3.1* KO mice in a three-chamber social interaction arena. During the habituation phase, mice were released into the central chamber and allowed to explore the arena for 10 min per day for 2 consecutive days (Fig. 5A). The time spent in each chamber was measured. Mice preferred to explore the two chambers with cylinders and spent comparable time in each chamber (time in chambers left vs right, paired two-tailed *t* test, WT: $t_{(8)} = -0.75$, $p = 0.47$, $n = 9$; KO: $t_{(8)} = -0.33$, $p = 0.75$, $n = 9$; Fig. 5B) with no difference between WT and KO mice, suggesting unbiased place exploration. To test the sociability, a dummy mouse made from Lego (Dummy Mouse) and an unfamiliar mouse (Novel Mouse 1) were placed into the cylinders in the left and right chamber, respectively (Fig. 5A). The test mouse was then free to explore all the chambers. Normal sociability is indicated by a clear preference to interact with the unfamiliar mouse (Novel Mouse 1) rather than the object (Dummy Mouse). Analysis of the close interaction time measured as time spent in the immediate vicinity of the chamber confirmed that both WT and KO mice preferably interacted with an unfamiliar living mouse rather than an object (close interaction time Dummy Mouse vs Novel Mouse 1, paired two-tailed *t* test, WT: $t_{(8)} = -6.43$, $***p = 0.0002$; KO: $t_{(8)} = -5.43$, $***p = 0.00062$; Fig. 5C). No genotype difference was observed in this test (close interaction time with Novel Mouse 1 WT vs KO, unpaired two-tailed *t* test, $t_{(16)} = 1.44$, $p = 0.17$), indicating normal sociability for KO mice. To assess whether mice could distinguish a newly introduced mouse from a previously encountered mouse, mice were subjected to social novelty test in which the Dummy Mouse was replaced with a second stranger mouse (Novel Mouse 2; Fig. 5A). Proper recognition is indicated by preference to the second novel mouse. Again, WT and also KO mice spent significantly more time interacting with the Novel Mouse 2 (close interaction time Novel Mouse 1 vs Novel Mouse 2, paired two-tailed *t* test, WT: $t_{(8)} = -2.61$, $*p = 0.03$; KO: $t_{(8)} = -4.40$, $**p = 0.0023$; Fig. 5D), suggesting a comparable and intact social recognition ability (close interaction time with Novel Mouse 2 WT vs KO, unpaired two-tailed *t* test, $t_{(16)} = -0.11$, $p = 0.92$; Fig. 5D). Notably, because the social recognition test was held 10 min after the sociability test, these data also demonstrate intact short-term memory for the *Arc/Arg3.1* KO mice, in agreement with our previous report (Plath et al., 2006).

Figure 5. Prenatal ablation of *Arc/Arg3.1* does not cause deficits in social engagement, working memory, or sensorimotor gating in adult mice. **A**, Illustration of habituation, sociability, and social novelty tests in the social interaction arena. **B**, WT and KO mice spent a comparable amount of time exploring the two chambers with the cylinder, with no place bias during the habituation phase. **C**, Both WT and KO mice preferably interacted with the Novel Mouse 1 over the Dummy Mouse during the sociability test. **D**, Both WT and KO mice preferably interacted with Novel Mouse 2 over Novel Mouse 1 during the social novelty test. All WT: $n = 9$, KO: $n = 9$. **E**, Illustration of Y-maze test. Visiting of arms in order 1–2–3 is an example of alternation, whereas 1–2–1 of non-alternation. **F**, Similar number of total arm entries in WT and KO mice. **G**, WT and KO mice made comparable percentage of spontaneous alternations. All WT: $n = 10$, KO: $n = 10$. **H**, Illustration of startle response to 120 dB auditory stimulation. **I**, Illustration of prepulse inhibition test. **J**, Strong startle responses were observed in both WT and KO mice upon 120 dB stimulation. **K**, Comparable PPI in WT and KO mice at various prepulse intensities. **L**, No difference of mean percentage of PPI in WT and KO mice. All WT: $n = 10$, KO: $n = 10$. Significance was tested with paired *t* test within groups and two-tailed two-sample *t* test between groups in **B–D**. Two-tailed two-sample *t* test was performed in **F**, **G**, **J**, and **L**. Two-way ANOVA with repeated measures was applied in **K**. Bars show mean $\pm$ SEM; n.s., not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Prenatal ablation of *Arc/Arg3.1* does not cause a working memory deficit in adult mice

