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# Layer-specific control of inhibition by NDNF interneurons

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**Neuronal processing of external sensory input is shaped by internally generated top–down information. In the neocortex, top–down projections primarily target layer 1, which contains NDNF (neuron-derived neurotrophic factor)-expressing interneurons and the dendrites of pyramidal cells. Here, we investigate the hypothesis that NDNF interneurons shape cortical computations in an unconventional, layer-specific way, by exerting presynaptic inhibition on synapses in layer 1 while leaving synapses in deeper layers unaffected. We first confirm experimentally that in the auditory cortex, synapses from somatostatin-expressing (SOM) onto NDNF neurons are indeed modulated by ambient Gamma-aminobutyric acid (GABA). Shifting to a computational model, we then show that this mechanism introduces a distinct mutual inhibition motif between NDNF interneurons and the synaptic outputs of SOM interneurons. This motif can control inhibition in a layer-specific way and introduces competition between NDNF and SOM interneurons for dendritic inhibition onto pyramidal cells on different timescales. NDNF interneurons can thereby control cortical information flow by redistributing dendritic inhibition from fast to slow timescales and by gating different sources of dendritic inhibition.**

interneurons | presynaptic inhibition | gating | information processing | predictive coding

## Significance

A prevailing theory in neuroscience is that different neuronal cell types serve different functions, which are supported by their unique properties. Inhibitory neurons in the outermost layer of the cortex—NDNF (neuron-derived neurotrophic factor) interneurons—are unique in their location, sparseness, and widespread release of inhibitory neurotransmitters. Yet, despite recent advances in their characterization, the role of NDNF interneurons in neural processing is largely unresolved. Using a combination of experiments and theoretical modeling, we show that NDNF interneurons control cortical circuits in an unconventional way. They release a neurotransmitter that selectively modulates the output of other interneurons by inhibiting their synaptic transmission rather than these interneurons themselves. This mechanism allows NDNF interneurons to regulate cortical information processing.

The neocortex receives a multitude of inputs that provide both sensory information and internally generated signals such as behavioral relevance (1, 2) or expectations (3, 4). These different information streams need to be filtered and integrated to form accurate sensory perceptions and produce appropriate behavioral responses. While sensory inputs are typically relayed from the thalamus ("bottom–up"), inputs from other cortical and subcortical areas carry memory- or context-related signals ("top–down;" 5–7) and mostly target the uppermost layer of the cortex—layer 1 (L1). Cortical L1 stands apart from other layers for its absence of excitatory cell bodies, instead containing the dendrites of pyramidal cells located in deeper layers (8–11) and inhibitory interneurons (INs; 12). With the identification of the genetic marker NDNF (neuron-derived neurotrophic factor) that selectively labels L1 INs (13), one class of L1 INs have become accessible for specific characterization and manipulation. However, how NDNF INs contribute to cortical computation remains an open question.

Inhibitory INs differ in their morphology, electrophysiology, peptide expression, and connectivity within the circuit. The most prevalent and well-studied IN types are parvalbumin-expressing (PV), somatostatin-expressing (SOM), and vasointestinal–peptide–expressing (VIP) INs. PV, SOM, and VIP INs form a characteristic connectivity pattern within the cortical microcircuit that is remarkably similar across sensory cortex and species (14–16). Their unique properties make them suitable for specialized functions (17–20). For example, SOM INs exert a powerful inhibition to the PC dendrite, controlling the propagation of input signals to the soma (21). PV INs, on the other hand, inhibit the perisomatic region of PCs and are thus implicated in providing stability by balancing excitatory inputs (22). VIP INs inhibit SOM INs, thus disinhibiting PCs. Since VIP INs are driven by top–down inputs, this disinhibition has been linked to behavioral state modulation and plasticity (19, 23–26).

Despite their strategic location among PC dendrites and top–down inputs, L1 INs have received less attention compared to other inhibitory INs, largely due to their sparse distribution and, until recently, the absence of a specific marker (13). NDNF INs in L1 receive top–down and neuromodulatory inputs in the mouse and human neocortex (7, 13, 27) but unlike VIP INs provide slow inhibition to PC dendrites (28–30). They can inhibit other INs but do not reciprocate the inhibition they receive from SOM INs (13, 30, Fig. 1*A*). Morphologically, NDNF INs are neurogliaform cells (13, 27, 29). A distinguishing feature of these cells is that they mediate volume transmission of Gamma-aminobutyric acid (GABA), which not only

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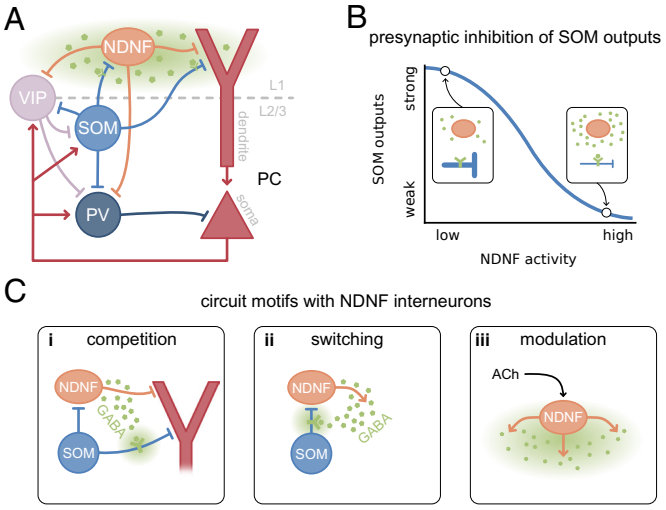


**Fig. 1.** Microcircuit model with NDNF INs mediating GABAergic volume transmission. (*A*) Schematic of the cortical microcircuit model with NDNF, SOM, PV, and VIP INs and two-compartment PCs. (*B*) Illustrative influence of NDNF activity on SOM outputs. *Insets* show low and high GABA release (i.e. NDNF activity) respectively. (*C*) Circuit motifs with NDNF INs and presynaptic inhibition.

induces slow postsynaptic effects but also targets presynaptic $GABA_B$ receptors (7, 31). Presynaptic inhibition via GABAergic volume transmission was recently identified as a mechanism to locally control inputs such as top–down projections to L1 (7, 32).

Motivated by these findings, we hypothesized that NDNF INs shape cortical processing by presynaptically modulating SOM IN outputs in L1 via GABAergic volume transmission (Fig. 1 *A* and *B*). Due to the location of NDNF IN output synapses (13), we propose that this modulation is limited to L1, implying that NDNF INs control the inhibition provided by SOM INs in a layer-specific way. To explore this hypothesis, we combine computational modeling and in vitro electrophysiology. First, we expand a model of the canonical cortical microcircuit by NDNF INs and GABAergic volume transmission. To validate our assumption that SOM outputs are presynaptically inhibited by ambient GABA, we performed whole-cell patch clamp recordings from genetically identified NDNF INs in mouse auditory cortex slices. Our experiments confirm that SOM synapses to NDNF INs in L1 are indeed modulated by presynaptic $GABA_B$ receptors. Using our microcircuit model, we show that this mechanism introduces functional motifs (Fig. 1*C*): i) Stimulating NDNF INs replaces SOM inhibition to PC dendrites with NDNF inhibition, creating a competition for the control of dendritic activity. ii) NDNF INs locally counteract the inhibition they receive from SOM INs, a motif that can amplify signals to NDNF INs and function as a bistable switch between NDNF INs and SOM outputs. Since NDNF and SOM INs mediate inhibition on different timescales this redistributes inhibition in time. iii) Neuromodulatory projections targeting NDNF INs can dynamically shape the signal processing in PCs. We show that modulating NDNF IN activity affects the relative balance of sensory (i.e. bottom–up) and top–down inputs to PCs, dynamically changing what PCs respond to in a predictive coding example.

