

Parvalbumin interneuron ErbB4 controls ongoing network oscillations and olfactory behaviors in mice

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eLife Assessment

This study provides **useful** information on the potential role of ErbB4 expression in parvalbumin-positive cells on olfactory behaviour and circuit dynamics in the olfactory bulb. The question is timely and novel, and findings could shed light on the critical role that ErbB4 may play in modulating olfactory bulb cell function and olfactory perception. Although the authors use a comprehensive set of experiments for their analysis, the evidence is **incomplete** as many of the experiments are underpowered and the model for selective knockout of ErbB4 in olfactory parvalbumin cells is not validated.

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Abstract

Parvalbumin (PV)-positive interneurons modulate the processing of odor information. However, less is known about how PV interneurons dynamically remodel neural circuit responses in the olfactory bulb (OB) and its physiological significance. This study showed that a reinforced odor discrimination task up-regulated the activity of ErbB4 kinase in mouse OB. ErbB4 knock-out in the OB impaired dishabituation of odor responses and discrimination of complex odors, whereas odor memory or adaptation had no alteration in mice. RNAscope analysis demonstrated that ErbB4-positive neurons are localized throughout the OB, whereas within the internal and external plexiform layers, ErbB4 mRNA are largely expressed in PV-positive interneurons. ErbB4 knock-out in PV interneurons disrupted odor-evoked responses of mitral/tufted cells, and led to increased power in the ongoing local field potential in awake mice. We also found a decrease in the frequency of miniature inhibitory postsynaptic currents and deficits in stimulus-evoked recurrent and lateral inhibition onto mitral cells, suggesting broad impairments in inhibitory microcircuit following PV-ErbB4 loss. Similarly, ErbB4 ablation in OB PV interneurons disrupted olfactory discrimination and dishabituation in mice. These findings provide novel insights into the role of PV-ErbB4 signaling in

inhibitory microcircuit plasticity, ongoing oscillations, and OB output, which underlies normal olfactory behaviors.

Introduction

Discriminating chemical cues in the environment is not only necessary for evaluating potential threats but also influences cognitive and emotional behaviors. The olfactory system dynamically adjusts its sensitivity to odors with changes in the environment and motivational and cognitive states. For example, odor learning improves performance on olfactory discrimination tests. Habituation and adaptation to successive exposures to an odor lead to decreased behavioral responses to it, whereas an unexpected odor enhances olfactory salience and stimulates behavioral dishabituation. Dysfunction in olfactory discrimination has been observed in the earliest stages of several neurodegenerative disorders and psychiatric diseases (Carnemolla et al., 2020 [↗](#); Walker et al., 2021 [↗](#)). Similarly, olfactory discrimination ability has significant predictive valence for future cognitive decline in healthy aging individuals (Sohrabi et al., 2012 [↗](#); Uchida et al., 2020 [↗](#); Yoshitake et al., 2022 [↗](#)). However, neural mechanisms that underlie the dynamic modulation of olfactory behaviors remain largely unknown.

The olfactory bulb (OB) is the first relay and processing station in the olfactory system. In the OB, odor signals are transformed into the informational output that is sent to the associated olfactory cortex. As the principal output neurons of the OB, mitral/tufted cells (M/TCs) receive glutamatergic excitatory inputs from olfactory sensory neurons and GABAergic inhibitory inputs from heterogeneous subpopulations of interneurons. An imbalance in excitation/inhibition onto MCs has been shown to impair olfactory discrimination (Lepousez and Lledo, 2013 [↗](#)). Furthermore, overlapping odor-evoked input patterns to the OB undergo reformatting to enhance discrimination of similar odors, and this reformatting depends on GABAergic interneurons (Gschwend et al., 2015 [↗](#); Li et al., 2018 [↗](#); Mohamed et al., 2019 [↗](#); Zavitz et al., 2020 [↗](#)). GABAergic interneurons in the OB mediate recurrent and lateral inhibition of MCs. Recurrent inhibition of MCs by granule cells (GCs) increases the frequency of odor-induced activity and facilitates odor discrimination (MacLeod and Laurent, 1996 [↗](#); Nunes and Kuner, 2015 [↗](#)). Interglomerular lateral inhibition sharpens or filters the odor-induced output signals of MCs, enhancing the contrast of odor representations and consequently the ability to discriminate similar odors (Egger and Kuner, 2021 [↗](#)). Overall, the OB possesses various types of interneurons to control different olfactory behaviors (Lyons-Warren et al., 2023 [↗](#); Pardasani et al., 2023 [↗](#); Takahashi et al., 2016 [↗](#)). Parvalbumin-positive (PV) interneurons form dendrodendritic contacts with the somata and dendrites of neighboring MCs (Huang et al., 2013 [↗](#); Kato et al., 2013 [↗](#); Matsuno et al., 2017 [↗](#); Miyamichi et al., 2013 [↗](#)). Unlike odor-specific responses of GCs and MCs, PV interneurons exhibit strong responses to broader odors (Kato et al., 2013 [↗](#)). M/TCs receives prominent input of PV interneurons from external plexiform layer (EPL) (Miyamichi et al., 2013 [↗](#)). However, the contributions of PV interneurons to olfactory behaviors and the associated molecular mechanisms are not understood.

The receptor tyrosine kinase ErbB4 is preferentially expressed by GABAergic neurons in cortical areas, but in subcortical regions, ErbB4 is also expressed by non-GABAergic neurons (Bean et al., 2014 [↗](#); Deng et al., 2019 [↗](#); Dominguez et al., 2019 [↗](#); Fazzari et al., 2010 [↗](#); Lin et al., 2018 [↗](#); Mei and Nave, 2014 [↗](#); Shi and Bergson, 2020 [↗](#)). ErbB4 kinases are expressed in neuroblasts of the subventricular zone and the rostral migratory stream to the OB, implicating ErbB4 signaling in olfactory interneuronal precursor differentiation and olfaction (Anton et al., 2004 [↗](#)). ErbB4 regulates synaptic transmission and plasticity in PV interneurons in the neocortex and hippocampus (Chen et al., 2022 [↗](#); Dominguez et al., 2019 [↗](#); Grieco et al., 2020 [↗](#); Lin et al., 2018 [↗](#)). Recently, we found a distinct ErbB4 signaling pathway in PV interneurons of the prefrontal cortex that hinders flexibility in odor-associated memory updating but has no influence

on the initial olfactory learning and memory (Cai and Shuman, 2022 [↗](#); Xu et al., 2022 [↗](#)). However, whether or not ErbB4 is expressed by PV neurons in the OB, and its effects on olfactory behaviors if so, are not clear.

In this study, we show that an odor discrimination task promotes the activation of ErbB4 in the OB. ErbB4 mRNA-positive neurons are localized throughout the OB layers, with most being PV-positive interneurons in the internal plexiform layer (IPL) and EPL. In this context, we evaluated the performance of PV-ErbB4 knockout mice in odor discrimination, habituation/dishabituation, and detection threshold tasks. We also explored the effects of ErbB4 in PV interneurons on the odor-evoked activity of MCs and ongoing network oscillations in awake mice, and on the inhibitory modulation onto MC output. Our findings reveal physiological roles and mechanisms of PV interneuron ErbB4 signaling in dynamic modulation of olfactory behaviors.

Results

Performing an odor discrimination task upregulates the activity of ErbB4 in the OB

First, we examined the levels of ErbB4 protein and its phosphorylated form (p-ErbB4) in mouse OB after a reinforced go/no-go odor discrimination task. Over 3 days (200 trials each day), adult mice were first trained to discriminate between a pair of simple odors (water-rewarded isoamyl acetate and unrewarded heptanone) and then learned to discriminate complex chemical cues (60%/40% binary mixtures of these odors) (Figures 1A [↗](#) and 1B [↗](#)). A similar learning task without odor discrimination (go/go task) was performed in the control group (Figure 1A [↗](#)). As shown in Figures 1C [↗](#) and 1D [↗](#), the level of p-ErbB4 in the go/no-go group was higher than that in the control group, suggesting a link between increased ErbB4 activity in the OB and olfactory discrimination ability.

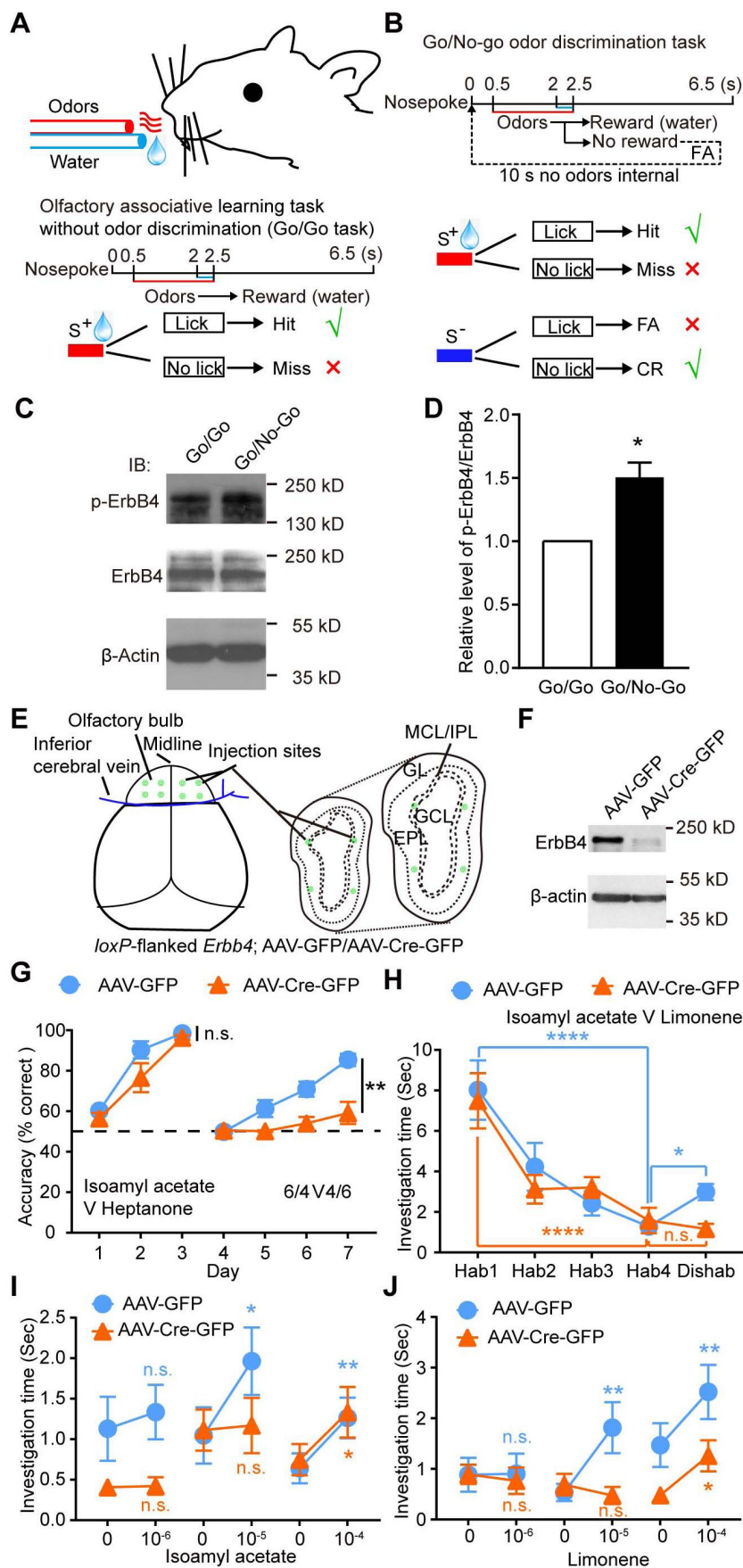


Figure 1.

ErbB4 in the OB is critical for odor discrimination and dishabituation

(A) Behavioral paradigm for olfactory associative learning without odor discrimination (go/go task). Mice inserted their snout into the sampling port to trigger odors. The schematic describes the timeline of a single trial. Mice learned to lick the metal tube to receive water in response to either of the odors in the pair (reward, hit).

(B) Timeline for a single trial in the go/no-go odor discrimination task. Mice learned to avoid licking the metal tube for the unrewarded odor (correct rejection, CR). Licking when presented with the unrewarded odor (false alarm, FA) led to no water reward and a timeout of up to 10 s. (C and D) ErbB4 activity in the OB was elevated after training on the reinforced go/no-go odor discrimination task. Relative p-ErbB4 and ErbB4 levels were normalized to their respective β -actin control groups in the western blot analysis ($n = 3$ mice per group, $t_{(2)} = 4.34$, $P = 0.0492$, paired t test). (E) Schema indicating virus injection sites. To specifically delete ErbB4 protein in the OB, AAV-Cre-GFP was injected into bilateral OB of neonatal *loxP*-flanked ErbB4 mice. (F) Reduced ErbB4 expression in the OB of a mouse injected with AAV-Cre-GFP. (G) Odor discrimination performance under the reinforced go/no-go task. The accuracy for simple odor pairs was similar for the control and experimental groups ($n = 8$ mice per group, $F_{(1, 14)} = 2.83$, $P = 0.1148$, two-way ANOVA). However, the accuracy for difficult odor mixtures (6/4 V 4/6) was reduced in AAV-Cre-GFP mice ($F_{(1, 14)} = 16.14$, $P = 0.0013$, two-way ANOVA). (H) Odor performance under a spontaneous habituation/dishabituation task. Both animal groups showed a decline in investigation time to isoamyl acetate over the habituation period ($n = 9$ AAV-GFP mice, $P < 0.0001$; $n = 10$ AAV-Cre-GFP mice, $P < 0.0001$, $F_{(3, 51)} = 21.83$, two-way ANOVA). However, AAV-GFP ($F_{(1, 17)} = 3.52$, $P = 0.0065$), but not AAV-Cre-GFP mice ($P = 0.6296$, two-way ANOVA), showed an increase in investigation time toward limonene in the dishabituation period. (I) Odor detection threshold to isoamyl acetate. For AAV-GFP mice, the sniffing time toward isoamyl acetate was significantly higher than that for mineral oil at concentrations of 10^{-5} and 10^{-4} , but not 10^{-6} . ($n = 12$ mice, $F_{(1, 21)} = 0.19$, 4.18 and 14.88, $P = 0.5689$, 0.0111, and 0.0090, two-way ANOVA). These results show that AAV-GFP mice were able to detect isoamyl acetate at a concentration of 10^{-5} . For AAV-Cre-GFP mice, the sniffing time toward isoamyl acetate was significantly higher than that for mineral oil at a concentration of 10^{-4} , but not at concentrations of 10^{-5} and 10^{-6} ($n = 11$ mice, $P = 0.9672$, 0.8713, and 0.0172, two-way ANOVA). These results show that AAV-Cre-GFP mice only detect isoamyl acetate at a concentration of 10^{-4} . (J) Similarly, AAV-GFP mice were able to detect limonene at a concentration of 10^{-5} ($n = 12$ mice, $F_{(1, 21)} = 0.03$, 4.77 and 12.96, $P = 0.9603$, 0.0011, and 0.0069, two-way ANOVA), whereas AAV-Cre-GFP mice only detected limonene at a concentration of 10^{-4} ($n = 11$ mice, $P = 0.7671$, 0.5490, and 0.0463, two-way ANOVA). Data are presented as means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, n.s. = not significant. EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; IPL, internal plexiform layer; MCL, mitral cell layer.