Deficits in working memory observed in patients are considered a cognitive impairment in schizophrenia (Lett et al., 2014). Therefore, we assessed mice behavior in the Y maze, which is frequently used to test working memory in rodents (Dember and Fowler, 1958; Lalonde, 2002; Sanderson and Bannerman, 2012). In this test, mice are free to move on a three-armed maze and naturally alternate between recently visited arms to avoid visiting the same arm repeatedly. The percentage of spontaneous alternations serves as a proxy of working memory (Fig. 5E). WT and KO mice

Figure 6. Prenatal ablation of *Arc/Arg3.1* does not alter the spontaneous locomotor activity and dopaminergic innervation but transiently increases the response to amphetamine. **A**, Spontaneous locomotor activity was comparable in WT and KO mice as indicated by total distance moved during 1 h open-field test (WT: $n = 12$, KO: $n = 8$). **B–F**, Locomotor activity following injection of amphetamine (AMP) at concentrations 1.5–2.5 mg/kg body weight. **B**, Injection of 1.5 mg/kg amphetamine did not increase locomotion in either WT or KO mice. **C**, Amphetamine at 2 mg/kg elicited a transiently increased locomotor activity in both WT and KO mice, that was significantly higher in the KO mice during the first 5 min (WT: $n = 8$, KO: $n = 9$). **D**, Comparable total path length in WT and KO mice during 1 h exploration after injection with 2 mg/kg amphetamine. **E**, Amphetamine at 2.5 mg/kg elicited a stronger but still transient increase of locomotion in WT and KO mice (WT: $n = 8$, KO: $n = 9$). **F**, Comparable total path length in WT and KO mice during 1 h exploration after injection with 2.5 mg/kg amphetamine. **G, H**, Quantification of TH-immunoreactivity shows comparable signal in (**G**) prefrontal cortex (WT: $n = 12$ slices from 4 mice, KO: $n = 11$ slices from 4 mice) and (**H**) striatum of WT and KO mice (WT: $n = 12$ slices from 4 mice, KO: $n = 12$ slices from 4 mice). Shown are mean $\pm$ SEM (**A–F**), and median, 25th and 75th percentile, min and max value (**G, H**). Significance was tested with two-way ANOVA with repeated measures in **A–C** and **E**. Bonferroni *post hoc* was performed to test the difference between groups in **C** and **E**; $*p < 0.05$. Significance was tested with two-tailed two-sample *t* test between groups in **D** and **F–H**; n.s., not significant.

made similar numbers of arm entries (unpaired two-tailed *t* test, WT vs KO: $t_{(18)} = 0.41$, $p = 0.69$, $n = 10$, 10, respectively; Fig. 5F), indicating a similar exploratory drive. WT and KO mice also exhibited a similar percentage of spontaneous alternations (unpaired two-tailed *t* test, WT vs KO: $t_{(18)} = -0.91$, $p = 0.37$, $n = 10$, 10, respectively; Fig. 5G), indicating intact and indistinguishable performance in this working memory test.

Prenatal ablation of *Arc/Arg3.1* does not affect sensorimotor gating ability in adult mice

