Post-weaning parvalbumin expression in left orbitofrontal cortex shapes sociability in female mice

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Abstract

The adolescent social experience is essential for the maturation of the prefrontal cortex (PFC) in mammalian species. However, it is still unclear which cortical circuits mature with such experience and how it shapes adult social behaviors in a sex-specific manner. Here, we found that the post-weaning social isolation during adolescence (PWSI) caused hypersociability in female mice only and impaired both the parvalbumin (PV) expression and social sniffing-induced activity in the left orbitofrontal cortex (OFC_L). Similarly, the CRISPR/Cas9-mediated knock-down of PV in the OFC_L enhanced sociability and reduced the social sniffing-induced activity in female mice via decreased excitability of PV⁺ neurons and reduced synaptic inhibition in the OFC_L. Moreover, optogenetic activation of OFC_L excitatory neurons enhanced sociability in female mice. Our data demonstrate that the adolescent social experience is critical for the maturation of PV⁺ inhibitory circuits in the OFC_L; this maturation shapes female social behavior via enhancing social representation in the OFC_L.

Keywords: CRISPR/Cas9 / hypersociability / orbitofrontal cortex / parvalbumin / post-weaning social isolation

Introduction

One of the hallmarks of postnatal maturation in the mammalian cortex is the maturation of GABAergic inhibitory circuits (Luhmann & Prince, 1991). Maturation is accompanied by an increase in the expression of both the parvalbumin (PV) in a subset of GABAergic neurons and the perineuronal nets (PNNs) surrounding the PV⁺ neurons (Celio & Blumcke, 1994; Huang *et al*, 1999). PV is a calcium-binding protein and a well-known genetic marker for fast-spiking GABAergic neurons in the cortex (Rudy *et al*, 2011). PV gene expression is upregulated in the sensory cortex in an activity-dependent manner (Krishnan *et al*, 2017; Sugiyama *et al*, 2008) after mammals are born. Like PV, PNNs also increase during cortical maturation and, in the adult cortex, surround most of the PV⁺ neurons (Ueno *et al*, 2017; Ueno *et al*, 2018). Along with the increase of PNNs in cortical circuits, other molecular changes reduce net plasticity and facilitate the maturation of the cortical network (Beurdeley *et al*, 2012). If mice are deprived of visual input during the critical period after eye-opening, via monocular deprivation or dark rearing, PV⁺ neurons are less developed, and PNNs are reduced in the primary visual cortex (V1) (Sugiyama *et al*, 2008). This deprivation, in turn, diminishes both the visual response of V1 neurons that receive input from the deprived eye (Sugiyama *et al*, 2008) and inhibition in the cortex (Dityatev *et al*, 2007). Therefore, the postnatal sensory experience is critical for the expression of PV and PNNs in PV⁺ neurons, as these are required for the maturation of PV⁺ inhibitory circuits and, eventually, for shaping sensory responses in the cortex (Hensch, 2005).

The maturation of the cortex occurs first in the sensory area, where the process is part of sensory experience, and later in the motor-frontal and the PFC (Hensch, 2005). Social experience is critical for the maturation of the PFC in both humans (Blakemore, 2008; Paus *et al*, 2008) and rodents (Makinodan *et al*, 2012) particularly during juvenile and adolescent periods. Patients with

autism or schizophrenia often show abnormal social cognition as well as dysfunction in the PFC (Bicks *et al*, 2015). In these patients, the expression of PV and PNNs is reduced in the dorsolateral PFC (Enwright *et al*, 2016; Wöhr *et al*, 2015). Thus, it is plausible that immature PV⁺ circuits in the PFC cause the psychiatric symptoms underlying abnormal social behavior in mammals. However, we still do not understand whether the social experience is required for PV⁺ inhibitory circuits in these PFC regions to mature. In particular, it is largely unknown whether the social experience affects the maturation of inhibitory circuits in the orbitofrontal cortex (OFC), one of the PFC sub-regions critical for social cognition (Adolphs, 2003; Amodio & Frith, 2006).

Mammalian social behavior is sexually dimorphic and important for maintaining a conspecific society. Social behavior begins to become more sex-specific during adolescence. Interestingly, how social information is processed and social behavior executed in the mammalian brain shows hemispheric asymmetry and lateralization (Marlin *et al*, 2015). Even in rodents, according to recent studies, the PFC functions asymmetrically in mediating social behaviors (Lee *et al*, 2015). However, whether such asymmetry in the PFC becomes increasingly pronounced in a sex-specific manner in response to social experience during adolescence is still unclear. In this study, we investigated whether social experience influences the maturation of PV⁺ neurons in the PFC of each sex and in both hemispheres. We found that, in female mice, social experience during adolescence is required for the maturation of PV⁺ neurons in the left OFC (OFC_L) and for shaping sociability. Using post-weaning social isolation (PWSI) and the CRISPR/Cas9 system, we revealed that social isolation during adolescence caused the defect in PV expression in the OFC_L of female mice, which led to hypersociability and reduced OFC_L activity during social contact.

Results

Post-weaning social isolation enhances sociability in female mice

Previous reports have shown that both short- and long-term social isolation affects mouse social behaviors (Makinodan *et al.*, 2012; Matthews *et al.*, 2016). However, the effects differ depending on the duration of isolation, and the data were collected only in male mice. To examine whether the effects of short- and long-term social isolation on the mouse sociability differ between the sexes, we performed the sociability test in both male and female mice. For the sociability test, we put two small chambers at diagonal corners in the maze, each of which contained a novel object (O) or a novel and sex-matched mouse (M), and measured relative times of mice sniffing or spending at or nearby the novel mouse compared to those times at the novel object as indices of the mouse sociability. We quantified the sociability in mice that had experienced three different post-weaning social conditions at postnatal day 80 (P80): group-housed (GH), socially isolated for 1-day (1dSI), or socially isolated for 60-days after weaning (post-weaning social isolation, PWSI) (Fig 1A and B). Both the sniffing time of 1dSI mice when they came into contact with a novel mouse and their time spent in the social interaction zone did not differ significantly from those of GH mice in both males and females (Fig 1C and D).

Notably, PWSI induced changes in sociability in a sex-specific manner: only female mice showed a significant increase in social sniffing time and social interaction time, while PWSI male mice showed normal sociability as the GH mice (Fig 1C and D). We also confirmed that the effect of PWSI on female mice was not simply due to hormonal changes that occur during the estrous cycle. First, we confirmed that the estrous cycle was not different between GH and PWSI female mice (Fig 1E). We next confirmed that the mouse sociability (both social sniffing index and social interaction index) that we measured was consistent across the estrous cycle in female mice (Fig

1F-1H). Therefore, our data indicate that long-term social isolation during adolescence affects social behavior in females more than in males, causing hypersociability in females.

PWSI reduces PV expression in the OFC_L of female mice

We next examined whether the social experience during adolescence is required for the maturation of PV⁺ inhibitory circuits in the OFC. We quantified the number of PV⁺ or PNN⁺ neurons in the OFC of the GH and the PWSI mice at P80. We collected data from male and female mice separately. We first immunostained both for the PV, a marker for fast-spiking GABAergic neurons, and the NeuN, a marker for all neurons (Fig 2A). We counted the number of immunostained neurons automatically in the three coronal sections that were collected across the center of the OFC (+2.1 ~ +2.34 mm from the bregma) per each hemisphere in each mouse (See Methods; Fig 2B and C). Interestingly, the number of PV⁺ and NeuN⁺ neurons in the OFC was significantly lower in the brains of PWSI female mice compared to the GH female mice, especially in the left hemisphere (Fig 2D-G). In male mice, however, the number of PV⁺ and NeuN⁺ neurons was not significantly different between GH and PWSI groups (Fig 2D-G). We next quantified the PNN⁺ neurons in the OFC by staining for *Wisteria floribunda agglutinin* (WFA), a marker for PNNs (EV1A). There were both PV⁺ and PV⁻ neurons surrounded by the PNNs in the OFC (Fig S1B). We found that the numbers of WFA⁺ neurons in the OFC were not different between GH and PWSI in both male and female mice (Fig S1B and C). Collectively, our data indicate that social experience during adolescence is crucial for the maturation of PV⁺ neurons, especially the PV expression, in the OFC_L of female mice.

PWSI mice experienced long-term social isolation from the time when they were weaned at P21 to the time when they became adults at P80. This period includes both early juvenile periods

before the onset of puberty and late adolescence after the onset of puberty. Since puberty leads to sexual dimorphism, we wondered whether the social experience after the onset of puberty is more important for the female-specific maturation of the OFC. We thus re-group-housed the isolated female mice at P50 and re-introduced them to social experience during late adolescence after the onset of puberty. We then measured the social behaviors and the number of PV⁺ neurons in the OFC_L of the re-group-housed female mice at P80 (re-GH; Fig 3A). Not like the PWSI mice, which showed an increase in sociability and a decrease of PV⁺ neurons in the OFC_L, re-GH mice did not show such changes compared to the GH mice (Fig 3B-D). These data indicate that social experience during late adolescence from P50 to P80 is important for the PV⁺ expression in the OFC_L and maturation of normal social behaviors in female mice.