## Results

Given the unique properties of NDNF INs and their strategic location among PC dendrites and top–down inputs in L1, we wondered how they contribute to cortical computation. To study how NDNF INs interact with the local circuit, we first introduced them into a classical cortical microcircuit model (14, 33). The rate-based model contains a population of excitatory PCs and the four main IN types PV, SOM, VIP, and NDNF (Fig. 1*A*). Each PC consists of two coupled compartments representing the soma and the dendrite, whereas INs consist of a single compartment (*Materials and Methods*). Connection strengths and probabilities between the neuron types are motivated by electrophysiological studies of cortical layer 1 to 3 (14, 15, see *Materials and Methods*) and established microcircuit models (26, 33–35). We incorporated GABAergic volume transmission from NDNF INs by modeling the GABA concentration in L1 (Fig. 1*A*, green cloud). We assumed that the GABA concentration increases with NDNF IN activity and mediates slow inhibition of the PC's dendrite. NDNF inhibition to PV and VIP INs is synaptic but weak, consistent with electrophysiological findings (30). To model presynaptic inhibition of SOM outputs in L1, we include a release factor that multiplicatively scales the strength of SOM synapses and decreases with the GABA concentration (Fig. 1*B* and *Materials and Methods*). We assume that GABAergic volume transmission is restricted to L1 (13, 31), affecting only the connections of SOM INs to the PC dendrite and NDNF INs, without impacting their connections to PV and VIP INs in lower layers, including layer 2 and 3.

**Experiments Confirm the Influence of NDNF INs on SOM Outputs.** Our main assumption is that SOM outputs in L1 are controlled by NDNF INs. In our model, SOM IN synapses to PC dendrites and to NDNF INs are presynaptically modulated by ambient GABA that is released by NDNF INs. A necessary prerequisite of the model is that the release probability of these synapses is modulated by presynaptic GABA receptors. The two main targets of SOM outputs in L1 are PC dendrites and NDNF INs. Synapses from SOM INs to PCs indeed express presynaptic $GABA_B$ receptors in the hippocampus, and SOM-induced inhibitory currents in PCs are markedly reduced by the application of the GABA agonist Baclofen (36). However, it is unknown whether synaptic transmission from SOM to NDNF INs in the auditory cortex is modulated by presynaptic $GABA_B$ receptors.

To directly address this assumption of the model, we performed electrophysiological recordings in the auditory cortex in vitro. To this end, we crossed mice expressing Cre recombinase under the SOM promoter with a strain expressing Flp recombinase under the NDNF promoter (13). Stereotactic injection of adeno-associated viral vectors (AAVs) into the auditory cortex was employed to achieve SOM IN-specific expression of the optogenetic activator ChR2, and NDNF IN-specific expression of tdTomato, a fluorescent marker protein (Fig. 2*A*). This allowed us to perform whole-cell patch clamp recordings from genetically identified NDNF INs in layer 1 of the auditory cortex in acute brain slices, while at the same time enabling optical stimulation of SOM INs with millisecond precision (Fig. 2*B*).

Optogenetic activation (0.5 ms pulses) of SOM INs caused robust inhibitory postsynaptic currents (IPSCs) in almost all NDNF INs tested (91%), consistent with the observed strong connectivity between these IN types (13). We minimized possible postsynaptic effects of $GABA_B$ receptor activation by using Cesium-based intracellular solution (7, *SI Appendix*, Fig. S1). Bath application of the selective $GABA_B$ receptor agonist Baclofen (10 μmol/l) strongly reduced the amplitudes of IPSCs (Fig. 2 *E* and *F*, *Right*, mean Ctrl. 344 pA, Baclofen 102

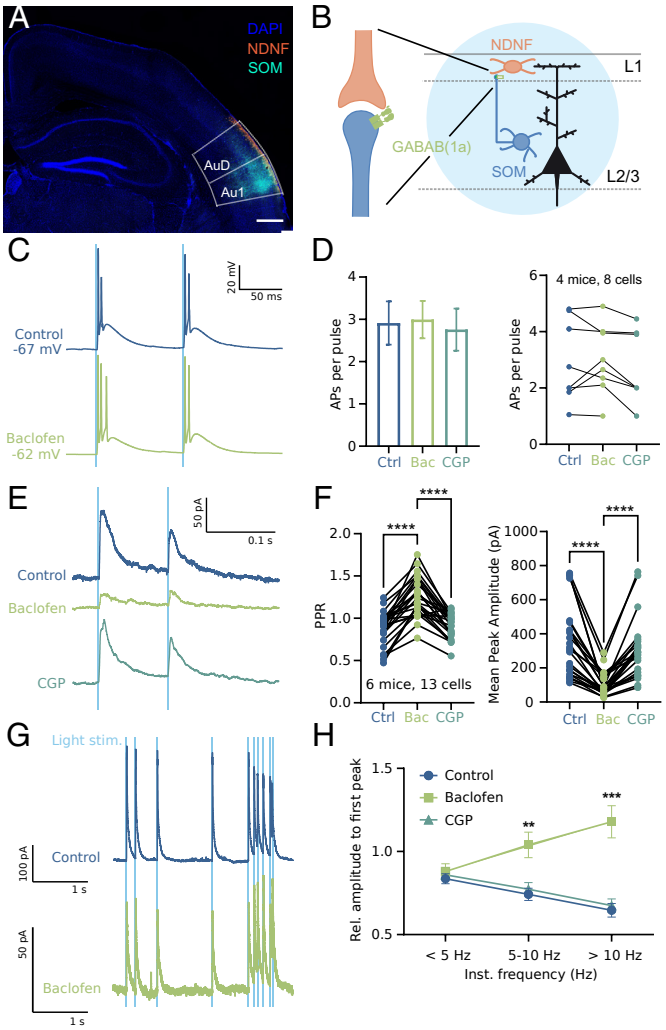


**Fig. 2.** Experiments confirm the influence of NDNF INs on SOM IN outputs. (*A*) Confocal microscope image showing expression of tdTomato in NDNF INs and ChR2-EYFP in SOM INs in the auditory cortex. (Scale bar, 500 µm.) (*B*) Hypothesis: NDNF INs in L1 modulate SOM IN inputs through presynaptic $GABA_B$ receptor-mediated inhibition as modeled by the application of Baclofen. The light blue circle represents optogenetic full-field stimulation to induce SOM INs activity. (*C*) Representative current clamp recording of a SOM IN during optogenetic stimulation in Control and Baclofen. (*D*) Neither Baclofen nor CGP55845 affects the number of evoked APs (first pulse), indicating that potential effects on synaptic transmission are not due to changed excitability of the SOM INs (*Left*: mean ± SEM, *Right*: individual recordings). (*E*) Representative IPSCs during paired pulse stimulation at 10 Hz (0.5 ms pulse). (*F*) Paired pulse ratio and mean peak amplitude for Control (ACSF), Baclofen, and CGP55845. (*G*) Representative IPSCs during naturalistic stimulation under each condition. (*H*) Normalized response amplitude, grouped according to instantaneous frequency of the stimulation. Data shown as averages of 10 sweeps for (*C*, *D*, *Right*, and *E*–*G*). Data shown as mean ± SEM for (*D*, *Left* and *H*). $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