ErbB4 in the OB is critical for odor discrimination and dishabituation

Next, we evaluated the performance of ErbB4 knockout mice in the go/no-go task. ErbB4 protein was specifically deleted in the OB by delivery of adeno-associated virus recombinants expressing Cre recombinase and green fluorescence protein (GFP) (AAV-Cre-GFP) into both sides of the OB of neonatal mice carrying *loxP*-flanked *ErbB4* alleles (Figure 1E and F). When learning to discriminate simple odor pairs (Figure 1G), both AAV-GFP and AAV-Cre-GFP mice reached an accuracy of above 90% within 3 days (~ 600 trials), suggesting that ErbB4 deletion in the OB has no influence on learning and memory or the sense of smell. However, when distinguishing the 60/40% binary mixtures of these odors, the AAV-GFP group showed an accuracy above 80% after the 4-day training program (~ 800 trials), but the AAV-Cre-GFP-injected mice showed a significant lower accuracy. This indicates that ErbB4 in the OB maintains the ability of mice to discriminate complex odors.

To further determine the role of OB ErbB4 in olfactory behaviors, we conducted a habituation/dishabituation test to assess the spontaneous behavioral responses of mice to repeatedly presented odors in the absence of any reward (**Figure 1H**). Both AAV-GFP mice and AAV-Cre-GFP mice habituated to four successive odor presentations with 2-min intertrial intervals: their behavioral responses to the same odor (isoamyl acetate) were progressively reduced. AAV-GFP mice rapidly dishabituated and increased their investigation time when a novel odor was presented (limonene, fifth trial), whereas AAV-Cre-GFP mice did not significantly alter their behavioral responses to the novel odor. Additionally, olfactory detection thresholds for isoamyl acetate and limonene were about 10^{-5} in AAV-GFP mice, but 10^{-4} in AAV-Cre-GFP mice (**Figures 1I** and **1J**). In the habituation and dishabituation tasks, the two odors were used at levels far above the thresholds.

These results demonstrate that ErbB4 in the OB contributes to olfactory discrimination and dishabituation, but not to olfactory memory and habituation.

ErbB4 is expressed in PV interneurons within the EPL and IPL layers of the OB

RNAscope analysis was performed to detect mRNA levels of ErbB4 in the OB of PV-*ErbB4*^{+/+} mice (generated by crossing PV-Cre mice with *loxP*-flanked *ErbB4* mice). As shown in **Figures 2A** and **2D**, ErbB4 mRNA was expressed in all five layers of the OB (green); ErbB4-expressing neurons accounted for 58.0%, 44.4%, 71.9%, 69.8%, and 71.2% of the total cells (DAPI, blue) in the GL, EPL, MCL, IPL, and GCL. PV interneurons (red) in the OB were mainly distributed in the EPL and IPL layers (**Figure 2A**); in these layers, ErbB4 tended to be located within PV interneurons (yellow) (**Figures 2A-2C**). Of the PV interneurons in the EPL and IPL, 69.4% and 100% expressed ErbB4, respectively (**Figure 2E**). Overall, ErbB4 expression in PV interneurons is highest within the IPL and lower within the EPL.

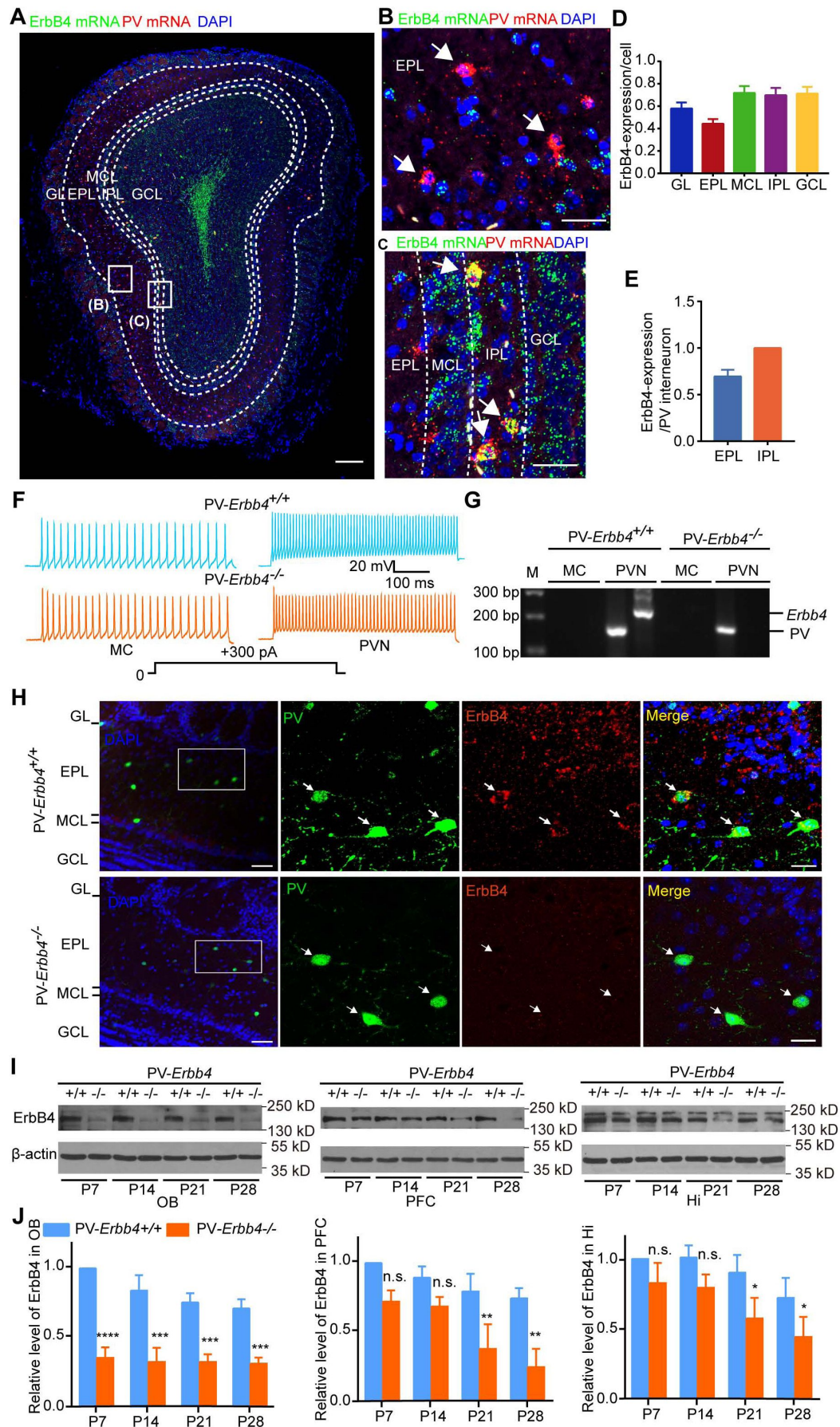


Figure 2.

ErbB4 proteins are largely expressed in PV interneurons in the OB

(A) *In vitro* imaging of ErbB4 mRNA in sections from PV-*ErbB4*^{+/+} mice (generated by crossing PV-Cre mice with *loxP*-flanked *ErbB4* mice). Double single-molecule fluorescence in situ hybridization of ErbB4 (green) and PV (red) in the OB. Scale bar, 200 μ m. (B) Magnified view of the EPL box from A. Scale bar, 50 μ m. (C) Magnified view of the IPL box from A. Scale bar, 50 μ m. (D) Summarized data showing the proportion of ErbB4-expressing neurons in different layers ($n = 24, 24, 24, 22$, and 24 fields from 4 mice). (E) Summarized data showing the proportion of the ErbB4/PV double-positive neurons relative to the total number of PV interneurons in the EPL and IPL ($n = 18$ and 5 fields from 4 mice). DAPI staining was used to determine the total number of cells. (F) *In vitro* electrophysiology experiments performed in slices from PV-*ErbB4*^{+/+} or PV-*ErbB4*^{-/-} mice (generated by crossing PV-Cre mice with *loxP*-flanked *ErbB4* mice). Representative examples of action potentials (APs) elicited by positive current injection (500 ms, 300 pA), recorded from an MC (left) and a fast-spiking PV interneuron (right). (G) Corresponding single-cell RT-PCR analyses showing that ErbB4 mRNA is detected only in PV interneurons (PVN) from PV-*ErbB4*^{+/+} OB. DI1000 was used as the size reference (M, 300, 200 and 100 base-pair fragments are indicated). (H) Specific deletion of ErbB4 in EPL PV interneurons of the OB. OB sections from PV-*ErbB4*^{+/+} and PV-*ErbB4*^{-/-} mice (P28) were stained with DAPI, anti-PV and ErbB4 antibody. Scale bars represent 50 and 20 μ m respectively. (I and J) Western blots showing that ErbB4 in PV-*ErbB4*^{-/-} OB was largely reduced from P7 onward, whereas ErbB4 in the PFC and hippocampus began to decrease only at P21. Relative levels were normalized to their respective P7 groups of control littermates ($n = 3$ mice per group, OB: $F_{(1, 8)} = 245.70$, $P < 0.0001$, $P = 0.0002$, 0.0006, and 0.0010; PFC: $F_{(1, 8)} = 61.20$, $P = 0.0532$, 0.1791, 0.0075, and 0.0021; Hi: $F_{(1, 8)} = 38.25$, $P = 0.2585$, 0.1005, 0.0139 and 0.0382, two-way ANOVA). Data are presented as means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.001$, n.s. = not significant. EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; Hi, hippocampus; MCL, mitral cell layer; ONL, olfactory nerve layer; PFC, prefrontal cortex.

To further ascertain ErbB4 mRNA expression in individual PV interneurons, we conducted patch-clamp electrophysiology followed by single-cell analysis of reverse transcription-polymerase chain reaction (RT-PCR). For this experiment, we used slices from mice in which ErbB4 was specifically knocked out in PV interneurons (PV-Cre mice crossed with *loxP*-flanked *ErbB4* mice; PV-*ErbB4*^{-/-}) and their control littermates (PV-*ErbB4*^{+/+}). In addition to their morphology, size, and location, MCs were characterized electrophysiologically as having low-frequency spike discharges (<100 Hz), whereas PV interneurons were identified by their high-frequency spike discharges (>100 Hz) (Figure 2F). The cytosolic contents from identified MCs and PV neurons were then subjected to RT-PCR, showing that ErbB4 mRNA was only present in PV interneurons from PV-*ErbB4*^{+/+} OB, but not in any of the MCs or PV interneurons from PV-*ErbB4*^{-/-} OB (Figure 2G).

Next, ErbB4 proteins were detected by immunofluorescence and western blot analysis. Within the EPL of PV-*ErbB4*^{+/+} mice, ErbB4 immunoreactivity was present in PV interneurons; this was not observed in the EPL of PV-*ErbB4*^{-/-} mice (Figure 2H). Western blots showed that the level of ErbB4 protein was largely reduced, but not abolished, in the OB, prefrontal cortex, and hippocampus of PV-*ErbB4*^{-/-} mice compared with their control littermates (Figures 2I and 2J). This suggests that ErbB4 is largely expressed in OB PV interneurons. Interestingly, ErbB4 deletion in the OB occurred at least two weeks prior to that in the prefrontal cortex and hippocampus (Figures 2I and 2J). Nissl staining showed that the overall lamina structures of the OB were not affected in PV-*ErbB4*^{-/-} mice (Figure supplement 1).

The above results demonstrate strong expression of ErbB4 in PV fast-spiking interneurons within the EPL and IPL layers of the OB.

ErbB4 in PV interneurons is necessary for olfactory behaviors

To determine the contribution of ErbB4 expressed in PV interneurons to olfactory behaviors, we conducted reinforced go/no-go tests of olfactory discrimination in PV-*Erbb4*^{+/+} and PV-*Erbb4*^{-/-} mice. Both groups learned to distinguish simple odor pairs with an accuracy of more than 90% within 600 trials (**Figure 3A**); for the difficult odor pairs, PV-*Erbb4*^{-/-} mice exhibited significantly lower accuracy than their control littermates (**Figure 3A**). During habituation/dishabituation tasks, both groups of mice habituated to the successive presentation of a familiar odor (isoamyl acetate, fourth trial) (**Figure 3B**); when a novel odor (limonene, fifth trial) was presented, PV-*Erbb4*^{-/-} mice did not increase their investigation time, unlike their control littermates (**Figure 3B**). Similarly, when the presentation of the odors was reversed, the PV-*Erbb4*^{-/-} group showed impaired dishabituation but the PV-*Erbb4*^{+/+} group did not (**Figure 3C**). The olfactory detection thresholds for isoamyl acetate and limonene were slightly higher in PV-*Erbb4*^{-/-} mice than in PV-*Erbb4*^{+/+} mice (**Figures 3D** and **3E**). We also tested mice with the buried food test, which depends on the animal's natural tendency to utilize olfactory cues for foraging, to confirm whether the mice could smell volatile odors. As shown in **Figure 3F**, both PV-*Erbb4*^{+/+} and PV-*Erbb4*^{-/-} mice found the buried pellets rapidly over three consecutive testing days. On trial day 4, they also easily located the pellet within a similar timeframe when the food pellet was made visible by placing it on the surface, suggesting that selective deletion of ErbB4 in PV interneurons does not alter the ability of mice to detect and locate odorous sources.

These findings reveal that ErbB4 signaling in PV interneurons serves as an essential regulator of olfactory discrimination and dishabituation behaviors.