PWSI disrupts social sniffing-induced activity in the OFC_L of female mice

Because PV⁺ neurons in the OFC_L were found to be less numbered following PWSI in female mice, we investigated whether the OFC_L neural response to social contact has been altered in head-fixed female mice by *in vivo* multichannel recordings (Fig 4A). During the recording, both GH and PWSI female mice showed both object-sniffing and social sniffing behavior under the head-fixed condition, and the number and duration of sniffing times did not differ between groups (Fig S2A-C). We identified neurons that showed significant sniffing-induced activity in the OFC (Fig 4B-D). Interestingly, in GH females, more neurons showed sniffing-induced activity in the OFC_L than in the OFC_R responses, and their responses were stronger when a mouse sniffed another mouse than when it sniffed an object (Fig 4C-F). More important, social sniffing-induced activity in the OFC_L of female mice disappeared after PWSI (Fig 4C, E and F). On the other hand, the sniffing-induced activity of the OFC_R did not differ between GH and PWSI female mice (Fig 4D).

These data indicate that PWSI disrupted social sniffing-induced neural activity and reduced social representation in the OFC_L.

PV⁺ expression, not PNNs in the OFC_L, shapes social behavior in female mice

As we found that PWSI impaired the PV expression in the OFC_L and increased social behavior, we next examined the causal relationship between the reduction of PV expression in the OFC_L and the increase in the sociability of female mice. To test this, we used the CRISPR/Cas9 system (Swiech *et al*, 2015) to remove the PV gene selectively in the nucleus and thus induce overall knock-down of PV expression in the OFC_L. We designed two pairs of single-guide RNAs (sgRNAs) to target the 4th and 5th exons of the PV gene (sgPV) and confirmed that those sgRNAs knocked out the PV gene by either deletion or insertion of the targeted sequences in more than 85% of the Cas9-transfected Neuro2a cells (Fig S3A-D). We then constructed the adeno-associated viral vectors (AAVs) to deliver the sgRNAs together with the tdTomato gene to neurons *in vivo* (Fig 5A; S3A). To knock out the PV gene in the PV⁺ interneurons *in vivo*, we injected the AAVs into the cortex of mice crossed between PV-Cre and Cas9^{fl/fl}-eGFP mice (sgPV; and Fig 5A; Fig S3E-G). In these sgPV mice, the neurons that express both eGFP and tdTomato were originally the PV⁺ neurons, but their PV gene was knocked out by Cas9 (Fig 5A). As a control, we injected the AAV that expressed only the tdTomato without sgRNAs to the crossed mice (tdTom). We first immunostained the PV and the PNNs in the injected brain samples and confirmed that the eGFP⁺/tdTomato⁺ neurons were selectively knocked down with PV, without any decrease of PNN expression (Fig 5B). We next performed the sociability test on the female mice that had been knocked down with PV in the OFC_L. These mice showed a significant increase in sociability (Fig 5C and D), similar to the PWSI females (Fig 1B-D).

We further examined whether the PNN expression surrounding PV⁺ neurons are also altered by the PWSI and influences the female sociability. We infused chondroitinase ABC (chABC), an enzyme that degrades PNNs by removing sulfate side chains of proteoglycans, into the OFC_L (Fig 5E). Treatment with chABC removed PNNs surrounding PV⁺ neurons without affecting PV expression (Fig 5F, left) and significantly decreased the number of WFA⁺ neurons in the OFC_L (Fig 5F, right). We then performed the sociability test on the female mice treated with chABC and found that they showed normal sociability (Fig 5G and H). Together, our data indicate that the PV expression, but not the PNN maturation, in the OFC_L was a key factor shaping social behavior of female mice.

Decrease in PV expression reduces social sniffing-induced activity via hyper-excitation of OFC_L neurons

We next investigated whether the decrease of PV expression in the OFC_L was responsible for the reduced sniffing-induced activity in the OFC_L of the PWSI female mice. We performed *in vivo* recordings in female mice injected with sgPV AAVs (**sgPV**) or injected with tdTomato AAVs (**tdTom**) in their OFC_L (Fig 6A-G). First, we found that the average baseline firing rates were significantly higher in the sgPV group compared to the tdTom group (Fig 6D and E). Furthermore, similar to PWSI, knocking down PV by sgPV decreased social sniffing-induced activity in the OFC_L of female mice (**sgPV**; Fig 6F and G). Conversely, the expression of tdTomato in the OFC_L did not alter the sniffing-induced activity of female mice (**tdTom**; Fig 6F and G). Knocking down PV in the OFC_L did not affect the sniffing behavior itself, as the number and duration of sniffs in the altered mice were similar to those in the tdTom control mice (Fig S1D and E). These data

indicate that the knock-down of PV increased overall firing activity while reducing the sniffing-induced activity in the OFC_L.

As we observed an increase in the baseline activity of OFC_L neurons by knocking down the PV, we next examined whether the knock-down of PV has altered the excitation-inhibition balance in the OFC_L of female mice. We first performed patch-clamp recordings of the PV⁺ neurons in the OFC_L by targeting tdTomato⁺ neurons of PV::Cas9 mice injected with sgPV or tdTom AAVs into the OFC_L (Fig 6H-L). We found that the knock-out of the PV gene by sgPV in PV⁺ neurons reduced the intrinsic excitability of those neurons (Fig 6I) without any changes in spontaneous excitatory synaptic currents (sEPSCs; Fig 6L). Other membrane properties, such as the half-width of spike waveforms and the input resistance, were normal in those neurons (Fig 6J and K). We next measured the spontaneous inhibitory postsynaptic currents (sIPSCs) in the OFC_L neurons, which were not labeled with the tdTomato in the sgPV and the tdTom mice. These neurons were regular spiking and thus putative excitatory neurons in the OFC_L (Fig 6H and M). The OFC_L excitatory neurons in the sgPV mice showed a significant decrease in both amplitudes and frequencies of spontaneous IPSCs compared to the tdTom control mice (Fig 6M). These data indicate that the decreased expression of PV in the OFC_L reduced the excitability of the PV⁺ neurons and, in turn, reduced inhibition of the neighboring neurons in the OFC_L of female mice.

Our recording data suggested that the decrease in inhibition upon knocking down the PV gene caused an increase of excitation, which led to a rise in the baseline activity of OFC_L and disruption in sniffing-induced activities (Fig 6D-G). We thus examined whether the abnormal increase of the OFC_L activity caused hypersociability in female mice. We expressed the channelrhodopsin-2 (ChR2) in the excitatory neurons of the OFC_L by injecting the AAV virus, which expresses the ChR2 fused to the eYFP under the CaMKII α promotor, to the wild-type

female mice (Fig 7A). We then delivered the light stimulation to the OFC_L by inserting a wireless optogenetic device that we developed (Kim *et al*, 2021; Qazi *et al*, 2021) to provide the 20Hz blue lights during the sociability tests (Fig 7B; see Methods). The stimulation was given on the second day of the sociability tests. We measured the first-day sociability (Day1) without the light stimulation and did not observe any differences in the sociability between the ChR2-eYFP and the eYFP groups. On the second day, we tested their sociability again but with the blue-light stimulation on the OFC_L (Day2). The eYFP control mice showed a significant decrease in their sociability on Day2 compared to Day1 potentially due to habituation (Fig 7C and D). On the other hand, the ChR2-eYFP-expressed mice showed higher levels of social sniffing behaviors than the control mice (Fig 7C), showing no such habituation on Day2 compared to Day1 (Fig 7D). Therefore, the optogenetic activation of the OFC_L resulted in less habituation in their social behavior and induced higher sociability on Day2 (Fig 7D). The light stimulation did not affect the locomotion in both groups (Fig 7E). Collectively, our data demonstrate that the reduced expression of the PV caused hyper-excitation of the OFC_L, which led to hypersociability in female mice.

Discussion

In this study, we found that social experience during adolescence is critical for shaping sociability in female mice. PWSI enhanced sociability in female mice while impairing the maturation of PV⁺ neurons and decreasing social sniffing-induced activity in the OFC_L. The knock-down of PV in the OFC_L of female mice induced the same phenotype as the PWSI mice: enhanced sociability and decreased sniffing-induced activity in the OFC_L. These effects were due to the imbalance between excitation and inhibition, as the PV knock-down caused an increase in excitation in the OFC_L. We further found that optogenetic activation of the OFC_L led to enhanced

sociability in female mice. Our data indicate that adolescent social experience is critical for balancing excitation with inhibition via maturation of PV⁺ inhibitory circuits in the OFCL to shape sociability in female mice. Our data also suggest that adolescent social experience functions differently in male and female mice, both in how the prefrontal cortex matures and how social behavior matures.

Importance of social experience during adolescence in shaping female social behavior

Social experience during adolescence is important for the maturation of the mammalian brain (Fuhrmann *et al*, 2015; Makinodan *et al.*, 2012). In humans, mistreatment or the lack of social care at a young age can increase the occurrence of neurological disorders and cause defects in brain function (Egeland *et al*, 1983; Hanson *et al*, 2010; Widom *et al*, 2007). Similarly, depriving rodents of social experience during adolescence caused defects in the maturation of their brains and behavior (Cooke *et al*, 2000; Schubert *et al*, 2009), and some of the physiological effects of social isolation were sex-specific (McCormick *et al*, 2008; Pisu *et al*, 2016). We used PWSI to deprive mice of social experiences during adolescence. Our data showed that female mice are more vulnerable to social isolation during late adolescence after the onset of puberty than male mice.