pA, CGP55845 301.1 pA; Ctrl vs. Baclofen $P < 0.0001$, Baclofen vs. CGP55845 $P < 0.0001$, Ctrl vs. CGP55845 $P = 0.6093$; Friedman test with Dunn's multiple comparisons test). Paired-pulse stimulation at 10 and 20 Hz further revealed an increase in paired-pulse ratio (PPR) under $GABA_B$ receptor activation (Fig. 2 *E* and *F*, *Left*, mean Ctrl. 0.8814, Baclofen 1.259, CGP55845 0.9109; Ctrl. vs. Baclofen $P < 0.0001$, Baclofen vs. CGP55845 $P < 0.0001$, Ctrl. vs. CGP55845 $P = 0.5325$; RM one-way ANOVA with Tukey's multiple comparisons test), consistent with presynaptic effects. Since both stimulation frequencies showed comparable effects, these data were pooled (see *SI Appendix*, Fig. S1 for individual plots). Moreover, both the effects on IPSC amplitude and PPR were completely reversed by the selective $GABA_B$ receptor antagonist CGP55845 (3 µmol/l). Both the decrease in IPSC amplitude and the increase in PPR suggest the presence of presynaptic $GABA_B$ receptors on synaptic terminals of SOM INs that target NDNF INs. In particular, the PPR is the most widely used metric to quantify changes in presynaptic release probability (37, 38). In line with this, direct recordings from ChR2-expressing SOM INs revealed that optogenetic stimulation elicits similar numbers of action potentials (APs) in all three conditions (Fig. 2 *C* and *D* and *SI Appendix*, Fig. S2, mean Ctrl 2.913 APs, Baclofen 2.994 APs, CGP55845 2.757 APs, all ns, one-way ANOVA with Tukey's multiple comparisons test), ruling out pharmacological effects on SOM IN excitability as a source for the observed effects on IPSC amplitude and PPR. Together, these results demonstrate that $GABA_B$ receptors dynamically and powerfully control the release probability at synaptic contacts from SOM INs to NDNF INs.

Importantly, presynaptic control can not only dynamically reconfigure the strength of a connection but also its frequency transfer function (38). We therefore investigated how presynaptic $GABA_B$ receptors control transmission under more naturalistic conditions. To this end, we used a spike train that was previously recorded in vivo (7) to define a naturalistic stimulation protocol comprising ten different instantaneous frequencies (Fig. 2*G*, ranging from 1 Hz to 26.77 Hz). The naturalistic stimulation revealed that pharmacological activation of presynaptic $GABA_B$ receptors indeed shifts the maximum of the frequency transfer function between SOM INs and NDNF INs from low (<5 Hz) under control and $GABA_B$ receptor antagonism to high during Baclofen application (Fig. 2*H*, Ctrl. vs. Baclofen $P = 0.0011$ for 5 to 10 Hz, Baclofen vs. CGP55845 $P = 0.0005$; Ctrl. vs. Baclofen $P = 0.0001$ for >10 Hz, Baclofen vs. CGP55845 $P < 0.0001$; RM two-way ANOVA with Tukey's multiple comparisons test). Together, these data demonstrate that presynaptic $GABA_B$ receptors robustly and dynamically control both the strength and the frequency transfer function at SOM IN connections to NDNF INs.

Our experiments on SOM-to-NDNF synapses together with earlier work on SOM-to-PC synapses (36) suggest that SOM outputs in L1 are indeed under the control of NDNF INs through GABAergic volume transmission. In the following, we investigate how this added level of computational flexibility at SOM synapses affects circuit function.

**Competition Between SOM- and NDNF-Mediated Dendritic Inhibition.** Having established that NDNF IN can modulate SOM outputs in L1 via GABAergic volume transmission, we asked how this mechanism affects the cortical microcircuit at the functional level. The two primary targets of SOM outputs in L1 are PC dendrites and NDNF INs. First, we focus on the role of modulating SOM outputs to PC dendrites, which are also inhibited by NDNF INs (Fig. 3 *A*, *Top*).

Increasing the activity of NDNF INs reduces the inhibition from SOM INs to pyramidal cell dendrites, replacing it with NDNF-mediated dendritic inhibition (Fig. 3 *A*, *Middle* graph). In other words, NDNF INs and SOM outputs compete for dendritic inhibition. This competition arises from presynaptic inhibition acting on synapses from SOM INs to PC dendrites, as NDNF INs do not directly inhibit SOM INs. Instead, NDNF activity increases ambient GABA levels, and GABA binds to presynaptic receptors located at synapses from SOM