PPI of the acoustic startle response is frequently used to detect sensorimotor gating deficits linked to psychiatric disorders (Braff et al., 2001; Swerdlow et al., 2008). Schizophrenia patients reproducibly display reduced auditory PPI (Mena et al., 2016; Haß et al., 2017; Swerdlow et al., 2018). PPI has also been adapted for use on rodent models as a biomarker of schizophrenia-like phenotypes (Swerdlow et al., 2008). Given the effectiveness and sensitivity of this paradigm for schizophrenia phenotype screening, we assessed the degree of PPI for the *Arc/Arg3.1* KO mice. In this test, an intense acoustic pulse alone leads to a strong startle response indicated by the peak amplitude of the whole-body acceleration measured by a piezoelectric sensor (Fig. 5H). Both WT and KO mice generated strong startle responses to the pulse stimulus (120 dB) with no difference between them, suggesting that KO mice had normal auditory perception like WT mice (unpaired two-tailed *t* test, WT vs KO: $t_{(18)} = 0.22$, $p = 0.83$, $n = 10$, 10, respectively; Fig. 5J). If a weaker pulse (henceforth the prepulse) is applied before the intense pulse, it leads to a reduced startle response (Fig. 5I). This inhibition effect produced by the prepulse presentation is termed PPI and reflects the sensorimotor gating ability of the mouse (Clapcote et al., 2007). *Arc/Arg3.1* KO mice consistently showed PPI at different prepulse intensities (two-way ANOVA with repeated measures, genotype: $F_{(1,18)} = 0.61$,

$p = 0.45$; prepulse intensity: $F_{(4,72)} = 41.53$, $p < 0.0001$; interaction: $F_{(4,72)} = 0.38$, $p = 0.82$; Fig. 5K) that were comparable to their WT littermates (unpaired two-tailed *t* test, WT vs KO: $t_{(18)} = -0.78$, $p = 0.45$, $n = 10$, 10, respectively; Fig. 5L). These data indicate that the sensorimotor gating ability is not diminished in the *Arc/Arg3.1* KO mice.

Prenatal ablation of *Arc/Arg3.1* does not alter spontaneous locomotor activity and only transiently increases the response to amphetamine

Additional biological traits identified in patients with schizophrenia are locomotor anomalies (Sano et al., 2012) that are traced to hypersensitivity to dopaminergic stimulation (Seeman et al., 2005). To test spontaneous locomotor activity, we first subjected *Arc/Arg3.1* WT and KO mice to a long-term open field test in which natural exploratory activity was recorded for 1 h. No difference was observed between WT and KO mice as indicated by a similar path length (two-way ANOVA with repeated measures, genotype: $F_{(1,18)} = 2.37$, $p = 0.14$; time: $F_{(10,180)} = 38.21$, $p < 0.0001$; interaction: $F_{(10,180)} = 1.36$, $p = 0.20$, WT: $n = 12$, KO: $n = 8$; Fig. 6A) throughout the testing time, demonstrating that KO mice were neither hyperactive nor hypoactive. To test locomotor activity upon dopaminergic stimulation, we injected independent cohorts of mice with amphetamine (1.5–2.5 mg/kg body weight, i.p.) to evoke dopamine release. Locomotor activity was recorded for 10 min before and 1 h after the injection. The lowest dose of amphetamine (1.5 mg/kg) did not elicit any increase of locomotion in neither WT nor KO mice (Fig. 6B). However, higher doses (2 and 2.5 mg/kg) elicited a transient increase of locomotion in both genotypes, which was slightly stronger in the KO mice during the first 5 or 10 min after injection, respectively (genotype: $F_{(1,15)} = 0.32$, $p = 0.58$; time: $F_{(11,165)} = 10.16$, $p < 0.0001$; interaction: $F_{(11,165)} = 2.84$, $p = 0.002$, WT: $n = 8$,

KO: $n = 9$; Fig. 6C; genotype: $F_{(1,15)} = 2.22$, $p = 0.16$; time: $F_{(11,165)} = 15.24$, $p < 0.0001$; interaction: $F_{(11,165)} = 1.31$, $p = 0.23$; Fig. 6E; Bonferroni *post hoc* test, $*p < 0.05$, WT: $n = 8$, KO: $n = 9$). However, no significant difference was detected in the total path length of WT and KO mice over the 1 h exploration after amphetamine injection (unpaired two-tailed t test, WT vs KO: $t_{(15)} = -0.57$, $p = 0.58$; Fig. 6D; $t_{(15)} = -1.49$, $p = 0.16$; Fig. 6F). These data suggest that the spontaneous locomotor activity was not altered in the *Arc/Arg3.1* KO mice, and the sensitivity to amphetamine was only transiently increased.