A recent study has shown that the 1dSI increased sociability in male mice (Matthews *et al.*, 2016). We also found male mice that had experienced 1dSI spent more time interacting with a novel mouse than did GH male mice (GH, 221.11 ± 17.93 s, 1dSI, 317.97 ± 31.46 s, p=0.023, student's t-test), but they also showed a tendency of increased interaction with an object (GH, 107.29± 9.88 s, 1dSI, 138.28 ± 23.36 s, p=0.256, student's t-test). Therefore, the social sniffing and social interaction indices, which indicate the ratio between social interaction and object interaction times, were comparable between GH and 1dSI male mice (Fig 1C and D). In female

mice, 1dSI had no specific effects on social and object interaction times. Collectively, our data demonstrate that long-term social isolation during adolescence affects social behaviors more strongly than the 1dSI specifically in females.

PWSI causes sensory deprivation because mice are prevented from interacting socially with other mice. Furthermore, PWSI causes stress in social mammals (Fone & Porkess, 2008), and social isolation lasting more than 1 day has been used to induce chronic stress in rodents (Fone & Porkess, 2008). It has been shown that both sensory deprivation and stress can impair the maturation of PV⁺ neurons in the sensory cortex as well as in the hippocampus (Czeh *et al*, 2005; Hu *et al*, 2010; Huang *et al.*, 1999). The OFC, which is classified as a secondary olfactory cortex, is one of the key areas involved in olfactory processing (Lipton *et al*, 1999) as well as in sniffing behavior (Kareken *et al*, 2004). Furthermore, the olfactory system is the most important way through which mice detect other mice (Radyushkin *et al*, 2009). The OFC has a strong connection with both the olfactory system and the hippocampus (Wikenheiser & Schoenbaum, 2016). Thus, it is possible that the olfactory deprivation and the stress that characterizes PWSI caused the decrease in PV expression in the OFC_L. Future studies are necessary to unravel which types of social olfactory cues and stress-related factors affect the maturation of PV⁺ neurons in the OFC_L and social behavior.

Our findings demonstrate that the OFC_L plays a key role in shaping social behavior, especially in female mice. Supporting this idea, an fMRI study in humans showed that in response to odorant cues with emotional valence, only females showed increased activity in their OFC_L (Royet *et al*, 2003). Thus, the female OFC_L seems to have evolved to recognize the valence of odorant cues, namely, those that characterize social cues in mammals. Our data further suggest that social experience shapes this function of the OFC_L during adolescence, particularly after the onset of

puberty, in females. Moreover, we found that PWSI caused a decrease in the activity of the OFC_L while enhancing sociability. Excessive sociability is a symptom of patients with Williams syndrome, a rare neurodevelopmental genetic disorder that shows abnormal activity patterns in the OFC (Mimura *et al*, 2010). Our data further demonstrate that PWSI induced hypersociability, which is similar to Williams syndrome, in female mice and that the normal maturation and activity of the OFC_L is important for maintaining an adequate level of sociability in females.

PV expression in the OFC is important for social behavior

The maturation of PV⁺ inhibitory circuits occurs by increasing the expression of PV and PNNs, balancing inhibition with excitation in the cortex; this balance is critical for shaping sensory responses (Hensch, 2004). Recent studies have examined the correlation between cognitive behaviors and PV expression in the PFC. For example, several mouse models of autism that show decreased sociability have shown reduced PV expression in the mPFC (Kobayashi *et al*, 2018) as well as in the striatum (Filice *et al*, 2016). Furthermore, early-life stress occasioned by maternal separation before weaning reduced PV expression in the OFC of female mice and impaired their flexibility (Goodwill *et al*, 2018). Interestingly, the reduced expression of PV in the dorsolateral PFC was observed in human patients with schizophrenia (Enwright *et al*, 2016; Nakazawa *et al*, 2012) and autism (Ferguson & Gao, 2018). However, none of these studies proved that PV expression in the PFC caused the deficits that characterize those cognitive behaviors. Although the whole-brain knock-out of PV caused the mice to show autism-like behavior (Wohr *et al*, 2015), we still do not know the specific function of the PV in sub-regions of the PFC. Our study used the CRISPR/Cas9 system to knock down the PV expression locally in the OFC_L. This method found that PV expression in the OFC_L is critical for shaping mouse social behavior (Fig 5).

PV is a calcium-binding protein that plays a key role in regulating the physiological properties of PV⁺ neurons. Electrophysiological studies have revealed that knocking down PV in fast-spiking interneurons in the striatum enhanced the fast-spiking properties of those neurons and increased the paired-pulse ratio of the inhibitory release to postsynaptic neurons (Ordaz *et al*, 2013) while decreasing the paired-pulse ratio of evoked excitatory inputs to those neurons (Wohr *et al.*, 2015). We further found that knocking down PV decreased the excitability of the inhibitory neurons, which in turn decreased synaptic inhibition on nearby excitatory neurons (Fig 6H-M). The reduced inhibition in the OFC_L, which was induced either by the knock-out of PV or by the optogenetic activation of excitatory neurons in the OFC_L, caused hypersociability potentially due to the disruption of overall social sniffing-induced activity in the OFC_L (Fig 6F and G). Moreover, reduced PV expression can also disrupt the network property of the OFC_L, similar to the previous report showing that the reduction of PV expression correlates with a decrease in the network oscillation in the PFC (Lodge *et al*, 2009). Maturation of network activity during adolescence may be critical for shaping social sniffing-induced activity in the OFC_L.

In a study of virgin female mice, PV and PNN expression in the auditory cortex was reported to be correlated with learning to retrieve pups (Krishnan *et al.*, 2017); this activity, which occurs in the left auditory cortex, is important for shaping maternal behaviors in female mice (Marlin *et al.*, 2015). Our data further support the idea that plasticity in PV⁺ neurons in the OFC_L is important for shaping female sociability and is also characterized by hemispheric asymmetry. Both genetic and epigenetic mechanisms have been identified as critical for regulating normal social behavior (Hensch, 2005), and thus social behaviors can be easily disrupted in a wide range of brain psychiatric disorders (Blakemore, 2008). Our study provides insight into how epigenetic

329 mechanisms, such as PV expression in the PFC, may underlie abnormal hypersociability that
330 characterizes certain psychiatric disorders.

Materials and Methods

Experimental model and subject details

We approved all experimental procedures from the KAIST Institutional Animal Care and Use Committee (IACUC-18-236). We maintained all mice ad libitum under light from 8 am to 8 pm and dark cycle from 8 pm to 8 am. All mice were weaned at postnatal day 21 (P21); mice were reared in double (28cm × 17cm × 14cm) cages with 3-5 mates for group housing or in single (20cm × 10cm × 14cm) cages alone for social isolation. We performed experiments on WT (C57BL/6J), PV-Cre (JAX, #008069), and the Rosa26-floxed STOP-Cas9 knock-in (JAX # 024857) mice.

Histology

We anesthetized mice with isoflurane and then perfused them with 1X phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA, w/v in PBS). Mouse brain samples were post-fixed for 4h at 4°C; after fixation, we incubated them in 30% sucrose solution (w/v in PBS) for 1-2 days at 4°C. Then, we embedded each brain sample in optimal cutting temperature medium (OCT, Tissue-Tek, Cat#4583) and froze each at -80°C. We sectioned frozen brains using a cryostat (Leica) and collected coronal sections either at 30 μm (for confirming injection sites) or 15 μm (for immunostaining) thickness. For confirming injection sites, brain sections were washed three times with 1X PBS and mounted in DAPI-containing mounting medium (VECTASHIELD®, cat#H-1200). For immunostaining, we washed the brain sections three times with 1X PBS and permeabilized them with 0.3% Triton X-100 in 1X PBS for 30 min. After the sections were incubated in a blocking solution (2% normal donkey serum in 1X PBS) for 2 hours at room temperature, we incubated them with primary antibodies (rabbit anti-PV, 1:500 dilution in

blocking solution, Swant, cat#PV25; mouse anti-NeuN, 1:500 dilution in blocking solution, Millipore, cat#MAB377) for 2 days at 4°C. Last, we incubated them with fluorescence-tagged secondary antibodies (Alexa Fluor 594 donkey anti-rabbit IgG for PV staining, 1:500 dilution in 1X PBS, Life Technology, cat#A21207; Alexa Fluor 647 donkey anti-mouse IgG for NeuN staining, 1:500 dilution in 1X PBS, Jackson ImmunoResearch, cat#715-605-151) and Fluorescein (FITC) labeled Wisteria Floribunda Lectin (WFA, WFL) for PNN staining (1:500 dilution in 1X PBS, Vector labs, cat#FL-1351) for 2 hours at RT before mounting them in clear mounting medium (DAKO, cat#S3023). We imaged fluorescent signals of mounted brain sections under a scanning microscope (ZEISS, Axio Scan.Z1).

Cell counting analysis

We matched the images obtained from the scanning microscope to the mouse brain atlas (Franklin & Paxinos) to confirm the anterior-to-posterior location of the coronal brain sections. We collected 15µm brain sections in every 60µm thickness across the frontal cortex, and we selected three sections in a row that include OFC areas (between +2.10mm ~ +2.34mm anterior from the bregma) for the immunostaining (see Fig 2C). We scanned the fluorescent images of the brain sections and put a 0.3 x 0.3mm² square as a region-of-interest at the center of the OFC (±1.3mm lateral from the bregma; depth, -2.5mm) to count the labeled neurons in the OFC. To quantify the number of PV⁺ and NeuN⁺ neurons, we used the automatic cell counting software (ImageJ). After converting the fluorescent images to the 8-bit grayscale images, we further transformed the grayscale images into binary images. For the binary transition, we used distinct thresholds at the grayscale for isolating the NeuN⁺ neurons (11% of total mean intensity) and the PV⁺ neurons (2% of total mean intensity). We then automatically counted the white-filled circles as cells if they satisfied the following conditions: 1) total size is above 60 x 60 pixels and 2) the

circularity is above 0.2. There was no significant difference between the number of cells counted automatically and the number of cells counted manually in a blind manner (see Fig 2B). To validate the knock-down efficiency of sgPV or chABC (see Fig 5), we manually and blindly counted the number of PV⁺ or WFA⁺ neurons within 0.5 x 0.5mm² regions-of-interest in three representative coronal sections covering OFC.