ErbB4 deletion in PV interneurons decreases odor-evoked responses in M/TCs in the OB

To investigate whether ErbB4 in PV interneurons modulates the activity of M/TCs, we recorded extracellular single-unit spontaneous and odor-evoked activity from M/TCs in the OB via tetrodes implanted in awake, head-fixed PV-*Erbb4*^{-/-} and PV-*Erbb4*^{+/+} mice (**Figure 4A**). Single units were identified by principal-component analysis scan clustering of the spikes. Both excitatory (upper) and inhibitory (lower) responses were observed upon odor presentation from an odor delivery system (**Figures 4B** and **4C**). **Figure 4D** shows the heat maps of odor-evoked firing rates of 240 M/TCs in PV-*Erbb4*^{+/+} mice (left) and 232 M/TCs in PV-*Erbb4*^{-/-} mice (right). As shown in **Figure 4E**, spontaneous firing in M/TCs was similar in PV-*Erbb4*^{+/+} and PV-*Erbb4*^{-/-} mice. However, odor-evoked firing of M/TCs was reduced by ErbB4 deletion in PV interneurons. Both the absolute value of odor-evoked changes and the normalized signal-to-noise ratio (SNR) were lower in PV-*Erbb4*^{-/-} mice than PV-*Erbb4*^{+/+} mice. We next analyzed the differences in neurons with excitatory responses, inhibitory responses, and no responses to odor presentation in PV-*Erbb4*^{+/+} and PV-*Erbb4*^{-/-} mice. **Figure 4F** and **4G** show the changes in firing for excitatory responses during odor presentation. Compared with the PV-*Erbb4*^{+/+} group, M/TCs in PV-*Erbb4*^{-/-} mice had significantly higher spontaneous firing. The odor-evoked firing rate was not changed by ErbB4 deletion in PV interneurons. The odor-evoked change in mean firing rate (Δ MFR), SNR, and normalized SNR were all decreased in PV-*Erbb4*^{-/-} mice. **Figures 4H** and **4I** showed the changes in firing for inhibitory responses during odor presentation. Compared with the PV-*Erbb4*^{+/+} group, M/TCs in PV-*Erbb4*^{-/-} mice had significantly lower spontaneous firing rates. The odor-evoked firing rate of M/TCs was also decreased by ErbB4 deletion in PV interneurons.

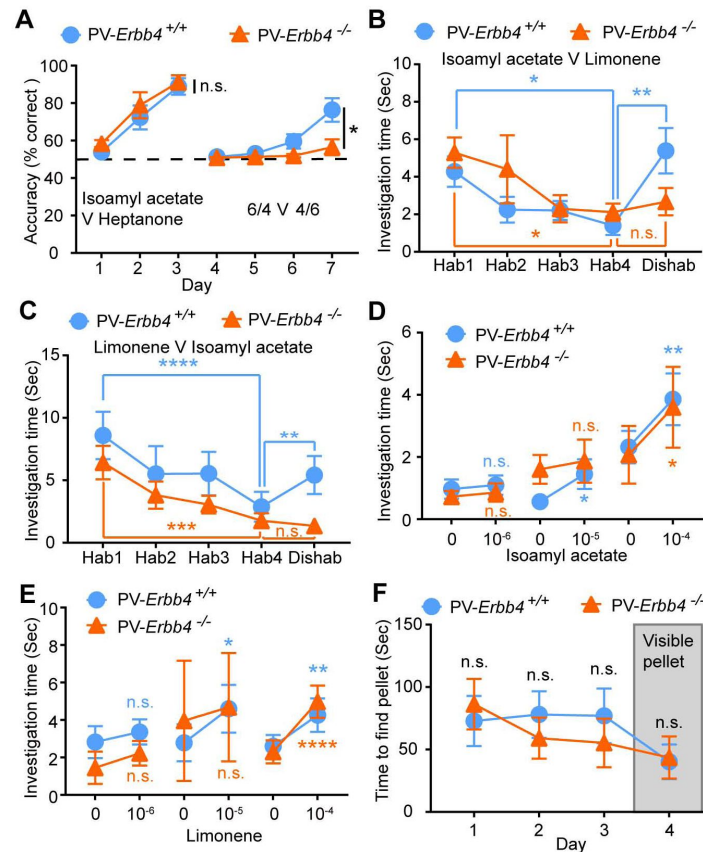


Figure 3.

ErbB4 in PV interneurons is critical for olfactory behaviors

(A) Odor discrimination under the reinforced go/no-go task in *PV-ErbB4*^{-/-} mice. The accuracy for simple odor pairs was indistinguishable ($n = 5$ and 6 mice, $F_{(1, 9)} = 0.70$, $P = 0.4260$, two-way ANOVA). However, the accuracy for difficult odor pairs was significantly lower in *PV-ErbB4*^{-/-} mice ($F_{(1, 9)} = 9.12$, $P = 0.0144$, two-way ANOVA). (B) Odor performance under the spontaneous habituation/dishabituation task. Both animal groups habituated to isoamyl acetate ($n = 12$ mice per group, $F_{(3, 66)} = 6.68$, $P = 0.0349$ and 0.0164 , two-way ANOVA). However, *PV-ErbB4*^{+/+} mice ($F_{(1, 22)} = 8.93$, $P = 0.0025$), but not *PV-ErbB4*^{-/-} mice ($P = 0.8451$, two-way ANOVA), dishabituated to limonene. (C) Odor performance under the reversed habituation/dishabituation task. Both animal groups habituated to limonene ($F_{(3, 54)} = 16.33$, $P < 0.0001$ and 0.0003 , two-way ANOVA). However, *PV-ErbB4*^{+/+} mice ($F_{(1, 18)} = 5.22$, $P = 0.0023$), but not *PV-ErbB4*^{-/-} mice ($P = 0.7890$, two-way ANOVA), dishabituated to isoamyl acetate. (D) *PV-ErbB4*^{+/+} mice were able to detect isoamyl acetate at a concentration of 10^{-5} ($n = 10$ mice, $F_{(1, 18)} = 0.60$, 3.74 and 16.69 , $P = 0.6069$, 0.0498 , and 0.0096 for 10^{-6} , 10^{-5} , and 10^{-4} , two-way ANOVA). *PV-ErbB4*^{-/-} mice only detected isoamyl acetate at a concentration of 10^{-4} ($n = 10$ mice, $P = 0.5764$, 0.5353 , and 0.0100 for 10^{-6} , 10^{-5} , and 10^{-4} , two-way ANOVA). (E) *PV-ErbB4*^{+/+} mice could detect limonene at a concentration of 10^{-5} ($n = 10$ mice, $F_{(1, 18)} = 1.75$, 7.95 and 32.51 , $P = 0.4540$, 0.0106 , and 0.0064 for 10^{-6} , 10^{-5} , and 10^{-4} , two-way ANOVA). *PV-ErbB4*^{-/-} mice only detected limonene at a concentration of 10^{-4} ($n = 10$ mice, $P = 0.2842$, 0.2709 , and < 0.0001 for 10^{-6} , 10^{-5} , and 10^{-4} , two-way ANOVA). (F) The latency for mice to locate the buried and visible food pellets did not differ between the groups ($n = 14$ and 11 mice, for the buried food pellet, $F_{(1, 23)} = 0.21$, $P = 0.9786$, 0.9279 , and 0.8873 ; for the visible food pellet, $P > 0.9999$, two-way ANOVA). Data are presented as means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, n.s. = not significant.

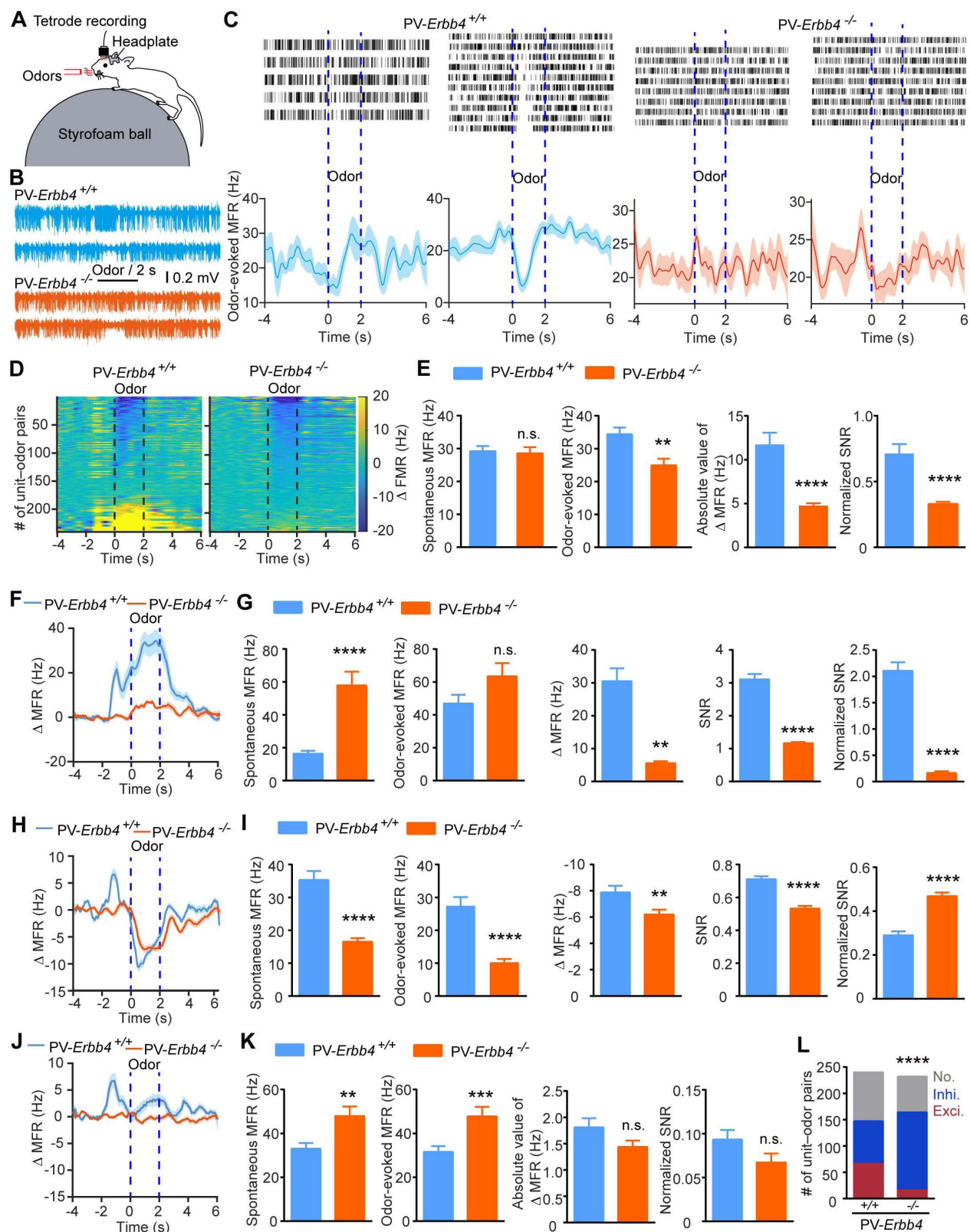


Figure 4.

Odor-evoked responses in M/TCs are decreased in PV-*ErbB4*^{-/-} mice

(A) Schematic of *in vivo* odor-evoked electrophysiological recordings in awake, head-fixed mice with ErbB4 knocked out in PV interneurons. (B) Representative raw traces of spike activity before (spontaneous), during (odor-evoked), and after 2-s odor stimulation in PV-*ErbB4*^{+/+} and PV-*ErbB4*^{-/-} mice. (C) Examples of raster plots (top) and peristimulus time histograms (PSTHs) of the firing rate (bottom) for odor-evoked excitatory (left) and inhibitory (right) responses in PV-*ErbB4*^{+/+} and PV-*ErbB4*^{-/-} mice. PSTHs were smoothed with a Gaussian filter with a standard deviation of 1500 ms. (D) Heat maps of the mean firing rate (MFR) across all unit-odor pairs in PV-*ErbB4*^{+/+} mice ($n = 240$ unit-odor pairs from 4 mice) and PV-*ErbB4*^{-/-} mice ($n = 232$ unit-odor pairs from 4 mice). (E) Quantitative analysis of the spontaneous firing rate ($t_{(470)} = 0.2665$, $P = 0.7899$), odor-evoked MFR ($t_{(470)} = 3.304$, $P = 0.0010$), absolute value of odor-evoked changes ($t_{(470)} = 5.046$, $P < 0.0001$), and normalized signal-to-noise ratio (SNR) ($t_{(470)} = 5.152$, $P < 0.0001$, unpaired t test) across all unit-odor pairs. (F) Odor-evoked excitatory changes in MFR (Δ MFR) for M/TCs recorded from PV-*ErbB4*^{+/+} mice ($n = 66$ unit-odor pairs from 4 mice) and PV-*ErbB4*^{-/-} mice ($n = 16$ unit-odor pairs from 4 mice). (G) Quantitative analysis of the spontaneous firing rate ($t_{(80)} = 7.666$, $P < 0.0001$), odor-evoked MFR ($t_{(80)} = 1.426$, $P = 0.1578$), odor-evoked Δ MFR ($t_{(80)} = 3.099$, $P = 0.0027$), SNR ($t_{(80)} = 5.909$, $P < 0.0001$), and normalized SNR ($t_{(80)} = 5.909$, $P < 0.0001$, unpaired t test) across excitatory unit-odor pairs. (H) Odor-evoked inhibitory Δ MFR for M/TCs recorded from PV-*ErbB4*^{+/+} mice ($n = 81$ unit-odor pairs from 4 mice) and PV-*ErbB4*^{-/-} mice ($n = 148$ unit-odor pairs from 4 mice). (I) Quantitative analysis of spontaneous firing rate ($t_{(227)} = 7.521$, $P < 0.0001$), odor-evoked MFR ($t_{(227)} = 7.228$, $P < 0.0001$), odor-evoked Δ MFR ($t_{(227)} = 2.724$, $P = 0.0070$), SNR ($t_{(227)} = 6.905$, $P < 0.0001$), and normalized SNR ($t_{(227)} = 6.905$, $P < 0.0001$, unpaired t test) across inhibitory unit-odor pairs. (J) Δ MFR for units with no response to odor in PV-*ErbB4*^{+/+} mice ($n = 93$ unit-odor pairs from 4 mice) and PV-*ErbB4*^{-/-} mice ($n = 68$ unit-odor pairs from 4 mice). (K) Quantitative analysis of spontaneous firing rate ($t_{(159)} = 3.129$, $P = 0.0021$), odor-evoked MFR ($t_{(159)} = 3.378$, $P = 0.0009$), absolute value of Δ MFR ($t_{(159)} = 1.627$, $P = 0.1057$), and normalized SNR ($t_{(159)} = 1.713$, $P = 0.0886$, unpaired t test) across “no response” unit-odor pairs. (L) Distribution of excitatory, inhibitory, and “no response” units in PV-*ErbB4*^{+/+} and PV-*ErbB4*^{-/-} mice ($\chi^2_{(2)} = 53.85$, $P < 0.0001$, Chi-Square tests). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, n.s. = not significant.