Prenatal ablation of *Arc/Arg3.1* does not disrupt dopaminergic innervation in the prefrontal cortex and the striatum

To further investigate whether prenatal ablation of *Arc/Arg3.1* could affect dopaminergic neurotransmission, we proceeded to directly measure dopaminergic innervation in the PFC (Fig. 6G) and striatum (Fig. 6H). We selected tyrosine hydroxylase, the enzyme for catalyzing dopamine synthesis as a marker, and performed immunofluorescent staining on both WT and KO slices. Dopaminergic fiber intensity was measured from confocal stack images. Interestingly, the dopamine axon density did not differ between WT and KO mice, neither in the prefrontal cortex (Mann–Whitney–Wilcoxon test, $U = 75$, $p = 0.61$, WT: $n = 12$ slices from 4 mice, KO: $n = 11$ slices from 4 mice; Fig. 6G) nor the striatum (Mann–Whitney–Wilcoxon test, $U = 89$, $p = 0.35$, WT: $n = 12$ slices from 4 mice, KO: $n = 12$ slices from 4 mice; Fig. 6H), suggesting unaltered dopaminergic projections in the absence of *Arc/Arg3.1*.

Absence of epileptic brain activity and reduced seizure susceptibility in *Arc/Arg3.1* KO mice

Comorbidity of schizophrenia and epilepsy are common in humans and animal models (Clarke et al., 2012). To further investigate the possibility that aberrant neural activity and enhanced seizure susceptibility might increase the risk of developing schizophrenia-like phenotypes in the KO mice, we performed telemetric electrocorticogram recordings combined with video- and activity-monitoring in freely behaving WT and KO mice for a total duration of 50 h (Fig. 7A–F). No behavioral abnormality, which could be associated with epileptic activity, was observed in any of the mice. Moreover, electrocorticograms from individual WT and KO mice (Fig. 7A) show similar patterns of electrographic activity. Notably, no continuous bands of low-frequency activity were observed in WT or KO mice, indicating the absence of epileptiform activity.

To investigate the possible occurrence of an inter-ictal activity, such as abnormally large single or multiple spikes in the electroencephalogram, we first detected putative K-complexes, which are large biphasic waves normally occurring in the cortex during sleep (Amzica and Steriade, 1997; Marini et al., 2004). Similar numbers of putative K-complexes were detected in WT

Figure 7. Absence of epileptic brain activity and reduced seizure susceptibility in *Arc/Arg3.1* KO mice. **A**, Two exemplary spectrograms of a WT (top) and a KO mouse (bottom) show ~50 h recording sessions, together with the corresponding activity patterns (below spectrograms). No continuous epileptiform or sharp-wave high-frequency bands were observed in any of the mice. **B, C**, Normal cortical events (K-complexes) were readily detected in WT (**B**) and KO (**C**) mice. The red horizontal lines indicate the detection threshold ($\times 2$ baseline mean), green and red vertical lines indicate the start and stop of the detected events, respectively. **D**, Similar numbers of putative K-complexes were found in WT and KO mice. Note that larger events reflecting possible epileptiform or ictal-like activity were not detected in any of the mice. **E**, Examples of putative REM epochs are shown. **F**, Normalized power spectra from two WT (black) and three KO mice (red) are shown, all revealing a theta and two separated gamma peaks and no abnormal peaks in the KO. **G**, A higher dosage of kainic acid is required to evoke generalized seizures in KO mice (WT: $n = 29$, KO: $n = 21$). $*p < 0.05$.

and KO mice (Fig. 7B–D). No event, larger than the putative K-complexes, which could reflect inter-ictal activity, was detected in any of the mice, confirming that both WT and KO mice were free of epileptiform or inter-ictal brain activity. We further analyzed REM-epochs of the recordings to detect possible abnormal activity during sleep. Power spectra of two WT and three KO mice all show expected theta and low- and high-gamma bands (Fig. 7E,F). Moreover, KO mice did not show an additional pronounced peak at low frequencies (1–30 Hz), which is typical of epileptiform and ictal activity (Peters et al., 2005; Shin et al., 2008; Jefferys, 2010). We then examined the susceptibility of WT and KO mice to kainic acid-induced seizures. Surprisingly, a significantly higher dosage of kainic acid was required to evoke seizures in the KO mice (WT: 27.2 ± 2.1 $\mu\text{g/g}$, $n = 29$; KO: 35 ± 0.3 $\mu\text{g/g}$, $n = 21$; unpaired two-tailed t test, $*p = 0.043$; Fig. 7G), suggesting a reduced susceptibility to seizure induction. In summary, our KO mice lacked any sign of epileptiform activity and were, in contrast, more resistant to induction of seizures.