Sociability test

We performed the sociability test at either P79-81 or at P50. Before behavior experiments, all mice were habituated 30-60 minutes in the habituation chamber. All mice were maintained in a group-housed condition or an isolated condition until the habituation. Mice were then moved to the sociability maze (50cm × 45cm × 20cm) where two containers were located diagonally at opposite corners. The sociability test consisted of two phases: 1) habituation in the sociability maze (empty containers, 10min) and 2) sociability test (an object container and a social container with a young, same-sex, and stranger mouse, 10min). We used a software, EthoVision[®]XT 11.5 (Noldus), to analyze interaction times. We analyzed mouse sociability by two indicators: time spent in interaction zones near the container (18cm × 18cm) and sniffing time. We calculated the social sniffing and social interaction indices as follows:

$$\text{Social sniffing index} = \frac{(\text{Social sniffing time})}{(\text{Object sniffing time}) + (\text{Social sniffing time})}$$

$$\text{Social interaction index} = \frac{(\text{Time in social zone})}{(\text{Time in object zone}) + (\text{Time in social zone})}$$

Measuring estrous cycle in female mice

We monitored estrous cycle of female mice using vaginal cytology (McLean *et al*, 2012). Vaginal smears from subject mice were collected right after the sociability test. Smears were placed on slides and stained with 0.1% crystal violet (Sigma Aldrich, cat#V5265). Each estrous cycle stage was determined based on the cell type that was mainly observed in the smear sample among the three types: nucleated epithelial cells, cornified squamous epithelial cells, and leukocytes. To examine the difference in estrous cycles between GH and PWSI, we performed the vaginal cytology for four consecutive days in each mouse.

***In vivo* extracellular recordings for measuring sniffing-induced activity**

We performed all recordings in the head-fixed and awake mice. We marked the top of left and right OFCs and implanted custom-designed head-plates on the skull of mice using dental cement and small screws. After 2-3 days of recovery, we habituated mice to be head-fixed for 20min per day for 3 days. After the habituation, we performed the recording in the OFC by making a small craniotomy on the marked spots and inserting 32 channel silicon probes (A1X32-Poly2-10mm-50-177 or A1X32-Poly3-10mm-50-177, Neuronexus) vertically into each OFC using a micro-drive manipulator (Siskiyou). We first recorded spiking activity in the OFC without presenting a stranger mouse for 10 min (object_{sniffing} session) and next continuously recorded neural activity while presenting a head-fixed stranger mouse in front of the recorded mouse for 10 min (social_{sniffing} session). During the recording, we monitored and recorded the sniffing behavior of the mouse by using a web camera (Microsoft, LifeCam HD-3000). All extracellular signals were recorded at a 30kHz sampling rate, filtered between 500~5000Hz, and amplified by a miniature digital headstage (CerePlex μ , Blackrock Microsystems). After the recording, we confirmed the recording sites by injecting red retrobeads (Lumafleur) into the recording areas and performing histology.

Automatic quantification of sniffing behaviors

We developed a Matlab code to automatically analyze sniffing events during object- and social sniffing sessions in head-fixed mice. First, we converted all the RGB image frames of a recorded video clip to a grayscale using the ‘*rgb2gray*’ function. Next, we chose the region of interest (ROI) including the nose and whiskers of the mouse being recorded, made a binary matrix with 1 inside the ROI and 0 outside the ROI of each pixel, and multiplied an original image by the binary matrix of each corresponding pixel along all the frames (ROI_1). We subtracted background brightness from ROI_1 to eliminate overall brightness fluctuations (ROI_2), and calculated deviations of brightness within the ROI by subtracting the mean brightness of each pixel (ROI ΔF). To detect sniffing events, we subtracted a moving average (F_0 , sliding window = 50 frames) from ROI ΔF (ROI $\Delta F - F_0$), and set a threshold by multiplying a median of absolute ROI $\Delta F - F_0$ by 4. If ROI $\Delta F - F_0$ in each frame crossed the threshold, we included the frame in a sniffing event.

Neural activity analysis

We performed the spike-sorting using Klusters software (<http://neurosuite.sourceforge.net/>) (Hazan *et al*, 2006). We grouped 32-channel data into eight groups and sorted single units by principal components analysis. We only isolated single units that show more than a 2 ms refractory period in their auto-correlogram. We performed all other analyses using custom-made codes in Matlab (Mathworks). We sorted sniffing trials in object sessions and social sessions by identifying sniffing events across the recording sessions. We only used trials that satisfied the following two criteria: 1) inter-sniffing interval (time between the offset of sniffing in current trial and the onset time of the next trial) > 5sec, and 2) sniffing-duration (time between the onset and the offset of

sniffing in one trial) > 1sec. To plot the peri-event time histogram (PETH) of all neurons as a population, we performed Z-scoring by averaging firing rates (Hz) of individual units across trials and normalized them by 1sec baseline activity (3 to 2 sec before the onset of sniffing). The normalized firing rates were convolved with a Gaussian filter ($\sigma = 50\text{ms}$) only for visualization purposes. To identify the neurons showing significant sniffing-induced activity, we statistically compared the firing rates of individual neurons during the 2 sec pre-period (3 to 1 sec before the onset of sniffing) and 2 sec post-period (0 to 2 sec after the onset of sniffing) of each sniffing trial by bootstrapping ($p < 0.01$, $n = 5000$). We quantified the sniffing-induced activity of neurons by averaging Z values during 0 to 2 sec after the onset of sniffing. We classified cell types of recorded neurons based on spike waveforms by quantifying spike widths of each isolated neuron (time between peak and trough of the spike, μs). If the spike width was below $350 \mu\text{s}$ or above $450 \mu\text{s}$, recorded neurons were classified as fast-spiking (FS) neurons or regular-spiking (RS) neurons, respectively. For the baseline activity analysis, we quantified the average firing rates (Hz) from 0 to 2 sec before the onset of sniffing.

Perineuronal net degradation

To remove the PNN, we reconstituted protease-free chABC (Amsbio) in PBS to a final concentration of 61U/ml . We injected female mice OFC_L with $0.5\mu\text{l}$ of PBS or chABC on postnatal day 70-75. Seven days after the injection, we performed behavior experiments and sacrificed the mice to confirm PNN degradation.

CRISPR / Cas9 experiments

Generating of the PX552-sgPV-tdTomato

To generate the AAV-tdTomato vector, we first replaced the eGFP gene in PX552 (Addgene # 60958) with tdTomato. The tdTomato template was obtained from pAAV-FLEX-tdTomato (Addgene # 28306). Next, we cloned two AAV-tdTomato plasmids, both of which induce the targeted mutations at the parvalbumin gene (sgPV). One virus expresses two sgRNAs targeting parvalbumin exon4: sgRNA1, 5'-AGACAAGTCTCTGGCATCTG-3' and sgRNA2, 5'-AAGGATGGGGACGGCAAGAT-3' (Cloned recombinant DNA fragment: AGACAAGTCTCTGGCATCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTCGATTCCCGGCTGGTGCAAAGGATGGGGACGGCAAGATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC, Bioneer & In-house PCR amplification), and the other virus expresses two sgRNAs targeting parvalbumin exon5: sgRNA3, 5'-GCTAAGTGGCGCTGACTGCT-3' and sgRNA4, 5'-GGCCGCGAGAAGGACTGAGA-3' (Cloned recombinant DNA fragment: GCTAAGTGGCGCTGACTGCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTCGATTCCCGGCTGGTGCAGGCCGCGAGAAGGACTGAGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC, Bioneer & In-house PCR amplification).

After cloning the AAV-tdTomato vectors, we transfected them with packaging plasmid into HEK293T (KCTC, cat# HC20005) to produce AAV-sgPV-tdTomato (exon4 and exon5) and AAV-tdTomato, using lipofectamine 2000 (Invitrogen, cat# 11668-019). After 72hr, we collected

AAV (AAV2) particles from the cell lysates and performed iodixanol gradient ultracentrifugation. Next, we concentrated the viral particles with Amicon Ultra Centrifugal Filter (Millipore) and quantified the concentration of viral particles by quantitative PCR (genome copies per ml; AAV-tdTomato, 3.92×10^{14} ; AAV-sgPV(exon4)-tdTomato, 3.07×10^{12} ; AAV-sgPV(exon5)-tdTomato, 7.17×10^{12}).

Validation of CRISPR/Cas9 in the Neuro2a cell

To identify the efficiency of sgPV in knocking down the PV, we transfected cloned AAV vectors with the Cas9-eGFP expressing vector (PX458) in the Neuro2a cells. After 72h, we collected cells and sorted tdTomato/eGFP double-positive cells using BD FACS Aria II (BD Biosciences). Next, we purified genomic DNA from sorted cells and performed PCR to amplify target sites of the PV gene, adding the index sequence for sequencing. Last, we subjected the samples to Miniseq (Illumina) and analyzed insertion and deletion frequencies using CRISPR RGEN Tools (<http://www.rgenome.net/>).