Interestingly, the odor-evoked Δ MFR also showed a significant decrement in PV-*ErbB4*^{-/-} mice. Although the SNR was decreased, the normalized SNR was increased in PV-*ErbB4*^{-/-} mice. **Figures 4J** and **4K** show the changes in firing for cells classes as having no response during odor presentation. Spontaneous firing in M/TCs was higher in PV-*ErbB4*^{-/-} mice than in PV-*ErbB4*^{+/+} mice. The odor-evoked firing rate of M/TCs was also increased by ErbB4 deletion in PV interneurons. The absolute value of odor-evoked changes (Δ MFR) and normalized SNR were not changed in PV-*ErbB4*^{-/-} mice compared with PV-*ErbB4*^{+/+} mice. These results show that odor-evoked excitatory and inhibitory responses were both compromised in PV-*ErbB4*^{-/-} mice. In other words, the absolute change in firing rate (Δ MFR) was reduced after ErbB4 ablation in PV interneurons regardless of the direction of the odor-evoked change. Furthermore, the distribution of types of odor-evoked responses was significantly altered in PV-*ErbB4*^{-/-} mice compared with PV-*ErbB4*^{+/+} mice (**Figure 4L**), suggesting compromised odor detection.

These results demonstrate that ErbB4 signaling in PV interneurons contributes to response intensity in M/TCs.

ErbB4 deletion in PV interneurons increases the power in the ongoing oscillations

Next, we investigated whether ErbB4 deletion in PV interneurons affects OB LFP oscillations. Raw LFP signals were divided into different frequency bands: theta (2 to 12 Hz), beta (15 to 35 Hz), low gamma (36 to 65 Hz), and high gamma (66 to 95 Hz) (**Figure 5A**). For all frequency bands of the

ongoing baseline LFP, the power was higher in PV-*ErbB4*^{-/-} mice than in PV-*ErbB4*^{+/+} mice (**Figures 5B–5I**). These results suggest that ErbB4 in PV interneurons controls the spontaneous ongoing network oscillations in the OB.

ErbB4 in PV interneurons regulates the OB output

To further evaluate the physiological role of PV interneuron ErbB4 in the regulation of the OB output, we recorded spontaneous and evoked excitatory firing rates from principle MCs (the output cells) in OB slices. Cell-attached recordings showed that the frequency of MC spontaneous action potentials (sAPs) was dramatically enhanced in PV-*ErbB4*^{-/-} mice compared with PV-*ErbB4*^{+/+} mice (**Figures 6A** and **6B**).

Furthermore, the frequency of evoked action potentials (eAPs) was also higher in PV-*ErbB4*^{-/-} mice (**Figures 6A** and **6B**). However, the ratio of eAP to sAP firing (i.e., the signal-to-noise ratio, SNR) was significantly lower in PV-*ErbB4*^{-/-} mice (**Figures 6A** and **6B**). More importantly, the SNR in response to stimuli at various intensities was lower in PV-*ErbB4*^{-/-} mice than in their control littermates (**Figures 6C** and **6D**), even though the overall current-evoked activity was elevated in PV-*ErbB4*^{-/-} mice compared with their control littermates (**Figures 6E** and **6F**).

These data indicate an involvement of PV interneuron ErbB4 in OB odor information processing.

ErbB4 in PV interneurons maintains inhibitory circuit plasticity in the OB

To investigate the neural mechanisms underlying the observed hyperexcitability in PV-*ErbB4*^{-/-} mouse MCs, we tested whether the effect of ErbB4 deletion in PV interneurons could be occluded by GABAergic blockage. Bicuculline (10 μM), a selective GABA_A receptor antagonist, elevated sAP firing in MCs from PV-*ErbB4*^{+/+} mice but did not further increase MC hyperexcitability in PV-*ErbB4*^{-/-} mice (**Figures 7A** and **7B**). Bicuculline also reduced the eAP:sAP ratio in PV-*ErbB4*^{+/+} MCs but not in PV-*ErbB4*^{-/-} MCs (**Figure 7A** and **7B**). These data indicate that the hyperexcitability in PV-*ErbB4*^{-/-} MCs depends on GABAergic downregulation. To further dissect the role of GABAergic transmission in MC hyperexcitability in PV-*ErbB4*^{-/-} mice, we recorded GABA_A receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) from MCs in whole-cell mode. As shown in **Figures 7C** and **7D**, the mIPSC frequency, but not the amplitude, were significantly reduced in PV-*ErbB4*^{-/-} mice, suggesting a presynaptic impairment of inhibitory transmission.

By contrast, neither the frequency nor the amplitude of glutamatergic receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) were altered in PV-*ErbB4*^{-/-} mice compared with their control littermates (**Figures 7E** and **7F**). These results suggest that ErbB4 in PV interneurons regulates olfactory information output by maintenance of GABAergic inputs to MCs in the OB.

We next examined the influence of specific PV-ErbB4 knock-out on recurrent inhibition of MCs. The prolonged hyperpolarization after AP firing that results from recurrent inhibition was recorded in the whole-cell patch-clamping configuration. Although the strength of recurrent inhibition is reported to depend on MC firing rates (Margrie et al., 2001), neither the amplitude nor the decay times of recurrent inhibitory postsynaptic potentials (IPSPs) showed significant alteration with the increment in AP frequency in PV-*ErbB4*^{-/-} mice (**Figures 8A** and **8B**). One possible explanation is that the loss of ErbB4 in PV interneurons severely impedes recurrent IPSPs. To test this hypothesis, we recorded recurrent IPSPs after a given frequency of APs in PV-*ErbB4*^{-/-} mice. As shown in **Figure 8C**, a current injection of 80 pA in PV-*ErbB4*^{-/-} mice elicited approximately the same firing rate as a current injection of 100 pA in PV-*ErbB4*^{+/+} mice.

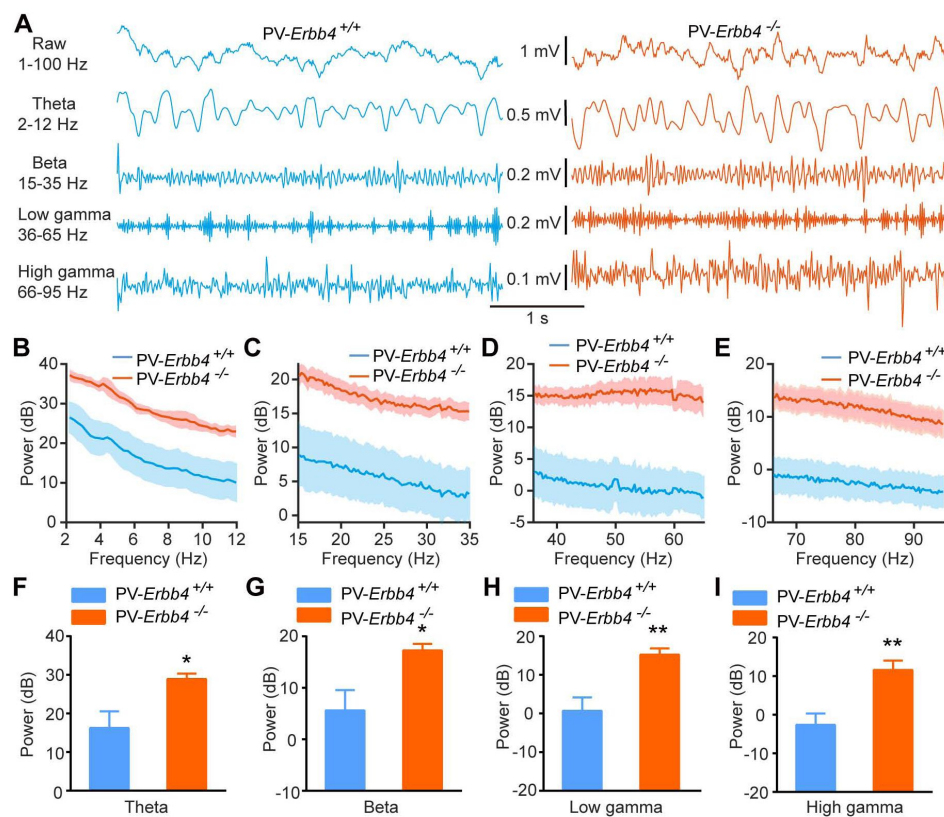


Figure 5.

The ongoing LFP in the OB is increased in *PV-ErbB4*^{-/-} mice

(A) Examples of ongoing LFP signals recorded in the OB from *PV-ErbB4*^{+/+} and *PV-ErbB4*^{-/-} mice. The five rows show the raw traces and the filtered theta, beta, low-gamma, and high-gamma signals. (B-E) Quantitative analysis of the averaged power spectra in the theta, beta, low-gamma, and high-gamma bands for the two groups. (F-I) Comparisons of power in the theta ($n = 5$ mice per group, $t_{(8)} = 2.80$, $P = 0.0233$, unpaired t test), beta ($t_{(8)} = 2.80$, $P = 0.0232$, unpaired t test), low-gamma ($t_{(8)} = 3.80$, $P = 0.0053$, unpaired t test), and high-gamma ($t_{(8)} = 3.73$, $P = 0.0058$, unpaired t test) oscillations in the OB in the two groups. * $P < 0.05$, ** $P < 0.01$.

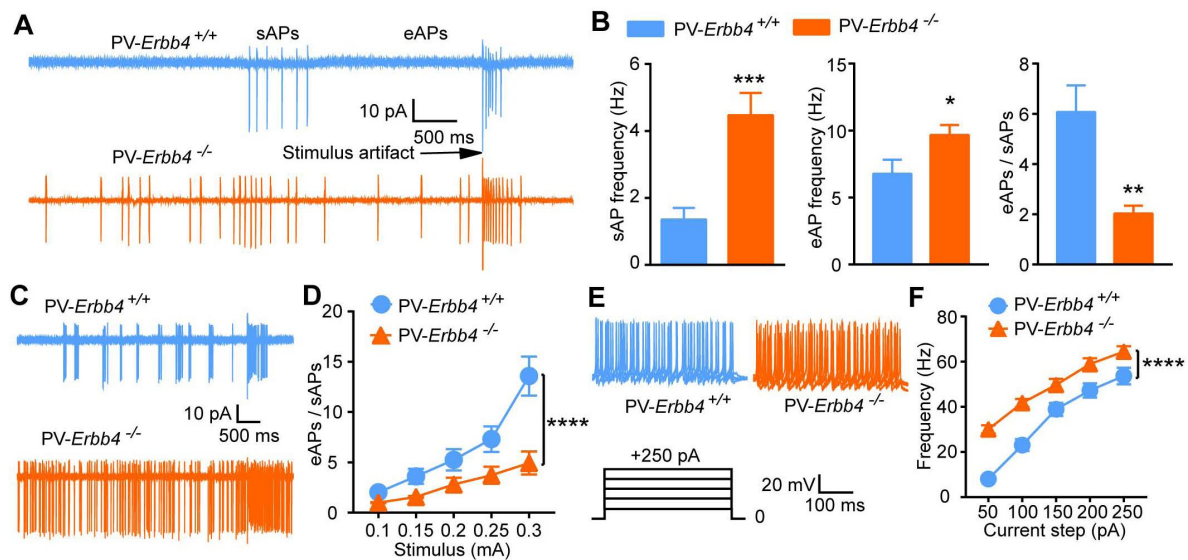


Figure 6.

PV-ErbB4^{-/-} mice have impaired information output from the OB

(A and B) Increased frequency of MC spontaneous action potentials (sAPs) and olfactory nerve-evoked APs (eAPs) in PV-ErbB4^{-/-} mice, but decreased ratio of eAPs to sAPs ($n = 9$ from 3 mice per group, sAPs: $t_{(16)} = 4.173$, $P = 0.0007$, eAPs: $t_{(16)} = 2.24$, $P = 0.0395$; ratio: $t_{(16)} = 3.68$, $P = 0.0020$, unpaired t test). (C and D) The eAP-to-sAP ratio was reduced in PV-ErbB4^{-/-} mice as the intensity of the stimulus increased ($n = 8$ from 3 mice per group, $F_{(1, 70)} = 32.39$, $P < 0.0001$, two-way ANOVA). (E and F) AP frequency elicited by injection of positive currents was higher in PV-ErbB4^{-/-} mice than PV-ErbB4^{+/+} mice ($n = 9$ from 4 and 3 mice per group, $F_{(1, 80)} = 78.9$, $P < 0.0001$, two-way ANOVA). Data are presented as means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

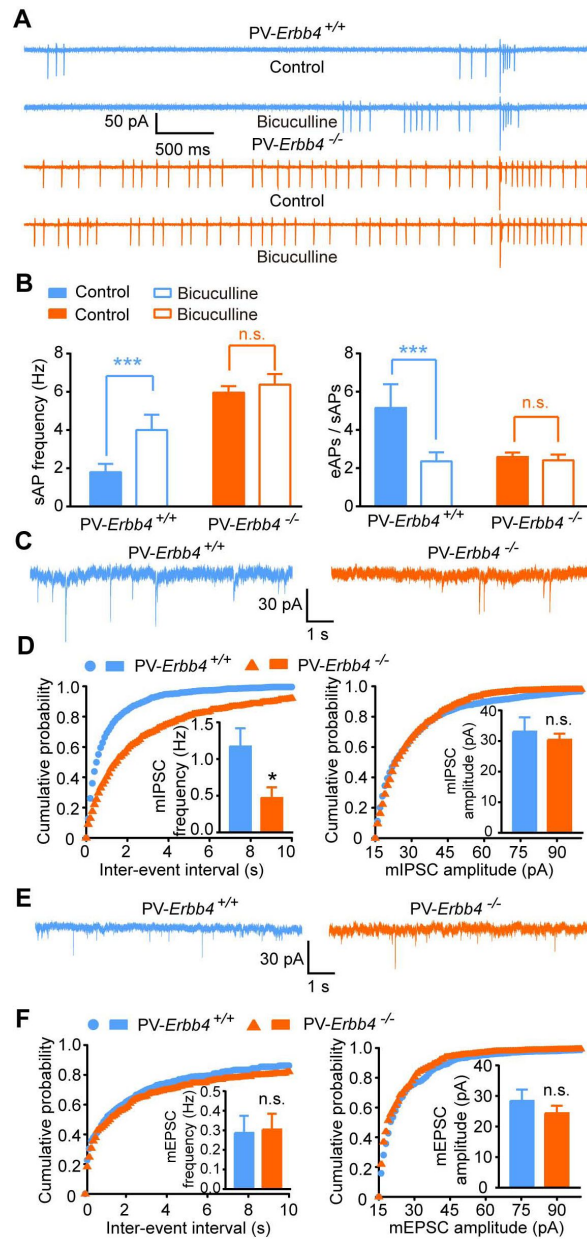


Figure 7.