Conditional deletion of *Arc/Arg3.1* late in development does not affect seizure susceptibility, social behavior, or amphetamine sensitivity

Although PFC activity was intact in late-cKO mice, absence of *Arc/Arg3.1* in the adult brain could reduce brain plasticity, similar to knockdown of *Arc/Arg3.1* in adult rats (Guzowski et al., 2000; Messaoudi et al., 2007) and thereby induce schizophrenia-like behaviors. To test this possibility, we subjected late-cKO mice to several behavioral tests. First, we examined the dosage of kainic acid required to evoke generalized seizures in WT and late-cKO

Figure 8. Conditional deletion of *Arc/Arg3.1* late in development does not affect seizure susceptibility, social behavior, and amphetamine sensitivity. **A**, Comparable dosage of kainic acid is required to evoke generalized seizures in WT and L-cKO mice (WT: $n = 12$; L-cKO: $n = 14$). **B–C**, Sociability and social novelty tests in L-cKO mice. **B**, Both WT and L-cKO mice preferably interacted with Novel Mouse 1 rather than the Dummy Mouse during sociability test. **C**, Both WT and L-cKO mice preferably interacted with Novel Mouse 2 rather than Novel Mouse 1 during social novelty test. All WT: $n = 7$, L-cKO: $n = 7$. **D**, Spontaneous locomotor activity was comparable in WT and L-cKO mice as indicated by similar path length during 1 h open-field test (WT: $n = 8$, L-cKO: $n = 8$). **E**, Indistinguishable locomotor response in WT and L-cKO mice when injected with 2.5 mg/kg body weight amphetamine intraperitoneally (WT: $n = 8$, L-cKO: $n = 8$). **F**, Similar total path length was generated in WT and L-cKO mice during 1 h exploration after injection with 2.5 mg/kg amphetamine. Bars show mean $\pm$ SEM. Significance was tested with two-tailed two-sample t test between groups in **A–C** and **F**. Paired t test was performed within groups in **B** and **C**. Two-way ANOVA with repeated measures was performed in **D** and **E**. n.s., not significant, $*p < 0.05$.

mice. Unlike KO mice, late-cKO mice developed generalized seizures in response to the same dosage of kainic acid as their WT-control littermates (unpaired two-tailed t test, WT vs L-cKO: $t_{(24)} = 0.20$; $p = 0.84$; WT: $n = 12$; L-cKO: $n = 14$; Fig. 8A), thereby exhibiting unaltered seizure susceptibility.

We next assessed the social behavior of the late-cKO mice to reveal a possible endophenotype of schizophrenia. Like KO mice, late-cKO mice preferred a living mouse over a Dummy Mouse (close interaction time Dummy Mouse vs Novel Mouse 1, paired two-tailed t test, WT: $t_{(6)} = 3.20$, $*p = 0.019$; L-cKO: $t_{(6)} = 2.82$, $*p = 0.03$; close interaction time with Novel Mouse 1 WT vs L-cKO, unpaired two-tailed t test, $t_{(12)} = -0.91$, $p = 0.38$; $n = 7$ and 7, respectively; Fig. 8B) and exhibited intact social preference to a second novel mouse (close interaction time Novel Mouse 1 vs Novel Mouse 2, paired two-tailed t test, WT: $t_{(6)} = -2.94$, $*p = 0.026$; L-cKO: $t_{(6)} = -2.78$, $*p = 0.032$; close interaction time with Novel Mouse 2 WT vs L-cKO, unpaired two-tailed t test, $t_{(12)} = 0.16$, $p = 0.88$; Fig. 8C). Additionally, late-cKO mice displayed spontaneous locomotor activity similar to their WT littermates (two-way ANOVA with repeated measures, genotype: $F_{(1,14)} = 1.13$, $p = 0.31$; time: $F_{(11,154)} = 9.71$, $p < 0.0001$; interaction: $F_{(11,154)} = 0.72$, $p = 0.72$, WT: $n = 8$, L-cKO: $n = 8$; Fig. 8D). Moreover, late-cKO mice did not display hypersensitivity when injected with amphetamine (2.5 mg/kg body weight, i.p.), as shown by indistinguishable path lengths of WT and late-cKO mice (two-way ANOVA with repeated measures, genotype: $F_{(1,14)} = 1.67$, $p = 0.22$; time: $F_{(11,154)} = 10.87$, $p < 0.0001$; interaction: $F_{(11,154)} = 0.52$, $p = 0.89$; Fig. 8E; total path length WT vs L-cKO, unpaired two-tailed t test: $t_{(14)} = -1.29$; $p = 0.22$;