Behavior and physiological experiments with CRISPR/Cas9 mice

To express Cas9 selectively in PV⁺ neurons, we crossed the PV-Cre mice (JAX #008069) with the Rosa26-floxed STOP-Cas9 knock-in mice (JAX # 024857). We injected AAV-tdTomato (tdTom) or AAV-sgPV-tdTomato (sgPV) to the crossed mice on the postnatal day 50-65. On postnatal day 78-85, we performed the behavior test and *in vivo* recording. To verify whether the AAV expression knocked down the PV effectively, we collected brain samples of the injected mice and performed immunohistochemistry as described above. We used the combination of the primary (rabbit anti-PV for PV staining, goat anti-GFP for Cas9 staining) and the secondary (Alexa Fluor 405 donkey anti-goat IgG and Alexa Fluor 647 donkey anti-rabbit IgG were used) antibodies.

Whole-cell patch-clamp recordings in brain slices

For electrophysiology experiments, brain samples were collected from anesthetized mice with isoflurane (Terrell) and sectioned (coronal, 300 μm thickness) using a vibratome (Leica VT1200). Brain extraction and sectioning were conducted in ice-cold dissection buffer containing (in mM) 212 sucrose, 25 NaHCO_3 , 5 KCl, 1.25 NaH_2PO_4 , 0.5 CaCl_2 , 3.5 MgSO_4 , 10 D-glucose, 1.25 L-ascorbic acid and 2 Na-pyruvate bubbled with 95% O_2 /5% CO_2 . The sectioned slices were incubated in a recovery chamber at 32 $^\circ\text{C}$ with normal ACSF (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 10 glucose, 2.5 CaCl_2 and 1.3 MgCl_2 , oxygenated with 95% O_2 /5% CO_2). After 60-min recovery at 32 $^\circ\text{C}$, slices were incubated for 30 min at room temperature (20–25 $^\circ\text{C}$). Slice was transferred to a recording chamber at 27–29 $^\circ\text{C}$ with circulating ACSF saturated with 95% O_2 and 5% CO_2 . Recording pipettes were made from thin-walled borosilicate capillaries (30–0065, Harvard Apparatus) with resistance 3.0–4.0 $\text{M}\Omega$ using a two-step vertical puller (PC-10, Narishege).

Whole-cell patch-clamp recordings were conducted using a MultiClamp 700B amplifier (Molecular Devices) and Digidata 1550 (Molecular Devices). During the recordings, hyperpolarizing step pulses (5mV, 40ms) were injected into the cell to monitor membrane resistance calculated by peak amplitude of the capacitance current. Data from cells that changed the resistance more than 20% were excluded. To measure the intrinsic excitability, recording pipettes (3.0–4.0 $\text{M}\Omega$) were filled with an internal solution containing (in mM) 137 K-gluconate, 5 KCl, 10 HEPES, 0.2 EGTA, 10 Na-phosphocreatine, 4 Mg-ATP, and 0.5 Na-GTP, with pH 7.2, 280 mOsm. Picrotoxin (Sigma, 100 μM), NBQX (Tocris, 10 μM) and D-AP5 (Tocris, 50 μM) were added to ACSF to inhibit postsynaptic responses. After gigaseal and rupture, currents were clamped, and RMP was maintained at -70mV. To measure the I-V curve, current inputs were

increased from -300 to 120pA in increments of 30pA per sweep with the time interval of 2 seconds. To measure the I-F curve, current inputs were increased from 0 to 660pA in increments of 60pA per sweep with the time interval of 10 seconds. For measuring sEPSCs in the OFC PV⁺ neurons, glass pipettes were filled with an internal solution composed of (in mM) 100 CsMeSO₄, 10 TEA-Cl, 8 NaCl, 10 HEPES, 5 QX-314-Cl, 2 Mg-ATP, 0.3 Na-GTP and 10 EGTA, with pH 7.25, 295 mOsm at a holding potential of -70 mV. Picrotoxin (100μM) were added to ACSF to block IPSCs. For measuring sIPSCs in the OFC excitatory neurons, recording pipettes were filled with an internal solution containing (in mM) 120 CsCl, 10 TEA-Cl, 8 NaCl, 10 HEPES, 5 QX-314-Cl, 4 Mg-ATP, 0.3 Na-GTP and 10 EGTA, with pH 7.35, 280 mOsm holding at -70 mV. Circulating ACSF were added NBQX and D-AP5 to block EPSCs. Data were acquired by Clampex 10.2 (Molecular Devices) and analyzed by Clampfit 10 (Molecular Devices). We confirmed the AAV expression by imaging the red fluorescence of the patched cells.

Wireless optogenetic activation of the OFC neurons *in vivo*

Design and fabrication of flexible optoelectronic neural probes

Flexible optoelectronic probes were designed using an electronic design automation software (Altium Designer 18.0, Altium Limited) and produced through a conventional photolithography process. Neural probes (0.1 mm-thick) were designed in a bilateral form, in which the right and the left probes (0.38 mm-wide and 4 mm-long each) were separated by 2.6 mm, in order to access both regions of the mouse brain. Copper traces and electrodes (18 μm-thick) were patterned on one side (i.e., top layer) of the polyimide substrate (25 μm-thick), while the other side (i.e., bottom layer) was covered with a copper layer (18 μm-thick) to stiffen the probe for penetration into brain tissue. The probe tip part, where the microscale inorganic light-emitting diodes (μ-ILEDs; TR2227, Cree; blue (470 nm), 270 × 220 × 50 μm³) were attached, was

not covered with a copper layer to allow light emission from both sides of the probe. The fabricated probes showed the optical intensity of $\sim 405 \text{ mW/mm}^2$ at the top layer and $\sim 45.3 \text{ mW/mm}^2$ at the bottom layer, both of which sufficiently exceeds the threshold intensity for ChR2 activation ($\sim 1 \text{ mW/mm}^2$). A 3-pin plug-n-play female pin connector (M50-3130345, Harwin) and two μ -ILEDs were mounted on the top layer electrodes after applying a low-temperature solder paste (SMDLTLFP10T5, Chip Quik Inc.) and soldered using a precision soldering iron (NASE-2C, JBC Soldering Tools) with the temperature of 250°C . After confirming that every component was robustly attached, probes were coated with Parylene C ($7 \mu\text{m}$ -thick) for waterproofing. Lastly, the probes were vertically bent and attached to a 3D-printed probe holder, followed by fixing them in place using epoxy (5 Minute Epoxy, Permatex).

Design of programmable wireless control module with detachable configuration

A Bluetooth Low Energy (BLE) based wireless control module was designed to be easily attached and detached with a bilateral optoelectronic probe and a battery through plug-n-play connection ((Lee *et al*, 2020; Qazi *et al*, 2019). This module controls the optical output of the μ -ILEDs through smartphone manipulation. The electronic circuit of the wireless module consists of a rechargeable Lithium Polymer (LiPo) battery (GM300910-PCB, PowerStream Technology; 12 mAh , $9 \times 10 \times 3 \text{ mm}^3$), a BLE System-on-Chip (SoC; EYSHSNZWZ, Taiyo Yuden; $8.55 \times 3.25 \times 0.3 \text{ mm}^3$), and various other electronic components (a linear voltage regulator, four decoupling capacitors, two resistors, and two indicator LEDs). The current supplied from the LiPo battery is regulated and stabilized by a voltage regulator (NCP4624DMU30TCG, onsemi) and decoupling capacitors, and flows to the BLE SoC to operate the module. Two indicator LEDs (VAOL-S4RP4, VCC; red (624 nm wavelength)) were placed at the output pins of BLE SoC, each connected in series with a resistor (RK73H1HTTC6041F, KOA Speer; $6.04 \text{ k}\Omega$), to visually show the current

operation status of the module during the animal experiment – for example, the indicator LED blinks every 5 seconds during optical stimulation. The wireless control module can be straightforwardly assembled with optoelectronic probes through 3-pin plug-n-play connectors (male: M50-3630342, Harwin; female: M50-3130345, Harwin) and assembled with LiPo battery through 2-pin connectors (male: M50-3630242, Harwin; female: M50-3130245, Harwin). Every component except the LiPo battery was soldered on the flexible printed circuit board (FPCB) of the wireless module ($9.3 \times 7.6 \times 0.1 \text{ mm}^3$), which were designed and manufactured through the same method for the μ -ILED probes. The module was wirelessly programmed over the air (OTA; Nordic Device Firmware Update (DFU) bootloader) to provide a pulse (20 Hz with 25 ms pulse width; i.e., 50% duty cycle) or continuous (i.e., 100% duty cycle) current to the connected bilateral μ -ILED probes. The BLE-based smartphone app was developed using a commercial app development software (Android Studio) and used to control the μ -ILED probes for wireless optogenetics.

Virus injection and probe implantation surgery

We injected the AAV-CAMKII α -eYFP (vector biolabs, VB1941) or AAV-CAMKII α -ChR2-eYFP (addgene) virus in the OFC_L (AP+2.35, ML $\pm$ 1.3, DV -2.5) of female WT mice between P50 to P60. For the injection, we anesthetized mice using 1.5% isoflurane in oxygen inhalation and performed stereotaxic surgery. Each injection contained 400nl of AAV- infused at a rate of 0.4 nl/sec using a microinjector. We recovered the mice for 7 days after the injection and implanted the wireless optogenetic LED probe was in the OFC.