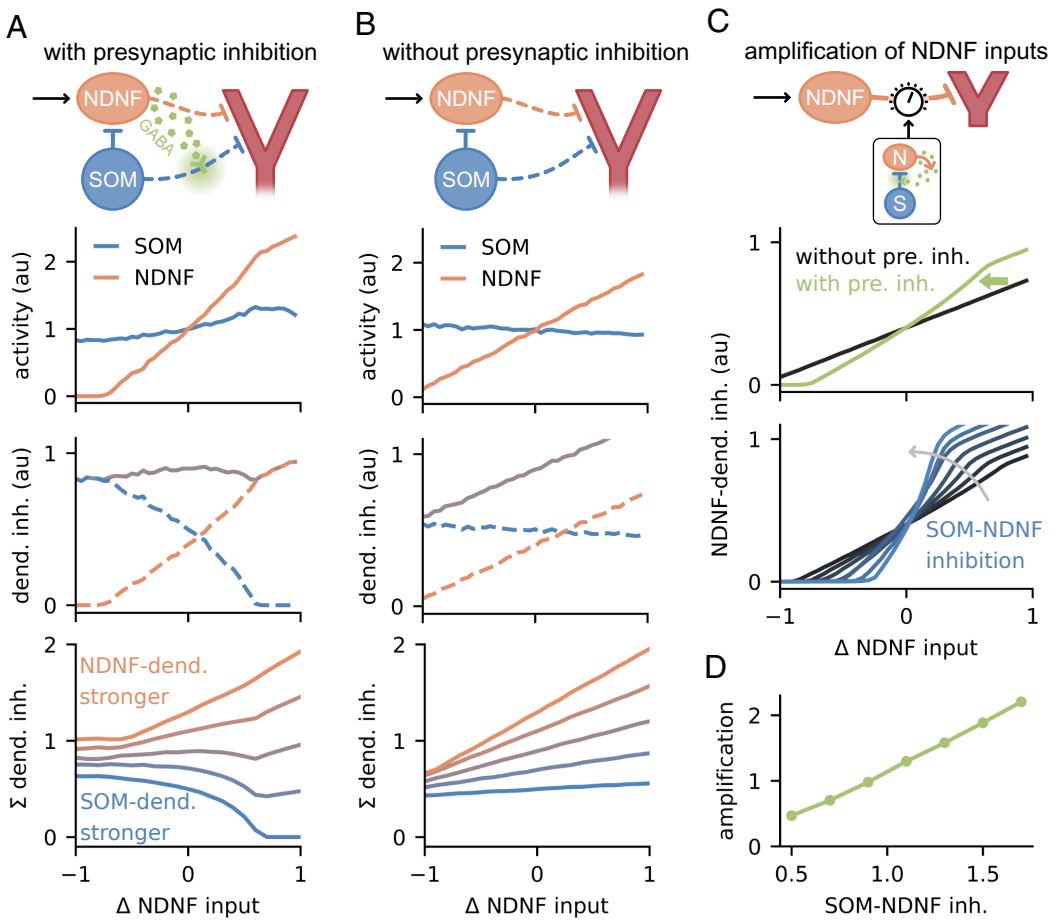


**Fig. 3.** Competition between SOM- and NDNF-mediated dendritic inhibition. (*A* and *B*) Model behavior with and without presynaptic inhibition for different levels of NDNF input relative to baseline. *Top*: Subcircuit consisting of SOM INs, NDNF INs, and PC dendrite. Second row: Activity of NDNF and SOM INs. Third row: Dendritic inhibition from SOMs (blue), NDNFs (orange), and both combined (gray). *Bottom*: Total dendritic inhibition for varying strengths of NDNF-to-dendrite inhibition. More orange colors indicate a stronger NDNF-to-dendrite synaptic weight and blue colors indicate a weaker weight. (*C*) *Top*: Illustration of the amplification of NDNF input by the NDNF-SOM motif. *Middle*: NDNF-dendrite inhibition with/without presynaptic inhibition as a function of NDNF input. *Bottom*: Same as above but for varying strengths of SOM-NDNF inhibition ($w_{NS}$ between 0.5 and 1.7). (*D*) Amplification of NDNF input as a function of SOM-NDNF inhibition. Amplification is quantified as the log ratio between the NDNF input–output slope with and without presynaptic inhibition (shown in *C*).

interneurons to PC dendrites. Consequently, SOM-mediated dendritic inhibition is reduced.

SOM INs and their outputs in lower layers are not directly affected (Fig. 3 *A*, *Top* graph), provided the effect of GABAergic volume transmission is restricted to cortical L1. Indirect effects on SOM INs can nevertheless occur due to recurrent interactions within the microcircuit, either through disinhibition via VIP INs (NDNF-VIP-SOM pathway) or changes in PC activity (NDNF-PC-SOM pathway, cf. Fig. 1*A* and *SI Appendix*, Fig. S7).

Whether the total dendritic inhibition is higher when NDNF or SOM inhibition dominates depends on their relative strength (Fig. 3 *A*, *Bottom*). When SOM-to-dendrite inhibition is stronger, stimulation of NDNF INs scales down SOM outputs and replaces them with weaker NDNF-to-dendrite inhibition, thereby decreasing the overall dendritic inhibition. Conversely, when NDNF-to-dendrite inhibition is stronger, stimulation of NDNF INs increases the overall dendritic inhibition.

Without presynaptic inhibition, stimulating NDNF INs does not modulate the SOM-to-dendrite inhibition, thus only increasing the overall dendritic inhibition (Fig. 3*B*). Monitoring dendritic activity in response to NDNF stimulation can therefore serve as an indicator of the strength of presynaptic inhibition on SOM outputs and the relative strength of SOM- compared to NDNF-mediated dendritic inhibition. Activation of NDNF INs decreases dendritic inhibition only if presynaptic inhibition and SOM-to-dendrite inhibition are sufficiently strong. Our model suggests that modulation of SOM-to-PC synapses by NDNF INs can gradually control the balance of SOM- and NDNF-mediated dendritic inhibition, introducing an effective competition between the two pathways that is restricted to L1.

**NDNF-Mediated Presynaptic Inhibition of SOM-to-NDNF Synapses Introduces a Mutual Inhibition Motif.** At first glance, NDNF INs seem to be at a disadvantage when competing for dendritic inhibition, because they are unidirectionally inhibited by SOM INs (13). However, our experiments revealed that SOM-to-NDNF synapses can also be modulated by NDNF-mediated presynaptic inhibition. This provides NDNF INs with an intriguing mechanism to counteract the inhibition they receive from SOM INs by effectively scaling it down (Fig. 3 *C*, *Top*). From a mathematical point of view, SOM-to-NDNF synapses and GABAergic volume transmission via NDNF INs form an unconventional and layer-specific "mutual inhibition" motif: SOM outputs inhibit NDNF INs and in return, NDNF INs presynaptically inhibit SOM outputs via GABAergic volume transmission (*Materials and Methods*).

Although this is not a classical mutual inhibition motif between inhibitory populations, we found that it displays

similar properties. Depending on the strength of the mutual inhibition, this motif can amplify small differences in the input and become bistable (33). Indeed, we find that presynaptic inhibition amplifies the NDNF-to-dendrite inhibition evoked by stimulating NDNF INs (Fig. 3 *C*, *Top* and *Middle*). The amplification increases with the SOM-to-NDNF inhibition (Fig. 3 *C*, *Bottom* and *D*), which—together with presynaptic inhibition—determines the strength of the mutual inhibition. In summary, the NDNF-mediated dendritic inhibition is amplified by an unconventional form of mutual inhibition between NDNF INs and SOM outputs.

**NDNF INs Can Act as a Switch for Dendritic Inhibition.** We wondered whether the NDNF-SOM motif could become bistable, similar to conventional mutual inhibition circuits (33). To test this, we provided transient input pulses to NDNF INs and observed their effect on the circuit (Fig. 4*A*). In a bistable circuit, transient inputs can change the network state more permanently than the input duration. We find that if the SOM-to-NDNF inhibition is sufficiently strong, positive input pulses lead to long-lasting increases and negative pulses to long-lasting decreases in NDNF activity (Fig. 4 *B*–*D*). Yet, for weak SOM-to-NDNF inhibition, transient inputs to NDNF INs do not have lasting effects (Fig. 4*E*), regardless of the pulse strength (Fig. 4*C*).

What is the underlying mechanism for this observation? Stimulation of NDNF INs (e.g., via positive pulses) causes an increase in the NDNF activity and thus a rise in the ambient GABA concentration. This weakens the SOM-to-NDNF synaptic transmission via presynaptic inhibition. With lower inhibition from SOM INs, NDNF INs can further increase their activity, resulting in even higher ambient GABA levels. Hence, transient inputs can permanently switch NDNF INs to an active or inactive state. Importantly, the switching does not affect SOM IN activity (Fig. 4 *D*, *Top*), unlike in a conventional mutual inhibition motif. Instead, the NDNF-SOM circuit exhibits winner-take-all behavior between NDNF IN activity and SOM outputs.

Because SOM outputs to the PC dendrites are also modulated by NDNF-mediated presynaptic inhibition, this "mutual inhibition" motif also switches the dominant source of dendritic inhibition between NDNF and SOM interneurons. (Fig. 4 *D*, *Bottom*).