Fig. 8F; all WT: $n = 8$, L-cKO: $n = 8$). These data further confirm that genetic ablation of *Arc/Arg3.1* by itself, before or during puberty, does not cause overt schizophrenia-like behavior in adult mice.

Adolescent social isolation of WT and *Arc/Arg3.1* KO mice does not affect social engagement and sensorimotor gating.

Studies in animal models show that environmental stressors during adolescence can result in schizophrenia-like behaviors when combined with genetic risk factors (Niwa et al., 2013; Li et al., 2018). We addressed the effect of *Arc/Arg3.1* gene-deletion and environment assault by subjecting *Arc/Arg3.1* KO mice and WT littermates to social isolation during puberty (Fig. 9A). Isolated adult WT and KO mice spent significantly longer times interacting with Novel Mouse 1 compared with Dummy Mouse (paired two-tailed t test, WT: $t_{(11)} = -2.99$, $*p = 0.012$; KO: $t_{(6)} = -2.91$, $*p = 0.027$, WT: $n = 12$, KO: $n = 7$; Fig. 9B). WT mice, tested for social recognition, exhibited a significant preference to the Novel Mouse 2 (paired two-tailed t test, WT: $t_{(11)} = -3.48$, $**p = 0.0051$; Fig. 9C). KO mice exhibited a similar preference, however this did not reach significance (KO: $t_{(6)} = -1.46$, $p = 0.19$; Fig. 9C). The interaction time with the

Novel Mouse 2 was not significantly different between WT and KO mice (unpaired two-tailed t test, $t_{(17)} = -0.26$, $p = 0.79$). These data show that isolated WT and KO mice maintain intact social preference despite prolonged isolation. The same cohort of mice was subjected to the PPI test to probe a second schizophrenia-like behavior.

Isolated WT and KO mice generated comparable startle responses to the pulse stimulus (unpaired two-tailed t test, WT vs KO: $t_{(17)} = 0.10$, $p = 0.92$; WT: $n = 12$, KO: $n = 7$; Fig. 9D) and consistently showed indistinguishable PPI rates at different prepulse intensities (two-way ANOVA with repeated measures, genotype: $F_{(1,17)} = 0.55$, $p = 0.47$; prepulse intensity: $F_{(4,68)} = 75.23$, $p < 0.0001$; interaction: $F_{(4,68)} = 1.40$, $p = 0.24$; Fig. 9E). The mean PPI rate was also similar between WT and KO mice (unpaired two-tailed t test, WT vs KO: $t_{(17)} = -0.74$, $p = 0.47$, $n = 12$, 7, respectively; Fig. 9F). Additionally, comparison of the startle responses of socially-isolated mice with the group-housed mice failed to detect any significant difference (all $p < 0.05$; data not shown).