Behavior test

To connect the small module part to the implanted probe on the mice head, the mice were briefly anesthetized with the isoflurane inhalation. After the module was connected, the mice have

10 min recovery phase in their home cage. On Day0, we habituated mice wearing a module for 15 min in the open field chamber. On Day1, we habituated the mice wearing a module in the sociability chamber for 5 min. We then presented a novel social target and a novel object for 5 min and recorded their approaching behaviors to measure the sociability of the mice. On Day2, we performed a sociability test with blue LED stimulation for optogenetic activation. We gave 20Hz blue light stimulation by 473nm LED connected to the probe during the 5 min sociability test session by using the custom-designed flexible optoelectronic neural probes. Interaction times of mice were analyzed on Day1 and Day2 as described previously. On Day3, we performed an open field test for 5 min with optogenetic stimulation and for 5 min without stimulation to measure the effect of light activation on the locomotion behavior. After the behavior test, we performed the histology of the brain samples from the mice to check the virus expression and probe implanted site. We collected data only if the virus was expressed more than 65% of the total volume of the OFC and the probe was targeted at the virus-expressed area.

Experimental design & Statistical analysis

All statistical analyses were performed using custom codes in IBM SPSS statistics 21 (IBM) and OriginPro 2019(Origin,). Normal distribution of data was verified using the Shapiro-Wilk normality test. For parametric tests, we used the Student's t-test to compare two groups, the one-way ANOVA with Fisher's least significant difference [LSD] post hoc test to compare multiple groups, and two-way ANOVA test to compare I-F curves of PV⁺ neurons. For nonparametric tests, we used the Wilcoxon signed-rank test to compare matched pairs, the Mann-Whitney *U* test to compare between two independent groups, and the Kolmogorov–Smirnov test to compare the distributions of two groups.

Data availability

This study includes no data deposition in external repositories. Further requests for data used in this study can be directed to the corresponding author (shleel@kaist.ac.kr).

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Author contributions

YSJ and DJ performed most of the experiments and contributed equally to this work; HK and EK performed and evaluated patch-clamp recording experiments; JHK helped to perform *in vivo* recordings and measure sniffing behavior; CYK and JWJ designed and fabricated the wireless-controllable optoelectronic neural probes; YO and SGK designed and cloned the AAV-sgPV vectors; YHL and CHK cloned the AAV-tdTomato vectors; SHL designed and supervised the study. YSJ, DJ, SHL wrote the manuscript with the contributions from all the authors.

Conflict of interest

643 The authors declare that they have no conflict of interest.

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788 **Figure Legends**

790 **Fig 1 - PWSI during adolescence enhances social sniffing behavior in female mice.**

791 A. Experimental design of the sociability test. Schematic of group-housing (GH), 1day social
792 isolation (1dSI), and post-weaning social isolation (PWSI). Note that the sociability behavior was
793 measured at P80.

B. Representative heat maps indicating mouse locations in the square chamber during the sociability test. In the chamber, a container containing a novel object (O) and a container containing the same-sex novel mouse (M) were located at diagonal corners. Heatmaps were calculated in EthoVision XT as mean duration of time spent in an area for each group.

C. The social sniffing index displays the social sniffing times divided by the total sniffing times. One-way ANOVA test, female: $F_{(2, 101)} = 8.431$, $***P < 0.001$, male: $F_{(2, 36)} = 0.015$, $P = 0.985$, Fisher's least significant difference post hoc test. Note that only the PWSI female mice showed a significant increase in the social sniffing behavior.

D. The social interaction index displays the time spent in the social zone divided by the total time spent in both object and social interaction zones. One-way ANOVA test, $F_{(2, 101)} = 3.496$, $*P = 0.034$, male: $F_{(2, 36)} = 0.109$, $P = 0.897$, Fisher's least significant difference post hoc test. Note that only the PWSI female mice showed a significant increase in social interaction behavior.

E. The number of estrous cycles per 4 days of GH ($n = 5$) and PWSI mice ($n = 5$). The duration of estrous cycles was not different between groups. Mann-Whitney U test, $P = 0.912$.

F. Representative images of cells in the vaginal smear that were stained with crystal violet. The estrous cycles of female mice were identified based on the shape of stained cells.

G. The social sniffing indices of females across the estrous cycle. One-way ANOVA test, $F_{(3,30)} = 0.927$, $P = 0.440$.

H. The social interaction indices of females across the estrous cycle. One-way ANOVA test, $F_{(3,30)} = 0.513$, $P = 0.677$.

Data information: All data are presented as mean $\pm$ SEM. In (C-D), Female GH, $n = 54$, 1dSI, $n = 14$, PWSI, $n = 36$; Male GH, $n = 15$, 1dSI, $n = 7$, PWSI, $n = 17$; In (G-H), Proestrus, $n = 10$,

816 Estrus, $n = 12$, Metestrus, $n = 6$, Diestrus, $n = 6$. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s.,
817 not significant.

818

Fig 2 - PV expression in the OFCL decreases in the PWSI female mice.

A. Representative image of the immunostained PV and NeuN in the OFC of a mouse brain coronal section marked with the regions of interest (ROI, 0.09 mm²). Scale bar, 1 mm.

B. Validation of the automatic cell counting method. Left; representative images of the PV (red) and NeuN (white) immunostained in the OFC_L of GH female mice. Left panels show original images used for manual counting, and right panels show automatically detected cell boundaries from original images. Bottom numbers indicate the counted cell numbers. Bars, average numbers

of NeuN⁺ and PV⁺ neurons in the OFC_L of GH female mice from the manual counting (gray) and automatic counting (black) ($n = 6$). Note that there is no difference between numbers from manual and automatic counting. Mann-Whitney U test (NeuN, $P = 0.200$; PV, $P = 1.000$).

C. Schematic brain images showing the selection of brain slices for cell counting. Three brain slices were selected between AP +2.1mm and +2.34mm with an interval of 60 μ m.

D. Representative fluorescence images of the PV (red) and the NeuN (white) immunostained in the OFC_L and OFC_R of P80 GH and PWSI female mice. Scale bars, 100 μ m.

E. The number of PV⁺ neurons in the OFC. PWSI decreased the number of PV⁺ neurons in the female OFC_L. Mann-Whitney U test (female left, $*P = 0.030$; female right, $P = 0.297$; male left, $P = 0.623$; male right, $P = 1.000$).

F. The number of NeuN⁺ neurons in the OFC. Mann-Whitney U test (female left, $*P = 0.024$, female right, $P = 1.000$, male left, $P = 0.903$; male right, $P = 1.000$).

G. The number of PV⁺ / NeuN⁺ neurons in the OFC. Mann-Whitney U test (female left, $*P = 0.045$; female right, $P = 0.298$; male left, $P = 0.540$; male right, $P = 0.903$).

Data information: All data are presented as mean $\pm$ SEM. In (E-G), Female, GH, $n = 6$ mice, PWSI, $n = 6$ mice; Male, GH, $n = 4$ mice, PWSI, $n = 5$ mice. $* P < 0.05$.

Fig 3 - Re-group-housing of PWSI female mice after P50 restores normal sociability and PV expression in the OFCL.

A. Schematic illustration of group-housing (GH) or re-group-housing (re-GH) conditions. Mice were isolated at P21 and re-group-housed at P50 in re-group-housing condition. Note that the sociability test and the histology were performed at P50 and P80.

B. Representative fluorescence images of the PV immunostained in the OFCL of GH and re-GH female mice at P50 and P80. Scale bars, 100μm.

C. The results of the sociability test of the GH and the re-GH mice at P50 and P80. Left, the social sniffing index. Wilcoxon signed-rank test within group (GH, $*P = 0.023$; re-GH, $P = 0.450$) and Mann-Whitney U test between groups (P50, $P = 0.555$; P80, $P = 0.212$). Right, the social interaction index. Wilcoxon signed-rank test within group (GH, $P = 0.068$; re-GH, $P = 0.625$),

858 Mann-Whitney U test between groups (P50, $P = 0.555$; P80, $P = 0.470$). GH, $n = 11$ mice, re-GH,
859 $n = 11$ mice

860 D. The number of PV⁺ neurons in the OFC. Mann-Whitney U test within group (OFC_L, GH, $**P$
861 $= 0.005$; re-GH, $*P = 0.030$; OFC_R, GH, $*P = 0.011$; re-GH, $*P = 0.014$), Mann-Whitney U test
862 between group (OFC_L, P50, $P = 0.901$; P80, $P = 0.749$; OFC_R, P50, $P = 0.934$; P80, $P = 0.285$).
863 GH, P50, $n = 11$ mice; P80, $n = 4$ mice; re-GH, P50, $n = 8$ mice, P80, $n = 6$ mice.

864 Data information: All data are presented as mean $\pm$ SEM. $**P < 0.01$, $*P < 0.05$, n.s., not
865 significant.

866

Fig 4 - PWSI reduces sniffing-induced activity in the OFC_L of female mice.

A. Experimental design for *in vivo* multichannel recording of the neurons responsive to social sniffing in the OFC of head-fixed mice.