From a functional perspective, why should it matter whether PC dendrites are inhibited by NDNF or SOM INs? NDNF and SOM INs display similar output connectivity patterns within the circuit, inhibiting PC dendrites, VIP, and PV INs. However, they receive different inputs and thus represent different signals: While SOM INs receive bottom–up sensory input (39), NDNF INs are targeted by top–down feedback inputs (7, 13).

To illustrate how switching between NDNF- and SOM-mediated dendritic inhibition can influence signal processing in PCs, we provided a time-varying signal (i.e., a sine wave) to SOM INs and repeated our switching experiment (Fig. 4*F*). We find that the switching has multiple simultaneous effects on PCs. In the PC dendrite, the sine signal is markedly weakened when NDNF INs are switched to a more active state because SOM synapses onto PC dendrites are inhibited presynaptically (Fig. 4 *F*, *Center*). When NDNF INs are switched back to a lower activity state, the dendritic oscillation is recovered. Notably, the somatic activity of the PCs shows a sinusoidal modulation for both low and high NDNF IN activity, but its sign is reversed when the NDNF state changes (Fig. 4 *F*, *Bottom Insets*). The reason is that SOM INs target PCs via two pathways. In addition

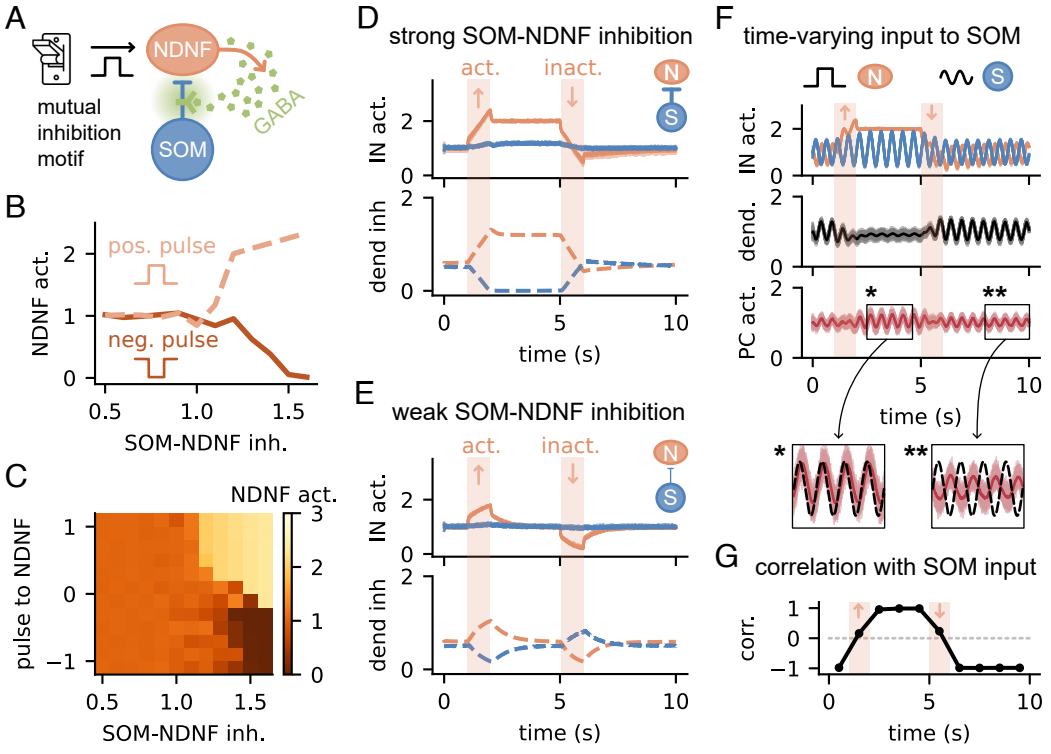


**Fig. 4.** NDNF INs can act as a switch for dendritic inhibition. (*A*) Schematic illustration of the switch between NDNF INs and SOM outputs. (*B*) Steady-state NDNF activity as a function of SOM-NDNF inhibition strength after a positive (dashed) or negative (solid) pulse to NDNFs. (*C*) Same as (*B*) but for different pulse strengths and signs. (*D*) Time course of SOM and NDNF activity (*Top*) and the dendritic inhibition they exert (*Bottom*) when NDNFs are switched on and off. SOM-NDNF inhibition is strong ($w_{NS} = 1.2$). (*E*) Same as (*D*) but for weak SOM-NDNF inhibition ($w_{NS} = 0.7$). (*F*) SOM and NDNF activity (*Top*), dendritic inhibition (*Center*), and PC activity (*Bottom*) in response to time-varying input to SOMs and pulses to NDNFs. (*G*) Correlation of PC activity with SOM input from (*F*).

to direct dendritic inhibition, SOM INs also inhibit PV INs in lower layers, which in turn inhibit the PCs perisomatically. This additional disinhibitory connection is not affected by presynaptic inhibition (cf. Fig. 1*A*). NDNF INs can therefore alter the balance between the inhibitory and the disinhibitory pathway. When NDNF INs are active, the disinhibitory SOM-PV-PC pathway dominates over the inhibitory SOM-dendrite connection. Conversely, for low NDNF activity, the direct SOM-dendrite inhibition dominates. The resulting signal inversion can be quantified by computing the correlation between SOM input and PC response, which flips from negative to positive when NDNF INs are switched on (Fig. 4*G*).

Collectively, these results demonstrate that the SOM-NDNF IN motif can be pushed to form a bistable switch for dendritic inhibition that dynamically changes the signals represented in PCs in response to transient inputs. This mechanism is particularly compelling when NDNF and SOM INs transmit different information to PCs such as bottom–up or top–down signals.

**Redistribution of Dendritic Inhibition in Time.** In addition to receiving different input signals, NDNF and SOM interneurons generate inhibition with distinct temporal dynamics. While SOM INs provide direct synaptic inhibition mediated by $GABA_A$ receptors, NDNF INs tend to inhibit PC dendrites via GABAergic volume transmission that targets both $GABA_A$ and extrasynaptic $GABA_B$ receptors (13, 28, 29, 31). Because metabotropic $GABA_B$ receptors act on slower timescales compared to ionotropic $GABA_A$ receptors, the postsynaptic currents elicited by NDNF INs show slower dynamics. In our model, this difference is captured by the GABA concentration that slowly increases with NDNF IN activity and mediates the inhibition to the dendrite (Fig. 5 *A* and *B*). The slow NDNF IN-mediated inhibition takes time to build up, which—in combination with other inhibitory pathways in the circuit—results in a multiphased response in the PCs (Fig. 5*C*). The faster $GABA_A$-mediated NDNF-PV-PC pathway causes a brief initial increase in PC activity, followed either by a further increase or a decrease depending on the strength of NDNF-to-dendrite inhibition (*SI Appendix*, Figs. S5 and S6). The termination of the stimulus can evoke a subsequent phase of PC response, resulting from the interplay between slow NDNF-dendrite inhibition, presynaptic inhibition of SOM outputs, and fast NDNF-PV-PC disinhibition (*SI Appendix*, Fig. S7).