Discussion

Schizophrenia is a strongly heritable neuropsychiatric disease believed to arise from polygenetic and environmental risk factors that converge to disrupt normal brain development and provoke behavioral, neurological, and cognitive aberrations. Recent studies identified copy number variations (Kirov et al., 2012; Fernández et al., 2017), single nucleotide polymorphisms (Huentelman et al., 2015), and rare mutations (Fromer et al., 2014; Purcell et al., 2014) associated with high schizophrenia risk in genes whose

products form complexes with *Arc/Arg3.1*. Here we used constitutive and conditional *Arc/Arg3.1* KO mice, which we previously generated (Plath et al., 2006; Gao et al., 2018) to investigate the impact of *Arc/Arg3.1* deletion on behavioral and neurological domains of schizophrenia. Examination of constitutive *Arc/Arg3.1* KO mice failed to identify any overt deficits in social interaction, working memory, sensorimotor gating, and locomotor activity. Reduced seizure susceptibility and decreased oscillatory activity in the PFC of the anesthetized KO and early-cKO mice are dissimilar to the excessive high-frequency cortical oscillations measured during resting state in schizophrenic patients (Spencer, 2011; Hirano et al., 2015). However, reduced brain oscillations during task performance have also been reported in human patients and animal models of schizophrenia (Lisman, 2012; Uhlhaas and Singer, 2015). Notably, late-cKO mice, in which *Arc/Arg3.1* was deleted late in postnatal development, exhibited normal behaviors and intact PFC oscillatory activity.

We hypothesize that early *Arc/Arg3.1* expression in the PFC of late-cKO mice supports the normal formation of synaptic networks and maturation of oscillatory activity.

The interplay between excitation and inhibition is a key determinant of gain control in recurrent cortical networks (Douglas et al., 1995) performing cognitive algorithms such as working memory and perception. The biophysical properties of excitatory and inhibitory synapses influence oscillatory power and frequency (Buzsáki and Wang, 2012). The faster sIPSCs and reduced gain in the KO PFC may contribute to the impaired oscillations and learning and memory deficits in the KO mice (Plath et al., 2006; Gao et al., 2018). The finding that the E/I balance was preserved in the KO PFC can explain why despite changes in PFC circuitry and LFP, KO mice do not exhibit schizophrenia-like symptoms or epilepsy and are, in fact, more resilient to seizures. In both diseases disrupted E/I balance might be particularly critical for pathology (Lisman, 2012; Uhlhaas and Singer, 2015).

Reduced gephyrin clustering and fast decaying sIPSCs could result from a change in subunit composition of GABA_A receptors, which is known to affect receptor kinetics and anchoring mechanism at the synapse (Fritschy et al., 2012). Reduced recurrent excitation to L5 PFC neurons without a concurrent reduction of sIPSC frequency could be a result of a loss of synaptic currents activated during recurrent but not spontaneous activity, for example NMDA receptor currents (Wang et al., 2008).

The normal spontaneous locomotion of the *Arc/Arg3.1* KO mice does not recapitulate hyperlocomotion observed in schizophrenic patients (Perry et al., 2010). Moreover, amphetamine-induced locomotion was far weaker and shorter in the *Arc/Arg3.1* KO compared with other rodent models with relevance to schizophrenia (van den Buuse, 2010; Young et al., 2016). In the absence of other dopaminergic or behavioral abnormalities, we speculate that the transient amphetamine response reflects direct or circuit-level modifications in serotonergic, adrenergic (van den

Figure 9. Adolescent social isolation does not affect social engagement or sensorimotor gating in adult *Arc/Arg3.1* KO mice. **A**, Illustration of time flow for social isolation and the following behavioral tests. Mice were socially isolated with individual housing from P35. **B**, Both WT and KO mice preferably interacted with the Novel Mouse 1 over the Dummy Mouse during the sociability test. **C**, Both WT and KO mice preferably interacted with Novel Mouse 2 over Novel Mouse 1 during the social novelty test. **D**, Strong startle responses were observed in both WT and KO mice upon 120 dB stimulation. **E**, Comparable PPI rate in WT and KO mice at various prepulse intensities. **F**, No difference of mean percentage of PPI in WT and KO mice. Significance was tested with paired *t* test within groups, and two-tailed two-sample *t* test between groups in **B** and **C**. Two-tailed two-sample *t* test was performed in **D** and **F**. Two-way ANOVA with repeated measures was applied in **E**. All WT: *n* = 12, KO: *n* = 7. Bars show mean ± SEM. n.s., not significant, **p* < 0.05, ***p* < 0.01. SI, social interaction.

Buuse et al., 2011; Schmidt and Weinshenker, 2014), or glutamatergic transmission (Coyle, 2006).