B. Schematic illustration of mouse brain sections marked with the recording sites in the OFC of GH or PWSI female mice. Blue shading, OFC; colored bars, recording sites confirmed by post hoc histology.

C. Neural responses associated with sniffing in the female OFC_L. The large pie charts, the OFC_L neurons showing social sniffing responses (Social, red), object sniffing responses (Object, blue), or both social and object sniffing responses (Both, green). Inc and dec, neurons showing significantly increased and decreased activities after the onset of sniffing, respectively ($P < 0.01$, bootstrap); No resp., neurons that show no responses. The small pie charts, the waveforms of the OFC_L neurons classified according to the waveform width. RS, regular-spiking neurons with the waveform width $>450\text{ms}$; FS, fast-spiking neurons with the waveform width $<350\text{ms}$; US, unclassified neurons with the waveform width between 350ms and 450ms. Heat maps, Z-scored activity of all neurons normalized by the pre-sniffing activity. GH: $n = 5$ mice, $n = 84$ units, PWSI: $n = 3$ mice, $n = 57$ units.

D. The same as (C) but for the OFC_R neurons. GH: $n = 4$ mice, $n = 74$ units, PWSI: $n = 4$ mice, $n = 60$ units.

E. Representative raster plots and peri-event time histograms of example OFC_L neurons from the GH female mouse (top, GH A #5) and from the PWSI female mouse (bottom, SI B #4) aligned by the onset of object sniffing (left, blue) and the onset of social sniffing (right, red).

F. Averaged sniffing-induced activity for 2 sec (Z score) of the top 20% of OFC_L neurons that show either increased or decreased responses after the onset of social sniffing. Bars, means $\pm$ SEM. Wilcoxon signed-rank test for each bar (Object_{sniffing}, Social_{sniffing}, $***P < 0.001$, $***P < 0.001$ for

893 GH Increased; $P = 0.083$, $**P = 0.004$ for PWSI Increased; $P = 0.478$, $***P < 0.001$ for GH
 894 Decreased; $*P = 0.045$, $**P = 0.004$ for PWSI Decreased). Wilcoxon signed-rank test within a
 895 group (Object_{sniffing} vs. Social_{sniffing}, $*P = 0.047$ for GH Increased; $*P = 0.023$, for PWSI Increased;
 896 $**P = 0.002$ for GH Decreased; $P = 0.120$ for PWSI Decreased); Mann-Whitney U test between
 897 groups (Object_{sniffing}, Social_{sniffing}, $*P = 0.019$, $***P < 0.001$ for Increased activity; $P = 0.452$, P
 898 $= 0.110$ for Decreased activity). GH, $n = 17$ units, PWSI, $n = 11$ units. $***P < 0.001$, $**P < 0.01$,
 899 $*P < 0.05$, n.s., not significant.

900

Fig 5 – CRISPR/Cas9-mediated knock-down of PV, but not PNN degradation, in the OFCL enhances sociability in female mice.

A. CRISPR/Cas9-mediated knock-down of PV in the female OFCL. Left and middle, representative fluorescence images of the OFCL of the female PV::Cas9-eGFP mice injected with the AAV-tdTomato (top, tdTom) or AAV-sgPV-tdTomato (bottom, sgPV). Red, tdTomato; green, Cas9-eGFP; blue, immuno-stained PV. Scale bars, 50µm. Right, a representative image of the OFCL of a PV::Cas9-eGFP mouse injected with the AAV-sgPV-tdTomato. Scale bar, 1000µm.

B. The number of PV⁺ and WFA⁺ neurons in the OFCL injected with the AAV-tdTomato (gray bars) or with the AAV-sgPV-tdTomato (red bars). tdTom vs. sgPV, $n = 5$ vs. $n = 6$. Mann-Whitney U test (PV⁺, $*P = 0.022$; WFA⁺, $P = 0.306$).

C. Social sniffing index of PV::Cas9 female mice injected with AAV-tdTomato (tdTom) and AAV-sgPV-tdTomato (sgPV). Mann-Whitney U test, $*P = 0.021$.

D. Same as (C) but for social interaction index. Mann-Whitney U test, $*P = 0.034$.

916 E. chABC-induced PNN degradation in the OFC_L of female mice. Left and middle, immunostained
 917 PV (red) and WFA (green) in the OFC_L of female mice injected with PBS (top) or chABC (bottom).
 918 Scale bars, 50μm. Right, representative fluorescence images of the PNN stained with the WFA.
 919 Note that the chABC injection selectively degraded PNNs in the OFC_L. Scale bars, 1000μm.

920 F. The number of PV⁺ and WFA⁺ neurons in the OFC_L injected with the PBS (gray bars) or the
 921 chABC (green bars). Mann-Whitney *U* test (PV, *P* = 0.609; WFA, **P* = 0.017), *n* = 7 for PBS, *n*
 922 = 7 for chABC.

923 G. Social sniffing index of mice injected with PBS (gray) or chABC (green). Mann-Whitney *U*
 924 test, *P* = 0.878

925 H. Social interaction index of mice injected with PBS (gray) or chABC (green). Mann-Whitney *U*
 926 test, *P* = 0.798.

927 Data information: All data are presented as mean ± SEM. In (C-D), female PV::Cas9-eGFP mice
 928 injected with AAV-tdTomato (tdTom, black and gray, OFC_L, *n* = 8 mice; OFC_R, *n* = 6 mice) or
 929 with AAV-sgPV-tdTomato (sgPV, red, OFC_L, *n* = 10 mice; OFC_R, *n* = 5 mice). In (G-H), social
 930 behavior in female mice injected with PBS (*n* = 8 mice) or chABC (*n* = 8 mice) into the OFC_L.
 931 **P* < 0.05, n.s., not significant.
 932

Fig 6 - Knock-down of PV reduces sniffing-induced activity and decreases synaptic inhibition in the female OFC_L .

936 A. Schematic illustration of *in vivo* multichannel recording in a head-fixed mouse.

937 B. Schematic illustration of coronal brain sections of *in vivo* recording sites in the OFC_L where

938 tdTomato (tdTom)- or PV sgRNA (sgPV)-expressing AAVs were injected.

939 C. Schematic illustration of analysis windows for baseline and sniffing-induced activities of OFC_L

940 neurons. Mean firing rates during 2 sec before the sniff onset and averaged Z scores during 2 sec

941 after the sniff onset were calculated as the baseline activity and the sniffing-induced activity,

942 respectively.

943 D. The cumulative distribution function of baseline firing rates in tdTom and sgPV mice.

944 Kolmogorov–Smirnov test, $**P = 0.006$.

945 E. Mean baseline firing rates of tdTom and sgPV mice. Mann-Whitney U test, $***P < 0.001$.

946 F. Neural responses associated with the sniffing in the OFC_L of tdTom- (top) or sgPV-(bottom)

947 injected PV::Cas9 female mice. Pie charts, the OFC_L neurons showing social sniffing responses

948 (Social, red), object sniffing responses (Object, blue), or both social and object sniffing responses

949 (Both, green). Inc and dec, neurons showing significantly increased and decreased activities after

950 the onset of sniffing, respectively ($P < 0.01$, bootstrap); No resp, neurons that show no responses.

951 Heat maps, Z-scored activity of all recorded neurons by the pre-sniffing activity. Note that both

952 the number of sniffing-responsive neurons and social sniffing-induced activity were reduced in

953 sgPV females. Heat maps, Z-scored activity of all neurons normalized by the pre-sniffing activity.

954 G. Averaged sniffing-induced activity for 2 sec (Z score) of the top 20% of OFC_L neurons that

955 show either increased or decreased responses after the onset of social sniffing. Wilcoxon signed-

956 rank test for each bar (Object_{sniffing}, Social_{sniffing}, $***P < 0.001$, $***P < 0.001$ for tdTom Increased;

957 $P = 0.154$, $**P = 0.006$ for sgPV Increased; $**P = 0.007$, $***P < 0.001$ for tdTom Decreased; P

958 $= 0.053$, $**P = 0.006$ for sgPV Decreased). Note that the increased neurons showed larger activity

changes after social sniffing than after object sniffing. Wilcoxon signed-rank test within a group (Object_{sniffing} vs Social_{sniffing}, *** $P < 0.001$ for tdTom Increased; ** $P = 0.008$ for sgPV Increased; $P = 0.141$ for tdTom Decreased; * $P = 0.011$ for sgPV Decreased). Social sniffing-induced activity was larger in tdTom females than in sgPV females. Mann-Whitney U test between groups (Object_{sniffing}, Social_{sniffing}, $P = 0.067$, ** $P = 0.004$ for Increased activity; $P = 0.720$, $P = 0.073$ for Decreased activity), tdTom, $n = 24$ units, sgPV, $n = 12$ units.

H. Physiological properties of PV⁺ neurons expressed with tdTom or sgPV. Schematic illustration of the whole-cell patch-clamp recordings of PV⁺ neurons (red) or excitatory neurons (blue) in the PV::Cas9 mice that had been injected with the AAVs expressing either tdTom or sgPV-tdTom into the OFC_L.

I. SgPV expression decreased the excitability of PV⁺ neurons. Current-firing (I-F) curves measured in the OFC_L PV⁺ neurons. sgPV expression caused a significant decrease in current-evoked spike counts in the PV⁺ neurons. Two-way ANOVA, $F_{AAV(1, 372)} = 14.528$, *** $P < 0.001$, $F_{current(11, 372)} = 121.158$, *** $P < 0.001$, $F_{AAV \times current(11, 372)} = 0.388$, $P = 0.960$.