Decreased GABAergic transmission mediates the OB output impairments in PV-ErbB4^{-/-} mice

(A and B) The increased sAP frequency and decreased ratio of eAPs to sAPs in MCs could not be further enhanced by bicuculline in PV-ErbB4^{-/-} mice ($n = 7$ from 3 PV-ErbB4^{+/+} mice, $n = 9$ from 3 PV-ErbB4^{-/-} mice; for sAP, $F_{(1, 14)} = 17.08$, $P = 0.0008$ and 0.5531 ; for ratio of eAPs to sAPs, $F_{(1, 14)} = 13.99$, $P = 0.0007$ and 0.9360 , two-way ANOVA). (C and D) The frequency but not the amplitude of MC mIPSCs was lower in PV-ErbB4^{-/-} mice ($n = 9$ from 7 PV-ErbB4^{+/+} and 6 PV-ErbB4^{-/-} mice; for frequency, $t_{(16)} = 2.45$, $P = 0.0263$; for amplitude, $t_{(16)} = 0.53$, $P = 0.6038$, unpaired t test). (E and F) Neither frequency nor amplitude of MC mEPSCs was different in PV-ErbB4^{-/-} mice versus PV-ErbB4^{+/+} mice ($n = 10$ from 5 PV-ErbB4^{+/+} mice, $n = 9$ from 7 PV-ErbB4^{-/-} mice; for frequency, $t_{(17)} = 0.14$, $P = 0.8899$; for amplitude, $t_{(17)} = 0.89$, $P = 0.3863$, unpaired t test). Data are presented as means $\pm$ s.e.m. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$, n.s. = not significant.

Remarkably, both the amplitude and the decay time constant of the subsequent recurrent IPSP were reduced in PV-*ErbB4*^{-/-} mice (**Figure 8D** [↗](#)). Thus, ErbB4 ablation in PV interneurons weakens recurrent inhibition evoked by the same MC firing intensity.

In addition, we attempted to isolate the broader lateral inhibition between MCs and detect the effect of PV interneuron ErbB4 ablation on it. The recorded MC corresponding to the target glomerulus was identified visually by Alexa 488 dye in the whole-cell patch pipette, as described previously (Hu et al., 2017 [↗](#)). A stimulating electrode was then placed into the glomerulus located 3–4 glomeruli (~ 400 μm) caudally from the target glomerulus. Before recording, we sectioned through the glomerular layer (GL) and GC layer (GCL) between the sites of conditioning stimulation and the target glomerulus to eliminate interglomerular and GC-mediated lateral inhibition (**Figure 8E** [↗](#)). As shown in **Figures 8F** [↗](#) and **8G** [↗](#), a burst of conditioning stimuli (40 pulses at 100 Hz) caused a reduction in the MC firing rate in PV-*ErbB4*^{+/-} mice, but not in PV-*ErbB4*^{-/-} mice. When we also cut through the EPL, this broader lateral inhibition was totally abolished in PV-*ErbB4*^{+/-} mice (**Figures 8H–8J** [↗](#)), indicating that the broader lateral inhibition was mediated by ErbB4-expressing PV interneurons.

The above data reveal an important contribution of PV interneuron ErbB4 to inhibitory circuit plasticity in the OB.

ErbB4 in OB PV interneurons is critical for olfactory behaviors

Finally, we evaluated the contribution of PV-ErbB4 in the OB to olfactory behaviors. ErbB4 expression in PV interneurons in the OB was reduced after *loxP*-flanked *ErbB4* mice were stereotactically injected in the OB with AAV-PV-Cre-GFP recombinants, but not when they were injected with the control AAV-PV-GFP group (**Figures 9A** [↗](#) and **9B** [↗](#)). ErbB4 reduction in OB PV interneurons impaired behavioral discrimination of complex odor mixtures in the reinforced go/no-go task (**Figure 9C** [↗](#)). Spontaneous dishabituation responses were also impaired in AAV-PV-Cre-GFP mice compared with the control AAV-PV-GFP group (**Figures 9D** [↗](#) and **9E** [↗](#)). Olfactory detection thresholds for isoamyl acetate and limonene showed a slight increase in AAV-PV-Cre-GFP mice compared with AAV-PV-GFP mice (**Figures 9F** [↗](#) and **9G** [↗](#)).

Thus, ErbB4 signaling in PV interneurons in the OB is required for olfactory discrimination and dishabituation in mice.

Discussion

ErbB4 expression is confined to PV interneurons in the cortex, hippocampus, basal ganglia, and amygdala (Bean et al., 2014 [↗](#); Fazzari et al., 2010 [↗](#)) and plays a role in mood (Chen et al., 2022 [↗](#); Chung et al., 2018 [↗](#); Y. Huang et al., 2021 [↗](#)) and cognitive behaviors (Cai and Shuman, 2022 [↗](#); Dominguez et al., 2019 [↗](#); Xu et al., 2022 [↗](#)). In the present study, we explore the distribution of ErbB4 in OB microcircuits and its functional properties in olfactory behaviors. We found ErbB4-positive neurons were distributed in all layers of the OB and were co-localized with PV interneurons in the IPL and EPL layers. Furthermore, ErbB4 in PV interneurons regulated OB oscillations and odor-evoked output by maintaining GABA release and lateral and recurrent inhibition of MCs. ErbB4 deficiency either in the OB as a whole or specifically in PV interneurons impairs olfactory behavioral performance. Our findings provide evidence that PV interneurons regulate the plasticity of MC inhibitory neurotransmission and broad ongoing fluctuations—and hence olfactory behaviors of discrimination and dishabituation—via ErbB4 signaling.

We found that in the EPL and IPL, ErbB4 mRNA and proteins are mainly distributed in PV interneurons. Our data do not rule out ErbB4 expression in other subtypes of interneurons, as suggested by other studies in which ErbB4-positive cells were located in all layers of the OB,

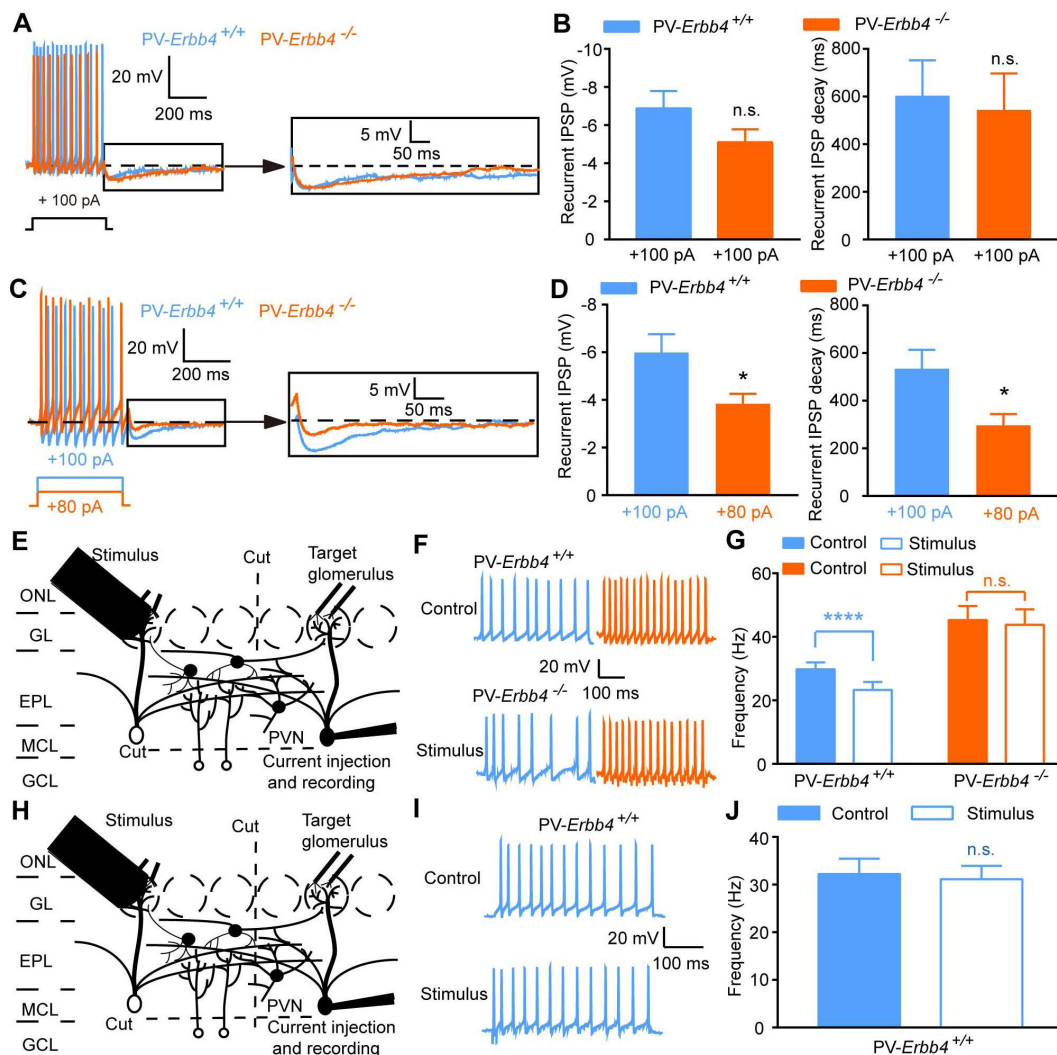


Figure 8.

Reduced recurrent inhibition and EPL lateral inhibition of MCs in PV-ErbB4^{-/-} mice

(A and B) Recurrent inhibition did not increase alongside MC hyperactivity in PV-ErbB4^{-/-} mice ($n = 9$ from 5 and 6 mice; for peak amplitude, $t_{(16)} = 1.57$, $P = 0.1351$; for decay time constant, $t_{(16)} = 0.28$, $P = 0.7821$, unpaired t test). (C and D) Recurrent IPSPs elicited by the same number of MC APs were smaller in PV-ErbB4^{-/-} mice. In PV-ErbB4^{-/-} mice, an 80 pA current induced ten APs, whereas in PV-ErbB4^{+/+} mice, a 100 pA current was needed to induce ten APs. Both the peak amplitude ($n = 10$ from 6 and 5 mice, $t_{(18)} = 2.46$, $P = 0.0242$, unpaired t test) and the decay time constant ($t_{(18)} = 2.56$, $P = 0.0198$, unpaired t test) of IPSPs evoked by the same number of APs were reduced in PV-ErbB4^{-/-} mice. (E) Schematic of the EPL lateral inhibition experimental configuration. Whole-cell recording of an MC (right side of the schematic) upon stimulus of an adjacent glomerulus (left side of the schematic). A cut was made through the GL and GCL between the sites of conditioning stimulation and target glomerulus to isolate EPL lateral inhibition. (F and G) EPL lateral inhibition was observed in the OB of PV-ErbB4^{+/+} mice but not PV-ErbB4^{-/-} mice ($n = 10$ from 7 PV-ErbB4^{+/+} mice, $F_{(1, 18)} = 26.48$, $P < 0.0001$; $n = 10$ from 6 PV-ErbB4^{-/-} mice, $P = 0.3426$, two-way ANOVA). (H-J) Cutting through the EPL as well abolished the EPL lateral inhibition in PV-ErbB4^{+/+} mice. (H) Schematic of the experimental configuration. (I and J) Representative and quantitative analysis of spike frequency in PV-ErbB4^{+/+} mice ($n = 9$ from 5 mice, $t_{(8)} = 0.80$, $P = 0.4468$, paired t test). EPL, external plexiform layer; GL, glomerular layer; GCL, granule cell layer; MCL, mitral cell layer; ONL, olfactory nerve layer; PVN, PV interneuron. Data are presented as means $\pm$ s.e.m. * $P < 0.05$, **** $P < 0.0001$, n.s. = not significant.

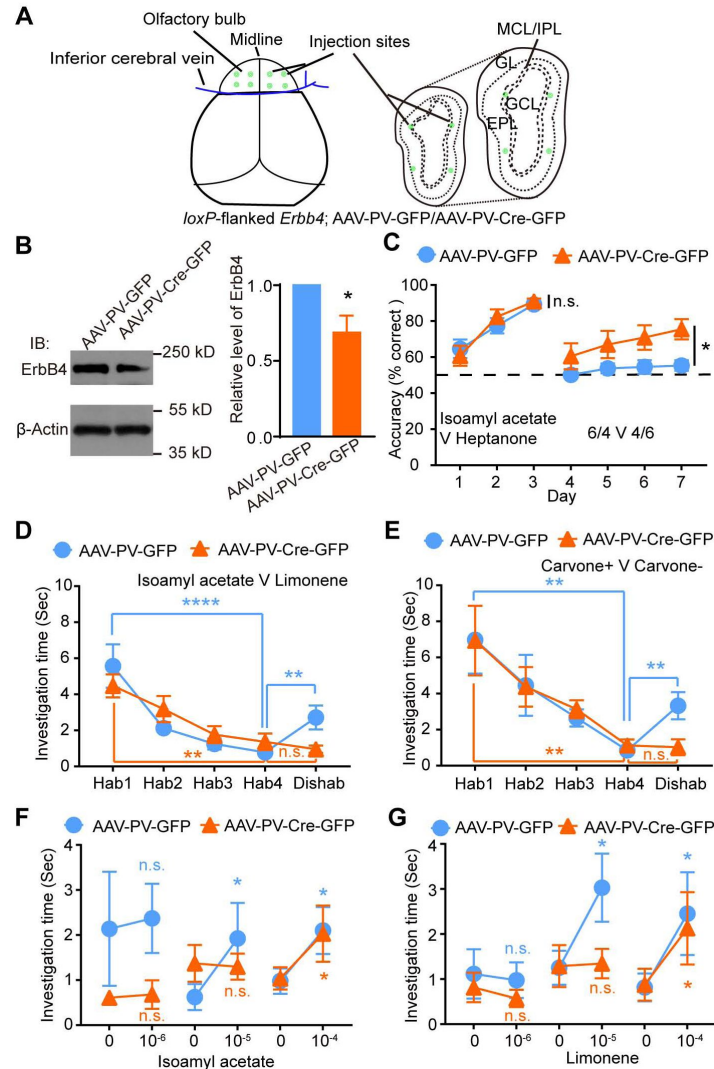


Figure 9.