To systematically study the downstream effects of NDNF compared to SOM stimulation, we provided pulses of varying lengths to NDNF or SOM interneurons. We find that the effect of these pulses depends both on their length and the circuit configuration. First, we focus on the circuit responses in our model with presynaptic inhibition (Fig. 5 *D*, *Left*). Stimulation of SOM INs generally decreases the PC activity, with longer stimulation evoking larger decreases (Fig. 5 *D*, *Left*). The PC response to NDNF IN stimulation is more complex and varies with the strength of the NDNF-to-dendrite inhibition (weak or strong compared to other weights in the circuit; see *Materials and Methods*). For weak NDNF-to-dendrite inhibition, PC activity increases with longer NDNF IN stimulation (Fig. 5 *D*, *Top Left*). The underlying mechanism is that activation of NDNF INs primarily leads to the disinhibition of PCs through two distinct pathways. First, NDNF INs reduce SOM-to-dendrite

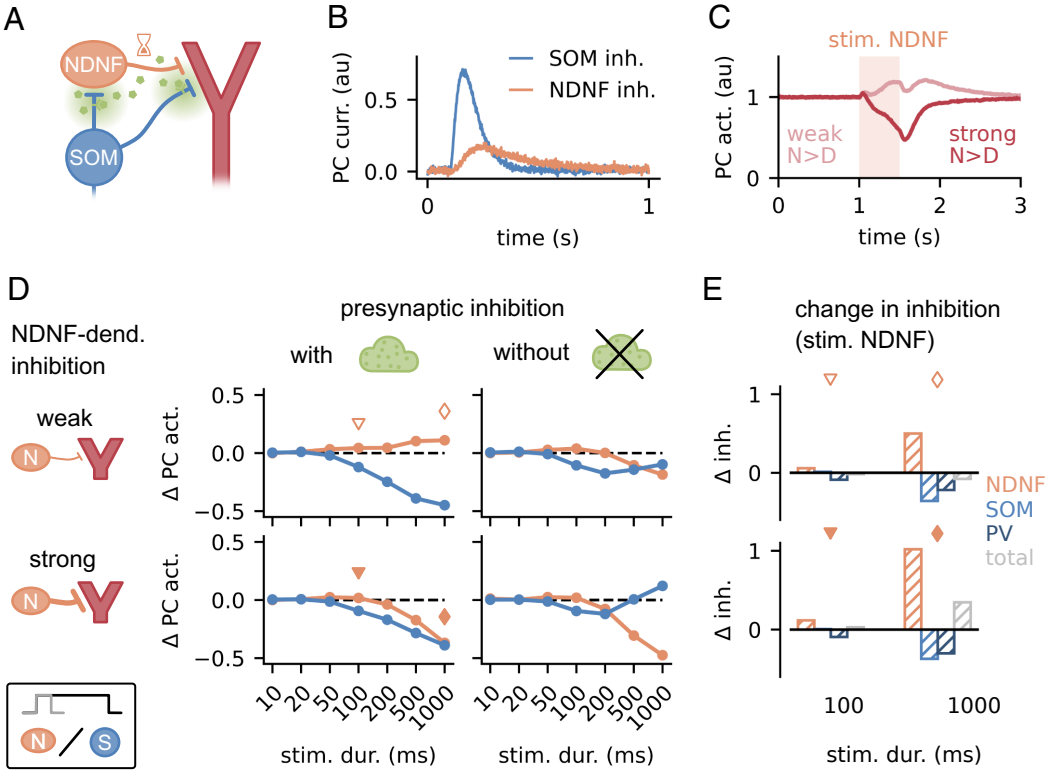


**Fig. 5.** Redistribution of dendritic inhibition in time. (*A*) Subcircuit consisting of NDNF INs, SOM INs, GABAergic volume transmission, and PC dendrites. (*B*) Inhibitory current in PCs in response to SOM and NDNF IN stimulation. (*C*) Mean PC activity in response to NDNF IN stimulation (orange box) for weak and strong NDNF-to-dendrite inhibition. (*D*) Response of PCs to constant NDNF and SOM IN stimulation of different durations. The same experiment is shown for weak/strong NDNF-dendrite inhibition and with/without presynaptic inhibition. (*E*) Contribution of inhibition provided to PCs by NDNFs, SOMs, PVs, and total inhibition when stimulating NDNF INs in (*D*), measured by the change compared to baseline. Triangles and diamonds denote corresponding data points from (*D*).

inhibition via presynaptic inhibition. Second, they disinhibit PC somata via the NDNF-PV-PC pathway (Fig. 5 *E*, *Top*, diamond; *SI Appendix*, Fig. S7). The importance of these pathways depends on the circuit parameters. When NDNF-to-dendrite inhibition is strong, this direct inhibitory contribution dominates the disinhibitory pathways (Fig. 5 *E*, *Bottom*), thus decreasing PC activity for longer NDNF IN stimulation. However, short stimuli can still cause a weak increase in the PC response (Fig. 5 *E*, *Bottom*, triangle), because the two pathways operate on different timescales. The direct synaptic NDNF-to-PV inhibition (i.e. PC disinhibition) is faster than the GABAergic volume transmission from NDNF INs that inhibits PC dendrites and SOM outputs in L1.

Our model predicts that stimulating NDNF INs with varying stimulus durations can be used to determine the relative strength of NDNF-to-dendrite inhibition. Long input stimuli should have opposite effects on PC activity for weak compared to strong NDNF-to-dendrite inhibition (cf. Fig. 5 *D*, *Left*, diamonds; *SI Appendix*, Figs. S5 and S6). Similarly, the PC responses to IN stimulation can be used to identify the presence or contribution of presynaptic inhibition in the circuit: Without presynaptic inhibition, NDNF INs predominantly inhibit PCs, because they do not counteract the SOM-mediated dendritic inhibition (Fig. 5 *D*, *Right*). Furthermore, NDNF INs cannot counteract the inhibition from SOM INs (cf. Fig. 4) such that stimulating SOM INs reduces NDNF IN activity and their inhibition of the dendrite. This implies that SOM IN stimulation counterintuitively increases the PC response when NDNF-to-dendrite inhibition is strong.

The model shows that stimulating NDNF and SOM INs can have diverse downstream effects depending on the relative balance of multiple inhibitory and disinhibitory pathways in the microcircuit. The stimulus duration plays a crucial role because NDNF INs mediate inhibition on longer timescales (28–30). The predictions from our model could be tested in future experiments by stimulating NDNF and SOM INs and using the PC responses as a unique signature to delineate relative pathway strengths and the contribution of presynaptic inhibition in the microcircuit.

**NDNF INs Enable Switching Between Prediction-Responsive and Mismatch Neurons.** We have shown that NDNF INs can control inhibitory pathways in L1 through GABAergic volume transmission and thereby modulate signal transmission to PCs. To illustrate how this layer-specific control could affect cortical processing at a computational level, we turned to a predictive coding example. The idea of predictive coding is that the brain aims to predict sensory information using internally generated predictions (3, 4, 40, 41). Deviations from predicted signals cause prediction errors that can be used to refine the inner model of the world and therefore improve future predictions. Prediction error (i.e. mismatch) responses have been widely observed (41, 42). For example, a subset of PCs in layer 2/3 of the rodent primary visual cortex specifically responds to mismatches between observed visual flow and the expected flow from motor commands (43, 44). Similar responses were found in the auditory cortex (45, 46). Recent theoretical work established how cortical microcircuits can give rise to mismatch responses, identifying the important role of multiple IN types to balance different sensory and prediction inputs (47, 48). However, these models did not consider NDNF INs.