J. Mean half-width of spikes (ms) measured in PV⁺ neurons expressed with the tdTom or the sgPV. Unpaired, Student's t-test, $P = 0.628$.

K. Mean input resistance (M Ω) measured in PV⁺ neurons expressed with the tdTom or the sgPV. Unpaired, Student's t-test, $P = 0.173$.

L. Spontaneous EPSCs measured in PV⁺ neurons of the OFC_L. Left bars, mean amplitudes (pA) of sEPSCs in PV⁺ neurons. Unpaired, Student's t-test ($P = 0.636$). Right bars, mean frequency (Hz) of sEPSCs in PV⁺ neurons. Unpaired, student's t-test ($P = 0.940$). tdTom = 10 cells (5 mice);

980 sgPV=10 cells (4 mice); female.

981 M. Spontaneous IPSCs measured in excitatory neurons of the OFC_L. Left bars, mean amplitudes
 982 (pA) of sIPSCs in Pyr neurons. Unpaired, Student's t test (** $P = 0.006$). Right bars, mean
 983 frequencies (Hz) of sIPSCs in Pyr neurons. Unpaired, Student's t test (* $P = 0.015$). tdTom = 16
 984 cells (3 mice); sgPV=17 cells (3 mice); female.

985 Data information: All data are presented as mean $\pm$ SEM. In (D-G), tdTom: $n = 6$ mice, $n = 122$
 986 units, sgPV: $n = 5$ mice, $n = 61$ units. In (I-M), tdTom = 16 cells (3 mice); sgPV = 20 cells (4 mice);
 987 female. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s., not significant.

988

Fig 7 – Optogenetic activation of OFCL caused hypersociability in female mice.

A. Virus expression in the female OFCL. Left, a representative image of the OFCL of a WT female mouse injected with the AAV-CAMKII α -ChR2-eYFP. Green, ChR2-eYFP; blue, DAPI. Right, schematic illustration of the OFCL in the WT female mouse injected with the AAV-CAMKII α -eYFP (eYFP) or AAV-CAMKII α -ChR2-eYFP (ChR2-eYFP).

B. Schematic illustration of wireless optogenetic manipulation. The wireless LED probe was implanted in the OFCL of a mouse. The smartphone app modulated light on and off by Bluetooth Low Energy (BLE). 20Hz 473nm light was delivered to the OFCL during the test session.

C. The social sniffing index of eYFP (open bars) and ChR2-eYFP (closed bars) mice measured on consecutive two days. For day1, light was not delivered (OFF), and the social sniffing index was

not different between eYFP and ChR2-eYFP groups. Unpaired, Student's t-test ($P = 0.516$). For day 2, light was delivered to the OFC_L (ON), and ChR2-eYFP group showed higher social sniffing index than eYFP group. Unpaired, Student's t-test ($*P = 0.030$); Paired, Student's t test within the group (eYFP, $**P = 0.007$; ChR2-eYFP, $P = 0.179$).

D. Social habituation index indicates the difference in social sniffing index between day 1 (OFF) and day 2 (ON). Unpaired, Student's t-test between groups ($*P = 0.036$); Wilcoxon signed rank test for each group (eYFP, $*P = 0.036$; ChR2-eYFP, $P = 0.179$).

E. Locomotion of mice (distance moved (cm) for 5 min) in the open field chamber was not affected by the optogenetic activation of the OFC_L. Paired, Student's t-test (OFF vs ON, eYFP, $P = 0.078$, ChR2-eYFP, $P = 0.602$). eYFP, $n = 5$, ChR2, $n = 3$; female mice.

Data information: All data are presented as mean $\pm$ SEM. Blue lightning sign, delivery of light stimulation (20Hz, 473nm). In (C-D), eYFP, $n = 6$; ChR2-eYFP, $n = 5$; female mice. $*P < 0.05$, n.s., not significant.

Supplementary Figure Legends

Supplementary Fig 1 – PWSI does not alter the PNN maturation in the OFC_L.

A. A representative image of a mouse coronal brain section that was immunostained for PV (red) and WFA (green) (scale bar, 1000 μ m).

B. Representative fluorescence images of the OFC_L immunostained with anti-PV and anti-WFA antibodies at P80 of GH and PWSI female and male mice.

C. The number of WFA⁺ neurons in the OFC left and right of male and female mice. Bars, means $\pm$ SEM. Mann-Whitney *U* test (GH vs. PWSI, female left, *P* = 0.46; female right *P* = 0.185; male

1024 left, $P = 0.337$; male right, $P = 0.442$); n.s., not significant. Females, GH, $n = 12$ mice, PWSI, n
 1025 = 11 mice; males, GH, $n = 7$ mice, PWSI, $n = 7$ mice.

1026

1028 **Supplementary Fig 2 - Sniffing behaviors of head-fixed mice during *in vivo* recording**

A. Left, a representative image of a mouse in a movie that was analyzed for sniffing behavior. ROI, region of interest that was used for analyzing the brightness changes in the video clip. Background, an area used for normalizing the brightness changes in the ROI. Right, a zoomed-in image of head-fixed mice under the social sniffing behavior. The right mouse was a subject for *in vivo* recording.

B. Representative sniffing events measured in GH or PWSI female mice. Gray trace, raw brightness changes in the ROI (ROI ΔF). Green trace, moving average in the ROI (F_0 , sliding window = 50 frames). Blue and red trace, normalized brightness changes in the ROI (ROI $\Delta F - F_0$). Circles, sniffing events were automatically detected by a Matlab-based image analysis code. Horizontal lines, sniffing events manually detected by an observer in the EthoVision XT software. Note that automatic counting methods detect sniffing events at similar times identified by manual counting. Blue, object sniffing; red, social sniffing.

C. Left, sniffing events automatically counted. Dots, individual data; lines, means $\pm$ SEM. Wilcoxon signed-rank test for within a group, Object vs. Social, GH, $P = 0.465$, PWSI, $P = 1.000$. Mann-Whitney U test for between groups, GH vs. PWSI, Object, $P = 0.857$, Social, $P = 0.629$. Right, sniffing events manually identified. Dots, individual data; lines, means $\pm$ SEM. Wilcoxon signed-rank test for within a group, Object vs. Social, GH, $P = 0.686$, PWSI, $P = 0.109$. Mann-Whitney U test for between groups, GH vs. PWSI, Object, $P = 0.393$, Social, $P = 1.000$. GH: $n = 4$ mice, PWSI: $n = 3$ mice.

D. Same as (B) but for sniffing events measured in tdTom or sgPV-expressed PV::Cas9 female mice.

E. Same as (C) but for tdTom and sgPV mice. Left, Wilcoxon signed-rank test for within a group, tdTom, $P = 0.463$, sgPV, $P = 0.892$. Mann-Whitney U test for between groups, Object, $P = 0.537$, Social, $P = 0.126$. Right, manual counting of sniffing bouts. Right, Wilcoxon signed-rank test for

1052 within a group, tdTom, $P = 0.528$, sgPV, $P = 0.131$. Mann-Whitney U test for between groups,

1053 Object, $P = 0.662$, Social, $P = 1.000$. tdTom: $n = 6$ mice, sgPV: $n = 5$ mice.

1054 Data information: All data are presented as mean $\pm$ SEM. n.s., not significant

1055

Supplementary Fig 3 - CRISPR/Cas9-Mediated Knock-down of PV.

A. Schematic illustration of the AAV-sgPV-tdTomato expression vector. The sgRNA vector contains the encoding sequence of tdTomato protein for the identification of transduced neurons.

We used two types of AAVs to target both exon 4 and exon5 of the PV gene.

B. DNA insertion (blue) and deletion (red) mutation ratio (Indel frequency) by the designed sgPVs targeting exon 4 and exon5 of the PV gene and cloned in the AAV-tdTom vector.

C and D. The gRNA sequences targeting PV exon4 (C) and exon5 (D), and representative mutation patterns detected by the sequencing of the PV locus of the infected Neuro2a cells. PAM sequence indicated by blue and red scissors show CRISPR/Cas9 cutting sites. Inserted nucleotides and deleted nucleotides are indicated in red. The total indel frequency and each type of indel frequency are labeled on the right of the mutated sequences.

E. Representative PV staining images of PV::Cas9 mice, which were injected with AAV-tdTomato or AAV-sgPV-tdTomato. Scale bars, 20 μ m. Red, tdTomato expressed by AAV vectors; green, Cas9-eGFP transgenically expressed in PV⁺ neurons; blue, immunostained PV proteins. Solid arrows indicate triple-positive cells, and dashed arrows indicate double-positive and PV-negative cells.

F. Quantification of PV expression levels in the retrosplenial cortex (RSC), injected with AAVs that deliver tdTom or sgPVs, compared to the Cas9-eGFP expression in PV::Cas9 mice. Numbers in columns indicate the number of Cas9 positive neurons analyzed. One-way ANOVA, $F_{(2, 101)} = 27.279$, $***P < 0.001$; Fisher's least significant difference post hoc test.

G. Quantification of PV⁺ cells among eGFP and tdTomato double-positive cells in the RSC injected with AAVs. Numbers in columns indicate the number of brain samples analyzed. One-way ANOVA; $F_{(3, 63)} = 6.478$, $***P < 0.001$; Fisher's least significant difference post hoc test.

1080 **Data information:** All data are presented as mean $\pm$ SEM. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

1081