ErbB4 in PV interneurons of the OB is critical for odor discrimination and sensitivity

(A) Schema indicating virus-injection sites. To specifically delete ErbB4 protein in the PV interneurons of the OB, AAV-PV-Cre-GFP was injected into the bilateral OB of neonatal *loxP*-flanked *ErbB4* mice. (B) Reduced ErbB4 expression in AAV-PV-Cre-GFP mouse OB ($n = 4$ mice per group, $t_{(3)} = 3.93$, $P = 0.0293$, paired t test). Relative levels were normalized to their respective control groups. (C) The accuracy in discriminating simple odor pairs was similar for the two groups ($n = 6$ and 7 mice, $F_{(1, 11)} = 0.06$, $P = 0.8147$). The accuracy in discriminating difficult odor pairs was significantly lower in AAV-PV-Cre-GFP mice ($F_{(1, 11)} = 5.74$, $P = 0.0355$, two-way ANOVA). (D) Both animal groups habituated to isoamyl acetate ($n = 10$ mice per group, $F_{(3, 54)} = 20.94$, $P < 0.0001$ and 0.0010 , two-way ANOVA). However, the AAV-PV-GFP ($F_{(1, 18)} = 3.95$, $P = 0.0045$), but not the AAV-PV-Cre-GFP mice ($P = 0.7107$, two-way ANOVA), dishabituated to limonene. (E) Both animal groups habituated to carvone+ ($n = 10$ mice per group, $F_{(3, 54)} = 9.92$, $P = 0.0019$ and 0.0035 , two-way ANOVA). However, AAV-PV-GFP ($F_{(1, 18)} = 6.72$, $P = 0.0025$), but not AAV-PV-Cre-GFP mice ($P = 0.9845$, two-way ANOVA), dishabituated to carvone-. (F) AAV-PV-GFP mice were able to detect isoamyl acetate at a concentration of 10^{-5} ($n = 10$ mice, $F_{(1, 18)} = 0.04$, 2.63 and 12.42, $P = 0.8309$, 0.0258, and 0.0164 for 10^{-6} , 10^{-5} , and 10^{-4} , two-way ANOVA), whereas AAV-PV-Cre-GFP mice only detected isoamyl acetate at a concentration of 10^{-4} ($n = 10$ mice, $P = 0.9484$, 0.8930, and 0.0312, two-way ANOVA). (G) AAV-PV-GFP mice detected limonene at 10^{-5} ($n = 10$ mice, $F_{(1, 18)} = 0.28$, 3.81 and 11.79, $P = 0.7894$, 0.0154, and 0.0131 for 10^{-6} , 10^{-5} , and 10^{-4} , two-way ANOVA) but AAV-PV-Cre-GFP mice only detected limonene at a higher concentration of 10^{-4} ($n = 10$ mice, $P = 0.6408$, 0.9349, and 0.0496 for 10^{-6} , 10^{-5} , and 10^{-4} , two-way ANOVA). Data are presented as means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, n.s. = not significant. EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; IPL, internal plexiform layer; MCL, mitral cell layer.

including in PV interneurons (Bean et al., 2014 [DOI](#); Bovetti et al., 2006 [DOI](#); Neddens et al., 2011 [DOI](#); Tan et al., 2022 [DOI](#)). ErbB4-deficiency in astrocytes and neural precursors alters the distribution and differentiation of interneurons in the mature OB and olfactory ability (Anton et al., 2004 [DOI](#); Moy et al., 2009 [DOI](#)). Our data show that ErbB4 deficiency in PV interneurons does not affect the overall lamina structure of the OB. This may be because ErbB4 regulation of interneuron migration relies on a critical time window (prior to E13.5) (Batista-Brito et al., 2023 [DOI](#); Mei and Nave, 2014 [DOI](#)). Our late-onset mutation of ErbB4 in the OB may not be early enough to cause the developmental phenotypes observed with early gene deletion.

In the OB, PV interneurons are excited predominantly by Ca^{2+} -permeable GluA2-lacking AMPA receptors and provide strong inhibitory input onto MCs (Kato et al., 2013 [DOI](#)). We recently found that ErbB4 is responsible for excitatory synaptic plasticity of AMPA receptors in the prefrontal cortex PV interneurons and olfactory associative reversal learning (Cai and Shuman, 2022 [DOI](#); Xu et al., 2022 [DOI](#)). Furthermore, transsynaptic binding of ErbB4–Neurexin1 β is critical for the activation of ErbB4 and extracellular signal-regulated kinase (ERK) 1/2 in PV interneurons, as well as brain-derived neurotrophic factor (BDNF) (Cai and Shuman, 2022 [DOI](#); Xu et al., 2022 [DOI](#)). In the present study, ErbB4 activity in the OB was increased after odor discrimination. ErbB4 deficiency in PV interneurons impaired odor discrimination and dishabituation. These findings reveal that ErbB4 in the OB is sufficient and necessary for proper olfactory function. Neurexin1 β is highly expressed in M/TCs and regulates inhibitory synaptic transmission in the OB (Wang et al., 2021 [DOI](#)). At the molecular level, we speculate that the association of ErbB4 with Neurexin1 β may facilitate the formation of GluA2-lacking AMPA receptor synapses onto OB PV interneurons, thus regulating GABA release. Thus, an NRXN1 β -ErbB4-ERK1/2-BDNF pathway may underlie olfactory processes.

The anatomical and physiological properties of the circuit between PV interneurons and M/TCs in the OB have been well characterized (Huang et al., 2013 [DOI](#); Kato et al., 2013 [DOI](#); Matsuno et al., 2017 [DOI](#); Miyamichi et al., 2013 [DOI](#)). PV interneurons exert linear and robust inhibition onto MCs, which is hypothesized to effectively change the odor response threshold of the MCs (Kato et al., 2013 [DOI](#); Miyamichi et al., 2013 [DOI](#)). We report here that knock-out of ErbB4 in PV interneurons decreased inhibition onto MCs and increased electrical-stimulation-elicited activity of MCs *in vitro*. However, the increment in spontaneous activity was larger than the increase in olfactory-nerve-evoked activity, indicating enhancement of background noise. Furthermore, ErbB4 deletion in PV interneurons decreased odor-evoked firing in M/TCs and changed the SNR *in vivo*. Thus, loss of ErbB4 in PV interneurons most likely elevates the odor detection threshold by interfering with the SNR in the OB. In the neocortex, PV interneurons have been implicated in the formation of gamma oscillations to reduce circuit noise and amplify circuit signals (Sohal et al., 2009 [DOI](#)). For example, selective deletion of ErbB4 from fast-spiking PV interneurons increases gamma oscillations in the hippocampus (Del Pino et al., 2013 [DOI](#)). Gamma oscillations in the OB are critical for odor discrimination (Lepousez and Lledo, 2013 [DOI](#)) and the generation of gamma rhythms in the OB requires inhibitory synaptic transmission in the EPL (Lagier et al., 2004 [DOI](#); Lepousez and Lledo, 2013 [DOI](#)). Although PV interneurons in the EPL show broad tuning to odors, ErbB4 deficiency in PV interneurons may disrupt gamma rhythms by reducing inhibitory synaptic transmission in the OB, leading to the impaired odor discrimination observed in the present study. Odor detection is a prerequisite of discrimination, so the impaired discrimination could also be due to the elevated detection threshold. Also, ErbB4 in PV interneurons broadly regulate olfactory-related functions through tuning all frequency bands of ongoing oscillations in the OB. Overall, our study reveals the functions of PV interneurons in the OB and the associated molecular mechanisms underlying the observed olfactory behaviors.

The spontaneous activity of MCs is driven mainly by inhibitory interneurons rather than excitatory olfactory sensory neurons (Duchamp-Viret and Duchamp, 1993 [DOI](#); Hu et al., 2021 [DOI](#); Hu et al., 2017 [DOI](#); Stakic et al., 2011 [DOI](#)). GCs are the most prominent GABAergic interneuron type and established play an established central role in the precise regulation of MC firing rates and synchrony through lateral and recurrent inhibitory mechanisms (Aghvami et al., 2022 [DOI](#)). PV

interneurons also form reciprocal synapses with MCs but provide strong, relatively broadly tuned inhibition and linearly control OB output (Huang et al., 2013 [DOI](#); Kato et al., 2013 [DOI](#); Matsuno et al., 2017 [DOI](#); Miyamichi et al., 2013 [DOI](#)). Here we observed decreases in mIPSC frequency, recurrent inhibition, and lateral inhibition in PV-*ErbB4*^{-/-} mice, suggesting that ErbB4 in PV interneurons plays an important role in regulating M/TC activity through recurrent and lateral inhibitory mechanisms. Another interesting finding is that the changes in spontaneous firing rate differed for odor-evoked excitatory and inhibitory responses *in vivo*, suggesting that ErbB4 in PV interneurons dynamically regulates the spontaneous activity of M/TCs in response to different odors. Our findings help unravel the circuit and molecular mechanisms underlying the modulatory effect of PV interneurons on M/TC odor responses in awake mice.

In the present study, we identified a spatially broad (300–400 μm in our experiments) lateral inhibitory circuit in the OB, requiring ErbB4 in PV interneurons. Thus, our findings raise the possibility that distinct classes of interneurons mediate interglomerular lateral inhibition within discrepant spatial dimensions. For example, short-axon cells usually have relatively long axonal arbors, spanning as much as 1000 μm (Aungst et al., 2003 [DOI](#)), that may effectively mediate the broadest (up to 600 μm) spatial dimension of lateral inhibition (Whitesell et al., 2013 [DOI](#)). By contrast, GCs connect only to their neighboring MCs (< 50–100 μm in distance); their lateral inhibition is narrowly tuned (Miyamichi et al., 2013 [DOI](#)). And here we found that PV interneurons mediate spatially moderate (300–400 μm) lateral inhibition via ErbB4. Future studies would benefit by defining the functions (e.g., contrast enhancement) of these distinct spatial dimensions of lateral inhibition in odor information processing. Both recurrent and lateral inhibition implement a “winner-takes-all” mechanism: Once the strongest input excites the principal cells, firings in the remaining inputs is inhibited (de Almeida et al., 2009 [DOI](#); Espinoza et al., 2018 [DOI](#); Karmakar and Sarkar, 2013 [DOI](#)). The regulation of recurrent and lateral inhibition by ErbB4 sheds light on how PV interneurons mediate this “winner-takes-all” inhibitory microcircuit.

In recent decades, impaired odor discrimination has been found in the earliest stages of neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease (Dan et al., 2021 [DOI](#)). Olfactory discrimination deficits develop prior to neuropsychological characteristics in patients with Alzheimer’s disease and have significant predictive valence for future cognitive decline in healthy aging individuals (Carnemolla et al., 2020 [DOI](#); Sohrabi et al., 2012 [DOI](#); Uchida et al., 2020 [DOI](#); Yoshitake et al., 2022 [DOI](#)). *ErbB4* has been independently identified as a susceptibility gene for Alzheimer’s disease and schizophrenia (Mei and Nave, 2014 [DOI](#); Swaminathan et al., 2012 [DOI](#)). Here we showed that selective loss of ErbB4 in OB PV interneurons impairs olfactory behaviors. Although ongoing fluctuation status predict behavioral alteration in health and disease, the contributions of its different frequency bands are less known (Iemi et al., 2022 [DOI](#)). This study links the broad ongoing fluctuations with OB output and hence controlling rapid recovery from olfactory adaptation and complex odor discrimination. Our findings will help us to better understanding the molecular and cellular mechanisms behind early olfactory dysfunction in neurodegenerative disease and schizophrenia and provide a potential strategy for treatment.

In summary, our study demonstrates a crucial role of ErbB4 in olfactory behaviors, involving ErbB4-expressing PV interneurons. Our results are most likely attributable to presynaptic impairments in the inhibitory microcircuits in the OB, which affect the processing of odor information and results in abnormal output signals from the M/TCs. The data presented may be promising not only for understanding physiological functions of ErbB4 in the OB but also for providing early diagnosis in Alzheimer’s disease and schizophrenia and potential therapeutic applications, particularly in patients with olfactory disorders.

Materials and methods

Reagents and animals

Rabbit polyclonal anti-ErbB4 antibody (sc-283, 1:2000, or 1:1000 for blotting, 1:100 for staining) was purchased from Santa Cruz, mouse monoclonal anti-PV antibody (P3088, 1:7000 for staining) was purchased from Sigma, anti- β -actin antibody (4970, 1:5000 for blotting) was purchased from Cell Signaling Technology, goat anti-rabbit IgG conjugated with Alexa Fluor 488 (A11089, 1:400 for staining) and goat anti-mouse IgG conjugated with Alexa Fluor 594 (A11037, 1:400 for staining) were purchased from Invitrogen. The Na^+ channel blocker tetrodotoxin (TTX, 1069) was purchased from Tocris and GABA_A receptor antagonist (+)-bicuculline (ALX-550-515) from Enzo. Other chemicals were sourced from Merck Sigma-Aldrich. ErbB4 conditional knockout mice (PV-*ErbB4*^{-/-}) were generated by crossing PV-Cre mice with *loxP*-flanked *ErbB4* mice (Garcia-Rivello et al., 2005 [↗](#); Wen et al., 2010 [↗](#); Xu et al., 2022 [↗](#)). Mice were housed under a 12-h light/dark cycle and had ad libitum access to water and food except for during the behavioral tests. All experiments were done with both sexes of mice. All experimental procedures were conducted in accordance with the guidelines described in the revised Regulations for the Administration of Affairs Concerning Experimental Animals (2011) in China and approved by the local Institutional Animal Care and Use Committee.

Western blot analysis

Tissues were homogenized in the homogenization buffer containing 50 mM Mops (pH 7.4), 320 mM sucrose, 100 mM KCl, 0.5 mM MgCl₂, 0.2 mM dithiothreitol, and phosphatase and protease inhibitors (20 mM sodium pyrophosphate, 20 mM β -glycerophosphate, 50 mM NaF, 1 mM each of EDTA and EGTA, sodium orthovanadate, p-nitrophenyl phosphate, PMSF and benzamidine, and 5 μ g/ml each of aprotinin, leupeptin, and pepstatin A). Homogenates (40 μ g of protein) were resolved by 7.5% SDS/PAGE and transferred to nitrocellulose membranes (Millipore), which were blocked with 3% bovine serum albumin (BSA) in Washing buffer (100 mM NaCl, 0.1% Tween-100, 10 mM Tris, pH 7.5). Membranes were incubated overnight with the primary antibody in 1% BSA then probed with the secondary antibody for 1h. Signals were visualized with Immobilon Western Chemiluminescent HRP Substrate. Immunoblotting quantification was performed in ImageJ (NIH).