Motivated by our findings, we speculated that NDNF INs can dynamically modulate the responses in prediction error circuits. Because NDNF INs are driven by feedback and neuromodulatory inputs (including cholinergic inputs; 7, 30), they could shape prediction error responses depending on context or behavioral state. To test this idea, we tuned our cortical microcircuit model such that PCs respond to prediction errors, extending the previous prediction error circuit to include NDNF INs (Fig. 6*A*). As in previous work, we assume that sensory input projects to PC somata, PV, and SOM INs. Conversely, top–down predictions cause inputs to PC dendrites and VIP INs.

We probed the responses of PCs to three different input combinations (Fig. 6 *A*, *Right*). In the "feedback" condition, top–down input accurately predicts sensory inputs. In the "mismatch" condition, there is a top–down prediction but no sensory input. Finally, in the "playback" condition, sensory input is present but not the associated top–down prediction. Mismatch neurons (44) should respond only when the prediction outweighs the sensory input (mismatch), but not when the sensory input is predicted or there is no prediction at all (feedback and playback; 47).

The PC responses in our predictive coding circuit with NDNF INs are consistent with those of mismatch neurons (Fig. 6 *B*, *Top*; 44, 47). PCs do not respond to conjunctive sensory and prediction input (feedback condition, Fig. 6 *B*, *Left*), because it is balanced out by the inhibitory pathways in the prediction error circuit: The total dendritic inhibition (SOM and NDNF-mediated) balances the prediction input at PC dendrites and the PV inhibition balances the sensory input at the PC somata (Fig. 6 *D*, *Left*). Sensory input alone (playback condition) does not evoke a PC response, because SOM INs maintain inhibition to PC dendrites and PV INs counteract the sensory input at the soma (Fig. 6 *B*, *Right*). Yet, in the mismatch condition, the prediction input activates VIP INs, which disinhibits PC dendrites and hence leads to a mismatch response (Fig. 6 *B*, *Center*).

What happens to the mismatch responses when the activity of NDNF INs is modulated, for instance, by cholinergic inputs to L1 (27)? Since NDNF INs control the inhibition of SOM INs to the dendrite, we expect them to influence the predictions arriving at the dendrites of PCs. We found that activation of NDNF INs causes PCs to respond in the feedback condition (Fig. 6 *C*, *Top*). At first glance, this response is not intuitive since SOM INs still increase their activity due to the sensory input (cf. Fig. 6 *B* and *C*). However, SOM- and NDNF-mediated dendritic inhibition is smaller compared to the control condition, because SOM outputs are inhibited by the NDNF INs (Fig. 6 *D*, *Right*). As a result, the total dendritic inhibition is outweighed by the prediction input, allowing PC dendrites to become active. PCs therefore respond to predictions regardless of sensory information instead of prediction errors. This behavior critically depends on the modulation of SOM IN outputs. Without presynaptic inhibition, activating NDNF INs does not qualitatively change the mismatch responses (*SI Appendix*, Fig. S8).

In summary, these findings provide evidence that NDNF INs can dynamically shape the prediction error circuit. In our model, whether PCs respond to mismatches or predictions can depend on the level of NDNF IN activity (Fig. 6 *B* and *C*). Gradually varying the input to NDNF INs enables a smooth transition between prediction and mismatch responses (Fig. 6*E*). NDNF INs receive feedback and cholinergic inputs that signal, for instance, arousal state (27, 49–51). We conjecture that the dynamic modulation of predictive coding responses can hence have behavioral relevance. During low arousal states (i.e. baseline NDNF activity), the circuit represents prediction mismatches, alerting the animal of deviations from its predicted sensory input.

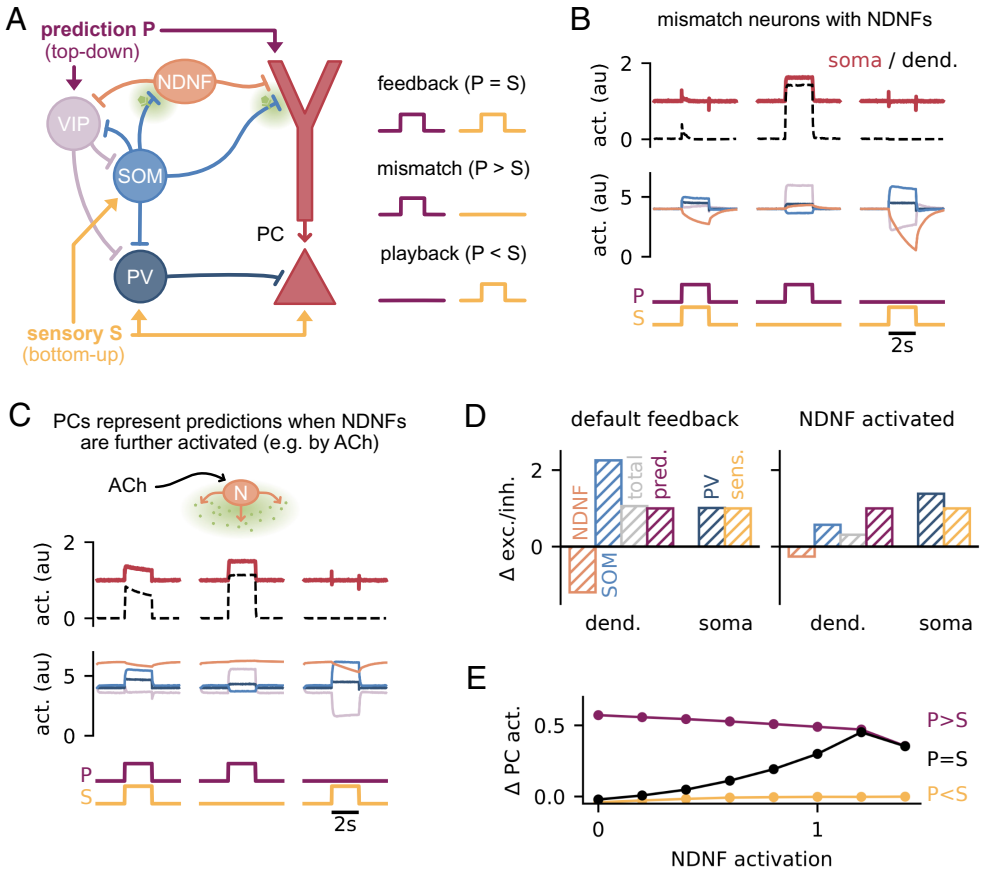


**Fig. 6.** NDNF INs enable switching between prediction-responsive and prediction error neurons. (*A*) Prediction error circuit with NDNF INs (*Left*) and different conditions for predictive and sensory input (*Right*). (*B*) Responses of PC dendrites and somata (*Top*) and four IN groups (*Center*) to different input configurations. Colors correspond to the schematic in (*A*). (*C*) Same as (*B*) but with NDNF INs activated, e.g. by cholinergic input. (*D*) Change in excitatory and inhibitory inputs to dendrite and soma during the feedback phase in the default condition (*Left*) and with NDNF INs activated (*Right*). Colors correspond to (*A*). (*E*) Change in PC activity as a function of NDNF activation for the three phases shown in (*A*).