Environmental stressors during adolescence are considered high-risk factors for schizophrenia, especially when combined with genetic aberrations. Social isolation during adolescence has been shown to induce depression and schizophrenia-like behaviors in mice harboring genetic mutations linked to schizophrenia, but not in WT mice (Niwa et al., 2013; Li et al., 2018). Subjecting *Arc/Arg3.1* KO mice to this stressor did not result in deficits in social behavior or PPI. These data indicate that *Arc/Arg3.1* deletions combined with subthreshold insults do not suffice to manifest schizophrenia-like behaviors.

Our findings are in disagreement with a recent study reporting overt schizophrenia-related phenotypes in a different *Arc/Arg3.1* KO mouse model (Managò et al., 2016). In this study, *Arc/Arg3.1*-deficient mice (*EGFP* knock-in–*Arc/Arg3.1* knock-out), displayed strongly impaired social interaction and PPI, as well as amphetamine hypersensitivity, and dopaminergic dysregulation. In stark contrast, using similar experimental procedures, we found no apparent schizophrenia-related phenotype in our KO mice. These contradicting observations might be reconciled and explained by the different strategies used for generating the two KO mouse models. The mouse model used by Managò et al. (2016) was generated by knocking into the *Arc/Arg3.1* open reading frame (ORF) a *d2EGFP* sequence terminated by a stop codon and followed by a floxed *neomycin* cassette, which has not been removed in the mutant mice (Wang et al., 2006). The *neomycin* cassette in these KO mice (*EGFP* knock-in–*Arc/Arg3.1* knock-out) could potentially produce adverse effects (Scacheri et al., 2001), such as disruption of neighboring gene expression (Pham et al., 1996), which is not seen in the WT littermates that do not carry the *neomycin* cassette. For example, it would be interesting to see whether the *neomycin* cassette could interfere with the expression of *JRK* and *LYNX1*, two epilepsy-related genes whose loci are close to the *Arc/Arg3.1* gene on mouse chromosome 15 (MGI: 88067, NCBI Gene: 11838). This interference might pro-

mote epilepsy (Toth et al., 1995; Smith et al., 2018) and facilitate schizophrenia pathogenesis. Indeed, a different study using the same *EGFP* knock-in–*Arc/Arg3.1* KO mice reported inter-ictal brain activity and higher seizure susceptibility (Peebles et al., 2010), in stark contrast to what we observed in our KO mice. Therefore, the *EGFP* knock-in–*Arc/Arg3.1* knock-out mice might serve as an interesting model for investigating the convergence of *Arc/Arg3.1* deletion and polygenetic dysregulation, if effects of the neomycin cassette can be isolated. In a less fortunate scenario, merely the expression of the selectable marker or the knocked in *EGFP* might have adverse effects on cell physiology or promote apoptosis (Müller, 1999; Jensen, 2012; Ansari et al., 2016).

Arc/Arg3.1 expression during early brain development (before puberty) shapes oscillatory activity in the PFC of adult mice similar to the effects we observed in the hippocampus (Gao et al., 2018). Our current findings reveal alterations in excitatory and inhibitory neurotransmission in the PFC as likely underlying mechanisms. These alterations may result from impaired *Arc/Arg3.1*-mediated plasticity during development, such as homeostatic synaptic scaling (Chowdhury et al., 2006; Shepherd et al., 2006), synapse pruning (Mikuni et al., 2013), or synaptic plasticity (Plath et al., 2006; Rial Verde et al., 2006). These mechanisms are disrupted in some neurodevelopmental disorders, including schizophrenia (Feinberg, 1982; Crabtree and Gogos, 2014).

Together, our findings demonstrate that the loss of *Arc/Arg3.1* *per se* does not induce schizophrenia-like behavior in adult mice. However, prenatal or perinatal deletion of *Arc/Arg3.1* alters cortical network activity without overtly disrupting the balance of excitation and inhibition in the brain and not promoting schizophrenia. Misregulation of *Arc/Arg3.1* rather than deletion could potentially tip this balance and thereby promote emergence of schizophrenia and other neuropsychiatric disorders.

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