Virus injections

Virus injections were performed as previously described (Kato et al., 2013 [↗](#)). Neonatal *loxP*-flanked ErbB4 mice (0–3 days old) were cryo-anesthetized in an ice-cold chamber and head-fixed in a stereotaxic device (RWD68513, RWD Life Science). Virus (AAV-Cre-GFP [55764361#] and AAV-GFP [55764363#] were from Obio Technology; AAV-PV-Cre-GFP [PT-0275] and AAV-Cre-GFP [PT-0154] were from BrainVTA) was delivered with titer 10¹² genome copies per milliliter. The injection coordinates were as follows (anteroposterior, mediolateral, and dorsoventral relative to the intersection of the midline and the inferior cerebral vein, in mm): (+0.2, $\pm$ 0.3, -0.9/0.7), (+0.2, $\pm$ 0.6, -0.9/0.7), (+0.5, $\pm$ 0.3, -0.7/0.5) and (+0.5, $\pm$ 0.6, -0.7/0.5). Virus (20 nl per site) were injected with a speed of 23 nl/s through a glass pipette. Behavioral tests were performed 10 weeks after virus injections.

Behavioral analysis

Behavioral analysis was carried out by investigators unaware of the genotypes of the experimental animals, to ensure that the study was blinded.

Go/no-go test

Mice were deprived of water then explored the set-up (Thinkerbiotech) and learned to lick a metal tube just below the odor port to obtain water, as previously described (Hu et al., 2021 [DOI](#)). Mice self-initiated trials by poking their nose into the sampling port, which interrupted an infrared light beam. All odors were diluted to 0.01% in mineral oil and further diluted 1:20 in air in the olfactometers. Odor pairs were presented for 2 s in a randomized order 0.5 s after trial initiation. Prior to training on the go/no-go task, mice were trained to perform a go/go task for two days. During the go/go task, if the mice licked within the odor presentation period when presented with either of the odor pairs, they received the water reward. Then the go/no-go task was performed to train mice to discriminate between a pair of odors to receive the water reward. Mice learned to lick the metal tube to receive water in response to the rewarded odor (hit) at the end of the trial (2.5-s total trial duration) and avoid licking the metal tube for the unrewarded odor (correct rejection). Licking when presented with the unrewarded odor (false alarm) led to no water reward and a timeout of up to 10 s. Performance “accuracy” was calculated as the percentage of correct trials (number of hits and correct rejections) over the total number of trials. The weight of mice under water restriction was strictly monitored (>85% of the initial body weight) during the entire test. Eight-channel semi-automated olfactometers, data acquisition, and analysis were all controlled through computer programs written in LabVIEW and Matlab (Thinkerbiotech).

Habituation/dishabituation test

Odors were presented by placing a cotton swab scented with an odor solution (150 μ l, 1:2000, freshly prepared with mineral oil before each experiment) above the floor of the animal’s home cage, as previously described (Hu et al., 2021 [DOI](#)). Mice were familiarized with a first odor in four successive trials (habituation) and then exposed to a novel odor on the fifth trial (dishabituation). The five successive 2-min trials were separated by 2-min intervals. Each trial was videotaped, and the investigation time of the swab was quantified offline. Sniffing behavior was defined as whenever the mouse had its nose within a 1-cm radius from the surface of the cotton swab. Lack of novel odor discrimination was considered to occur when mice spent as much time investigating the swab during the dishabituation trial as in the fourth habituation trial.

Odor detection threshold test

Odor detection thresholds were obtained according to procedures described previously (Nicolis di Robilant et al., 2019 [DOI](#)). Odors (isoamyl acetate and limonene) were freshly diluted with mineral oil to different concentrations (10^{-6} , 10^{-5} , and 10^{-4} in 150 μ l) and applied to a cotton swab, then placed above the floor of the animal’s cage along with mineral oil on the other side. Each trial was conducted for 2 min separated by 2-min intertrial intervals. Mice were deemed able to detect the odor when they spent more time sniffing the diluted odor than the mineral oil, and the minimum concentration detected by mice was taken as the threshold.

Buried food pellet test

All chow pellets were removed from the home cage 24 h before testing. Mice had free access to water. Before each experimental trial, 10–12-week-old mice were familiarized with the test cage for 10 min. On each trial, a single mouse was placed in the test cage (46 $\times$ 30 $\times$ 16 cm) to recover a 0.2-g food pellet that was buried 0.5-cm below the surface of the bedding material. The location of the food pellet was selected at random. The time between when the mouse was placed in the cage and when it grasped the food pellet with its forepaws or teeth was recorded and defined as the latency. Mice were allowed to consume the food pellet and were then returned to their cages. The food pellet was presented to the mouse for consumption if not be found within 300 s, in which case the latency was recorded as 300 s. A visible food pellet test was conducted as a control: the procedures were identical except the pellet was placed randomly on surface of the bedding.

RNAscope

To examine the expression of the ErbB4 gene, RNAscope was performed using an RNAscope Fluorescent Multiplex Kit (Advanced Cell Diagnostics, ACDBio) per manufacturer's recommendations. Briefly, the OB was cut into 20- μ m coronal sections. Sections were serially thaw-mounted onto 20 slides (Fisherbrand) through the entire OB and then air-dried for 1 h at room temperature prior to storage at -80°C . RNAscope probes were obtained from ACDBio for ErbB4 (Cat #318721) and PV (Cat #421931-C2). Sections were counterstained for the nuclear marker DAPI (Abcam, Cat #104139).

Immunohistochemistry

Mice (4 weeks old) were perfused transcardially with 4% paraformaldehyde. Dissected brains were postfixed overnight and dehydrated in 30% sucrose for 24 h at 4°C , then the OBs were cut into 40- μ m-thick sections horizontally on a vibratome (Leica). OB sections were processed for double immunofluorescence in 0.1 M PBS (pH 7.4) as follows: three washes in 0.1 M PBS, 10 min each; blocking in 0.1 M PBS containing 10% normal goat serum, 1% BSA, and 0.25% Triton X-100 overnight at 4°C ; primary antibody incubation for 40 h at 4°C in blocking solution; three washes; secondary antibody for 90 min at room temperature in blocking solution; three washes; rinsing with PBS and mounting with a mounting medium (Vectashield, Vector Laboratories).

Immunoreactivity was imaged by Alexa 488- and Alexa 594-conjugated goat anti-mouse IgG. Sections were analyzed with a confocal microscope (Zeiss L710) at 20 $\times$ and 60 $\times$ magnification. Cresyl violet staining was carried out as described previously. The OCT-embedded OBs were cut into 20- μ m sections. OB sections were stained with 0.1% cresyl violet for 35 min, dehydrated with ascending grades of alcohol, then cleared with xylene, mounted, and observed under an Olympus microscope.

Slice preparation and patch-clamp electrophysiology

Acute OB slices were prepared as described previously (Hu et al., 2021 [DOI](#); Hu et al., 2017 [DOI](#)). In brief, animals were heavily anesthetized with ketamine/xylazine (140/20 mg/kg, intraperitoneally) and decapitated, then the OB was quickly removed and immersed in ice-cold and oxygenated (95% O_2 /5% CO_2) ACSF containing (in mM: 124 NaCl, 3 KCl, 2 CaCl_2 , 1.3 MgCl_2 , 25 NaHCO_3 , 1.25 NaH_2PO_4 , and 10 glucose). Horizontal slices (300 μ m) were cut with a VT1000s vibratome (Leica), recovered at 37°C for 30 min and then maintained at 25°C .

OB slices were transferred to the recording chamber, which was warmed to 33°C and superfused with oxygenated ACSF (2 mL/min). Neurons were visualized with infrared optics using an upright microscope equipped with a 60 $\times$ water-immersion lens (BX51WI, Olympus) and an infrared-sensitive CCD camera. MCs and PV interneurons were identified by their fluorescence, morphology, size, location, and electrophysiological characterization.

For cell-attached mode, recording pipettes with resistances of 6–8 $\text{M}\Omega$ were pulled from borosilicate glass capillaries (P97, Sutter Instrument Co) and filled with ACSF. To record olfactory-nerve-evoked responses, monophasic square pulses (200 μ s) with an intensity of 0.2 mA or gradual increasing intensities of 0.1, 0.15, 0.2, 0.25, and 0.3 mA were delivered through a concentric electrode that was placed on the outermost layer of the OB, near the recorded MC. The frequency of firing was defined as number of action potentials/time window. A 5-s window prior to olfactory-nerve stimulation was used to assess the spontaneous firing rate, and the first 1-s window after stimulation of the olfactory nerve was used to assess the evoked response. The signal-to-noise ratio (SNR) was defined as the ratio of evoked responses to spontaneous firing in the 5 s prior to olfactory nerve stimulation.

To record whole-cell action potentials and mEPSCs, pipettes were filled with a solution containing 140 mM K-methylsulfate, 4 mM NaCl, 10 mM HEPES, 0.2 mM EGTA, 4 mM MgATP, 0.3 mM Na₃GTP, and 10 mM phosphocreatine (pH was adjusted to 7.4 with KOH). MCs were held at -65 mV in the presence of 20 μM bicuculline and 1 μM TTX during recording of mEPSC.

To isolate mIPSCs, MCs were held at -65 mV in the presence of 50 μM AP5, 20 μM NBQX and 1 μM TTX with a CsCl-base intracellular solution containing (mM): 135 CsCl, 10 HEPES, 0.2 EGTA, 2 Na₂ATP, 0.3 Na₃GTP, and 10 glucose. Miniature events in a 5-min recording period were analyzed in Mini Analysis software (Synaptosoft, ver. 6.07).

Lateral inhibition recordings were obtained according to procedures described previously (Arevian et al., 2008 [\[1\]](#); Hu et al., 2017 [\[2\]](#); Whitesell et al., 2013 [\[3\]](#)). Briefly, a concentric bipolar stimulating electrode was placed into the caudal glomerulus located 3–4 glomeruli (~400 μm) caudally from the one correlated with the recorded MC. The intensity of the stimulation was moderate (100 μA) to avoid stimulating the glomerulus of the recorded MC, which would invariably evoke excitatory postsynaptic potentials (EPSPs). Pulses of 200-μs duration were delivered at 100 Hz and synchronized with the recording by a Master-8 stimulator (AMPI). Alexa 488 dye (100 μM) was added to the intracellular solution to visualize cell morphology. Signals were acquired with a MultiClamp 700B amplifier (Molecular Devices), filtered at 2 kHz, and sampled at 10 kHz with a Digidata 1440A interface (Molecular Devices) and Clampex 10.2 software (Molecular Devices). Data were accepted when the series resistance stayed within 15% of the initial value. The spontaneous and miniature events in a 5-min recording period were analyzed in Mini Analysis software (Synaptosoft, ver. 6.07).

Single-cell RT-PCR

At the end of the recording session, gentle suction was applied to aspirate the cytoplasm into the pipette while maintaining a tight seal. After complete incorporation of the soma, the pipette was carefully removed from the neuron to perform an outside-out patch recording, then quickly removed from the bath. The harvested contents were expelled into a PCR tube. RT was performed in a final volume of 20 μl using a QuantiTect Reverse Transcription Kit (205311, Qiagen). Next, outer and nested primer sets were used for two consecutive rounds of PCR, as described previously (Vullhorst et al., 2009 [\[4\]](#)). For the first round of PCR, all targets were amplified simultaneously using outer primer pairs for PV and ErbB4 (first set PCR primer sequences: GCCTGAAGAAAAGAACCCG and AATCTTGCCGTCCCCATCCT for PV, CCAGCCCAGCGTTCTCAGTCAG and GTATTTTCGGTCAGTTCTTTAATCC for ErbB4, 20 pmol of each) in a final volume of 100 μl for 21 cycles. Each cycle comprised a 94°C denaturation for 30 s, 60°C annealing for 30 s, and 72°C extension for 30 s. For the second round of PCR, PV and ErbB4 were separately reamplified using specific nested primer sets for 35 cycles of PCR, as described above, with 2 μl of the first PCR product as template (second primer sequences: CGGATGAGGTGAAGAAGGTGT and TCCCCATCCTGTCTCCAGC for PV, CTGACCTGGAACAGCAGTACCGA and AGGCATAGCGATCTTCATATAGT for ErbB4). Products were assayed on 2% agarose gels stained with Gel Red, with dl1000 as a molecular weight marker.

Implantation of electrodes for in vivo electrophysiological recordings

After being deeply anesthetized, mice were secured in a stereotaxic frame (RWD Instruments, Shenzhen, China). The fur on the surface of the scalp from the midpoint between the ears to the midline of the orbits was removed. For spike and LFP recording, tetrodes (single-wire diameter, item no. PF000591, RO-800, 0.0005"/12.7 μm, coating 1/4 hard PAC, Sandvik) were implanted into the hole drilled above the OB (4.28 mm anterior from bregma, 1.0 mm lateral from the midline). The tetrodes were lowered into the brain after the dura mater was punctured. Each tetrode was connected to a 16-channel electrode interface board (EIB-16, Neuralynx). Signals were sent to a headstage and amplified by a 16-channel amplifier (Plexon DigiAmp) and monitored in real time

to ensure optimal placement within the ventral MC layer. A stainless-steel screw was inserted into the parietal bone (1 mm posterior to bregma and 1 mm from the midline) and connected to the ground as the reference electrode. Finally, a custom-designed aluminum head plate was sealed to the skull surface with dental cement to enable head fixation during recordings. Mice were returned to their home cages to recover for at least a week.

Electrophysiological recordings in awake mice

Recordings began at least one week after electrode implantation. Mice were head-fixed with two horizontal bars fixed to the headplate by two screws and were able to walk on an air-supported floating polystyrene foam ball (Thinkerbiotech, Nanjing, China). For spike recordings, the signals from the tetrodes were filtered at 300–5000 Hz and sampled at 40 kHz. Odor stimulus event markers were recorded alongside the spike recording. For local field potential (LFP) recordings, the signals were amplified by 2000×gain, bandpass filtered at 0.1–300 Hz, and sampled at 1 kHz (Plexon DigiAmp).

Odor application for electrophysiological recordings in awake mice

A standardized panel of eight odors (isoamyl acetate, 2-heptanone, phenyl acetate, benzaldehyde, dimethylbutyric acid, n-heptane acid, n-pentanol, and 2-pentanone, purchased from Sinopharm Chemical Reagent) were presented by an odor delivery system (Thinkerbiotech). All odors were dissolved at 1% (v/v) dilution in mineral oil. During odor stimulation, a stream of charcoal-filtered air flowed over the oil at a total flow rate of 1 L/min and the vapour-phase concentration was further diluted to 1/20 by the carrier airflow. The mice were first anesthetized with isoflurane for about 20 s and then rapidly transferred to the polystyrene foam ball. Before the start of *in vivo* electrophysiological recordings, mice were allowed 10 min to acclimate to the fixation and recover from the isoflurane. Odor presentation was controlled by the odor delivery system coupled to a solenoid valve driven by a digital-to-analog converter.

Each odor was presented for 2 s and the inter-trial interval was about 24 s.

Off-line spike sorting and statistics of the unit data

Spikes were identified and sorted from the raw data in Offline Sorter V4 software (Plexon). Spikes were detected and sorted when they had an amplitude larger than five times the standard deviation of the noise. Principal component analysis of the data was performed to separate different units. When <0.75% of the inter-spike intervals were <1 ms, a unit was classified as a single unit. Data from 4 s before until 6 s after the onset of the odor stimulation event were extracted, and the spike firing rate was averaged within 100-ms bins to generate a peristimulus time histogram (PSTH). The spontaneous firing rate and the odor-evoked firing rate were calculated by averaging the spikes recorded during the periods 4–2 s before odor stimulation and 2 s after the start of odor stimulation, respectively. To determine whether the odor evoked a significant response, we used a Wilcoxon signed-rank test to compare the spontaneous (baseline) firing rate with the odor-evoked firing rate across all trials for each unit–odor pair. The particular unit–odor pair was defined as nonresponsive if the *P* value was >0.05. Conversely, if the *P* value was <0.05 the unit–odor pair was defined as responsive. The unit–odor pairs were further categorized as showing an excitatory response (if the odor-evoked firing rate was greater than the spontaneous firing rate) or an inhibitory response (if the odor-evoked firing rate was lower than the spontaneous firing rate). Subtracting the baseline firing rate from the odor-evoked firing rate yielded the Δ mean firing rate (MFR). The normalized signal-to-noise ratio (SNR) was defined as follows: $\text{normalized SNR} = |(\text{odor-evoked firing rate}/\text{the baseline firing rate}) - 1|$.

LFP signal analysis

LFP signals were analyzed with a Matlab script and divided into different frequency bands: theta (2 to 12 Hz), beta (15 to 35 Hz), low gamma (36 to 65 Hz), and high gamma (66 to 95 Hz). The spectral power of each frequency resolution was calculated over 4-s window. The spectral power of all frequencies within the bandwidth was averaged.

Statistical analysis

Data are presented as the mean $\pm$ standard error of the mean (SEM). Unpaired or paired *t* tests were used to compare data from two groups. A Chi-square test was used for comparison of two constituent ratios. One or two-way ANOVAs were used to for comparisons between more than two groups (western blots, behavioral and electrophysiological studies). All tests were two-sided and considered to be statistically significant when $P < 0.05$. Statistical analyses were performed in GraphPad Prism.

Additional information

Competing interest

The authors declare no competing interests.

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Author Contributions

Bin Hu, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing—original draft; Chi Geng, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft; Feng Guo, Ying Liu, Data curation, Formal analysis, Investigation, Methodology; Ran Wang, Investigation, Methodology; You-Ting Chen, Methodology; Xiao-Yu Hou, Conceptualization, Resources, Supervision, Funding acquisition, Validation, Project administration, Writing—review and editing.

Ethics

All experimental procedures were conducted in accordance with the guidelines described in the revised Regulations for the Administration of Affairs Concerning Experimental Animals (2011) in China and approved by the local Institutional Animal Care and Use Committee.

Data availability

All data generated or analyzed during this study are included in the manuscript and supporting files.

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Reviewer #1 (Public review):

Summary:

The study by Hu et al. investigated the role of olfactory ErbB4 in regulating olfactory information processing. The authors demonstrated that ErbB4 deletion impairs odor discrimination, sensitivity, habituation, and dishabituation by using an impressive combination of techniques from morphological to electrophysiology (both slice and in vivo) and from viral injection to cell-type-specific mutation to behavioral analysis. The findings underscore the crucial role of ErbB4 in olfactory PV neurons in modulating mitral cell function and odor perception.

Strengths:

This study contains a pretty comprehensive set of experiments.

Major concerns:

(1) Line 151 page 7, "PV-ErbB4^{+/+} mice (generated by crossing PV-Cre mice (Wen et al., 2010) with loxP flanked *ErbB4* mice". Does this mean mice carrying PV-Cre and ErbB4 floxed allele? Or with the WT allele? This is confusing. Figures 2B and 2C, ErbB4 expression was evident in many cells that were not positive for PV. What are the identities of those cells? Are they important?

(2) In Figure 4, the authors performed tetrode recordings in awake head-fixed animals. Although individual neuron spikes could be obtained by spike-sorting, this is not a "single-unit" experiment due to the nature of this approach.

What is the odor used in Figure 4? How did the authors clean up the odor to limit the stimulation within 2 seconds? In what layer were the tetrodes placed? What is the putative cell type presented in Figure 4C? If Figure 4C is a representative neuron recorded, the odor-induced suppression of spike activity seems to be impaired in PV-ErbB4^{-/-} animals. However, Figure 4D shows that suppressed neurons were similar between the two types of animals. Such comparisons among individual mice are difficult for in vivo electrophysiological experiments because the recorded cell type and placement of electrodes would be different. The authors should apply ErbB4 inhibitors to the same animals and compare the effects before and after. This would ensure the recoding of the same population of neurons.

(3) At a glance in the heatmap in Figure 4D, excited neurons were reduced in PV-ErbB4^{-/-} mice, but not inhibited neurons. This was different from Figure 4L. The authors need to have a criteria or threshold to show how they categorized each population.

(4) Figure 4D, 4F and 4J seemed to be inconsistent. In Figure 4D before odor, there was no clear increase in the spontaneous activity in PV-ErbB4^{-/-} mice; in Figure 4F-4G and 4J-4K, clearly, there was a high spontaneous activity in PV-ErbB4^{-/-} mice.

(5) What are the neurons recorded in Figure 6E-6F? If they were MCs, loss of ErbB4 in PV neurons should not alter their intrinsic electrical properties. Rather GABAergic inputs could be altered. Indeed, the authors presented a reduction of GABAergic inputs from PV neurons to MCs.

(6) Figure 8E-8H, a better experiment would be specifically expressing ErbB4 or PV neurons. In Figure 8F and Figure 8I, was it the excitability after the current injection? Why not perform the spontaneous activity recording?

Reviewer #2 (Public review):

Summary:

Hu et al investigate the role of PV neurons and their expression of *Erb4* in olfactory performance through a series of behavioral tests, selective knockout experiments, and in vivo and in vitro electrophysiology. Knockout of *Erb4*, either in PV cells or the whole OB, resulted in impairment of discriminating complex odors. The authors present data that inhibition is impaired in MCs, which is likely underlying the abnormal odor-evoked responses of MCs in vivo and the impaired behavioral responses.

Strengths:

Overall, a key strength of this manuscript is the breadth of experiments to test the role of PV *Erb4* expression on circuit dynamics and behavior. The behavioral experiments were clear and sufficiently powered.

Weaknesses:

The major drawback of this manuscript is the lack of depth and rigor in experiments. Some experiments are preliminary, underpowered, and not quantified. As a result, many conclusions of the manuscript are weakly supported in its current form and would require significant revisions to address these shortcomings. Major weaknesses that should be addressed are as follows:

AAV-PV-Cre-GFP is not described or validated. Is this the S5E2 enhancer or something else? What is the specificity and efficacy of this approach in selectively knocking out *Erb4* in PV neurons? Reduced *Erb4* expression in the entire OB with PCR does not validate the selectivity of this approach. At a titer of 10^{12} , it is unlikely to be specific. Even a small amount of off-target Cre expression will knock out the gene in non-PV cells, so the authors should show whether the gene is knocked out at the single cell level from PV and non-PV cells. Without validation of this approach, this experiment is no different than the AAV-Cre-GFP experiments.

Figure 1D - three mice per group is insufficient. There is no control group error (the same as Figure 9). Why is it a paired t-test when there is a control group? The authors should be comparing go/go vs. go/no-go. The methods for normalization are unclear and are likely to hide the fact that $n=3$ is insufficient to capture a difference without extra measures to normalize the data.

The analysis of LFP is limited. During what period was this quantified? Are there any differences in task-related LFP changes? Also related to in vivo electrophysiology, the authors should show examples of isolated units, including their waveforms and how units were clustered and assigned to M/TCs.

The authors use 80pA and 100pA to elicit equivalent AP spiking in MCs to determine if recurrent inhibition differs, but do not actually show that AP spiking is the same across groups. This should be quantified.

There seems to be a prominent increase in the firing of MCs in PV-*Erb4*^{+/+} mice before odor presentation, but not in PV-*Erb4*^{-/-} mice. What is the significance of this?

There is a disconnect between the in vivo firing rates of MCs and ex vivo firing rates. In slice, the authors note that the spontaneous activity of MCs is elevated in the KO, but this is not observed in vivo, where conditions are physiological. Therefore, it is unclear whether the

concept of signal-to-noise changes in slice (higher spontaneous, lower evoked), indeed translate to something in vivo. It would be important to know what the PV cells are doing in vivo. Perhaps they have low firing rates prior to odor onset, which may explain the lack of observed difference in baseline FRs in MCs. The authors should have this data in their tetrode recordings, which would offer insight into when inhibition is recruited.

Since PV neurons are required for gamma oscillations, why is it that KOs have higher gamma oscillations? Is it indeed the case that PV cells have a hypofunctional phenotype in this model? Again, recording from PV cells in vivo would help make sense of this.

A clearer picture of how PV cell inhibition changes with *ErbB4* KO would be achieved with optogenetically evoked IPSPs, rather than changes in mini frequency.

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Reviewer #3 (Public review):

Summary:

The authors investigate the role of *ErbB4* in parvalbumin (PV) interneurons within the olfactory bulb (OB) and its regulation of odor discrimination behavior in mice. They demonstrate that odor discrimination increases *ErbB4* kinase activity and that the loss of *ErbB4* in the OB impairs the dishabituation of odor response and discrimination of complex odors. The study also characterizes the expression of *ErbB4* in the OB, showing it is enriched in PV neurons. Furthermore, the authors utilize a mouse model in which *ErbB4* is knocked out in PV neurons and perform a variety of behavioral, electrophysiological, and local field potential (LFP) recording experiments to characterize alterations in olfactory bulb activity. They then use a model in which *ErbB4* is specifically knocked out in PV neurons in the OB and show that this manipulation disrupts odor-related behaviors in mice.

Strengths:

The study's strengths lie in its use of a diverse range of techniques, including RNAscope, IHC, and Western blotting, to assess the presence of *ErbB4* in PV neurons within the OB. Additionally, the authors employ various behavioral tests to evaluate the effects of *ErbB4* manipulation in different mouse models, alongside comprehensive electrophysiological experiments and LFP recordings to examine the impact of these manipulations on OB physiology.

Weaknesses:

While the data presented in this paper are interesting, several major concerns reduce my enthusiasm for this study, as outlined below:

(1) In reviewing Figure 1C/D, there are several concerns regarding the clarity and interpretation of the data:

a) While the Western blot for *ErbB4* in other figures (Figure 1F, 2I) of the manuscript shows a clear single band, the blot presented in Figure 1C (for both p-*ErbB4* and total *ErbB4*) shows multiple bands, which is unexpected. This discrepancy raises concerns about the consistency of the results.

b) The data presented in Figure 1D uses only 3 mice per group, and the reported p-value of 0.0492, while technically significant, is very close to the threshold. This raises concerns about the robustness of the finding, especially given the small sample size. Additionally, the p-*ErbB4* band intensity in the Go/No-Go condition in Figure 1C does not appear to show a clear

increase over the Go/Go condition, which is not congruent with the bar graph in Figure 1D showing a 50% increase in p-ErbB4/ErbB4 levels.

c) It is a standard practice in many journals to include full, uncropped Western blot images as supplementary material. This transparency helps ensure that no bands are selectively shown or omitted and increases confidence in the presented data.

(2) In Figure 2, the authors used the anti-ErbB4 antibody sc-283 from Santa Cruz to assess the expression of ErbB4 in PV neurons and the absence of its expression in PV-ErbB4 knock-out mice. However, this particular antibody has been shown to produce non-specific bands in Western blotting and also generate non-specific labeling in IHC. This non-specificity has been demonstrated in Vullhorst et al. (2009, J Neurosci), raising significant concerns about the reliability of the data generated using this antibody.

(3) In reviewing the statistical analysis for the series of odor discrimination tests, there could be a potential issue with the clarity of the significance testing. Although the figure legend reports the F and p values from the two-way ANOVA, it is unclear whether these values represent the main effects or the results of a post hoc test. Additionally, it is not clear whether the asterisk in the figures reflects significance from a post hoc test or from the overall ANOVA. The methods section does not explicitly state whether a post hoc test was performed to assess differences between the knockout and control groups. Given that the tests were conducted across multiple days or conditions, a post hoc test that can adjust for multiple comparisons would be necessary to accurately identify where specific differences between the groups exist.

(4) Throughout the manuscript, the authors use different mouse models, including ErbB4 knockout specifically in the OB (AAV-Cre-GFP), ErbB4 knockout in PV interneurons throughout the brain (PV-ErbB4^{-/-}), and ErbB4 knockout in PV interneurons within the OB (AAV-PV-Cre-GFP). For Figures 4 and 5, the authors use the PV-ErbB4^{-/-} model to examine odor-evoked activity and neural oscillations within the OB. Since the knockout affects PV interneurons across the entire brain, it is difficult to disentangle whether the observed changes in the OB are due to local effects or broader network alterations elsewhere in the brain.

(5) While the electrophysiological experiments shown in Figures 6-8 provide valuable insights into the reduced inhibition to MCs in PV-ErbB4 knockout mice, it appears that the authors did not record from PV interneurons themselves. Since PV interneurons are central to the proposed mechanism, directly recording them would provide critical information on how the ErbB4 knockout affects their intrinsic properties, synaptic inputs, and firing behavior. Without these direct recordings, the conclusions about the specific role of PV neurons in regulating MC activity remain somewhat indirect. Prior studies have established that knockout of ErbB4 in PV interneurons reduces mEPSC frequency in PV neurons (Del Pino et al., 2013).

(6) In Figure 9, the authors knock out ErbB4 in PV neurons in the OB with AAV-PV-Cre-GFP and show with western blotting that ErbB4 expression is reduced in the mouse injected with AAV-PV-Cre-GFP. However, it is not clear whether ErbB4 was selectively knocked out in PV neurons without the quantification from IHC assays.

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