However, during high arousal (i.e. elevated NDNF activity), the circuit assigns more relevance to internal signals such as predicted stimuli, providing a mechanism for recognizing expected stimuli more rapidly (52).

## Discussion

We showed that NDNF INs can modulate cortical information processing by controlling the outputs of SOM INs in L1. NDNF INs release ambient GABA, which targets presynaptic $GABA_B$ receptors and thereby inhibits synaptic transmission (7). We validated experimentally that this mechanism affects the synapses from SOM to NDNF INs by performing optogenetic stimulation, patch-clamp recordings, and pharmacological manipulations in slices of the mouse auditory cortex. Together with evidence of presynaptic inhibition of SOM-to-PC synapses (36), these findings support our hypothesis that SOM outputs in L1 are controlled by presynaptic inhibition. In a cortical microcircuit model that includes NDNF INs, we explored the effects of presynaptic inhibition of SOM outputs on the circuit dynamics and function. We found that NDNF INs can control inhibition in a layer-specific way, by targeting SOM outputs. NDNF INs also form a competitive circuit motif with SOM INs, in which NDNF INs counteract the unidirectional inhibition from SOM INs by presynaptically inhibiting the SOM inputs they receive. The motif can amplify small signals and form a bistable switch that enables shifting between NDNF- and SOM-mediated inhibition to the dendrite, thus dynamically changing the signal processing in PCs. Stimulation of NDNF INs can have diverse downstream effects depending on the temporal dynamics of the stimulation and relative connection strengths. Finally, we illustrated the functional relevance of NDNF INs in a predictive coding example. Modulating the activity of NDNF INs, e.g. by cholinergic inputs, shapes the representation of predictions and prediction errors in the circuit. Our results demonstrate that by controlling SOM inhibition in a layer-specific way, NDNF INs increase the functional flexibility of cortical circuits.

**Layer-Specific Control: Plausibility and Functional Implications.** In our model, NDNF INs have a layer-specific inhibitory effect. The main assumption is that presynaptic inhibition via GABAergic volume transmission affects SOM synapses within L1, an inhibition that selectively targets SOM outputs rather than SOM neurons themselves. This specificity is particularly intriguing for Martinotti cells that project an axon to L1 while their somata lie in deeper layers (L2/3 or L5 39, 53). If GABAergic volume transmission is confined to cortical L1, the activity and thus the outputs of these cells in lower layers remain unaffected. This includes their projections to PV INs, which are thought to be essential to maintain an excitation/inhibition balance at the soma of PCs (22). Similarly, the layer-specificity of the mechanism enables controlling inputs to PC dendrites separately from the soma. As the soma typically receives bottom–up sensory inputs while the dendrite receives top–down contextual and behavioral

input (5, 6, 12, 54), this layer-specificity provides a nuanced control over cortical information processing relevant for cognitive functions such as predictive coding (Fig. 6).

But how specific is GABAergic volume transmission? We conjectured that ambient GABA released by NDNF INs only acts within L1, the resident layer of NDNF INs. To mediate presynaptic inhibition, GABA released by NDNF INs must reach presynaptic $GABA_B$ receptors at, for instance, SOM output synapses. One challenge for this diffusive form of signaling is uptake mechanisms that actively remove neurotransmitters around synapses and release sites (55). Thus, the amount of released neurotransmitters must be sufficient to overcome reuptake and diffuse to nearby synapses. The diffusion of GABA is further limited by physical obstacles, L1 being densely packed with dendritic and axonal arbors (12). NDNF INs (morphologically neurogliaform cells) are particularly well suited to drive GABAergic volume transmission, because they have a high density of GABA release sites that are often not associated with a synapse (56). Therefore, a single action potential can cause large slow postsynaptic inhibitory currents (31, 53, 57). As the NDNF IN axons as well as their output synapses are largely constrained to L1 (13, 29), and GABA diffusion is limited physically as well as by reuptake mechanisms (31), it is unlikely for GABAergic volume transmission to exert a meaningful effect below L1.

While VIP interneurons are predominantly located in layer 2 to 3 (53), they can reach lower L1 (29). Hence, GABAergic volume transmission may affect the synapses of SOM to VIP INs at the border of L1 if they express presynaptic $GABA_B$ receptors. Another potential target of presynaptic inhibition in L1 is the synapses from NDNF INs to the dendrite (31). We found that the competition and bistability between SOM and NDNF INs is robust to GABAergic volume transmission targeting SOM-to-VIP or NDNF-to-dendrite synapses (*SI Appendix*, Figs. S3 and S4). Therefore, our results do not critically rely on modulating only a specific subset of synapses within L1.

In our model, the ambient GABA concentration and its effect on presynaptic release probability is homogeneous within L1. This assumption was motivated by the observation that NDNF IN axonal arbors extend over large horizontal distances in L1 (29, 56, 57) and that their activity tends to be correlated (51), suggesting that—despite the sparseness of NDNF INs—ambient GABA release is relatively uniform across the cortical microcircuit. We did not model the spatial distribution of cells within the cortical circuit beyond their home layer (L1 or L2/3). Future work could explore the role of spatially heterogeneous GABAergic volume transmission in a model with spatial structure.

**NDNF and SOM INs: Competing Master Regulators.** In the model, NDNF INs exert a powerful and unique control over the cortical microcircuit. Our work supports the notion that NDNF INs serve as "master regulators" of the cortical column (30), a role that has also been ascribed to Martinotti-type SOM INs (57). Both NDNF and SOM INs inhibit many other cells in the circuit, yet they form different connectivity patterns with different output mechanisms (presynaptic or postsynaptic) and timescales (7, 13, 57), suggesting that they operate in different ways (57). These properties can result in distinct downstream circuit effects (Fig. 5). Furthermore, SOM INs tend to receive local recurrent and feedforward inputs that contain sensory information (39), whereas NDNF INs are targeted by top–down feedback inputs that carry contextual, behavioral state, or memory-related signals (7, 13, 30). Although NDNF INs can respond to sensory stimulation (auditory and visual), their sensory responses are sensitive to previous experience and behavioral states (13, 51). SOM and NDNF INs thus regulate local circuitry based on distinct signals. Notably, we showed that these two "master regulators" may interact via a unique mutual inhibition motif, creating a dynamic interplay that flexibly regulates the cortical microcircuit depending on behavioral states, for instance. Understanding how this interplay influences cortical signal processing to guide behavior will require future theoretical work and experimental studies in vivo.

**Limitations of Our Theoretical and Experimental Approach.** The goal of this study was not to develop a detailed physiological model of the L1 circuit, but rather to delineate potential functional roles of NDNF interneurons in controlling information flow in L1. Therefore, we focused on key features of the cortical circuit model and made several simplifying design choices. Inhibitory INs were modeled as single-compartment rate neurons, describing the activity of each neuron by its firing rate. Therefore, the model does not consider the timing of spikes or electrophysiological differences between the INs. NDNF INs tend to show a late-spiking behavior (29, 30), distinct from the low-threshold and adaptive spiking of SOM INs or the fast-spiking of PV INs (53). Including the electrophysiological properties of the different INs would add another layer of complexity to the circuit model. While our results should still hold in a spiking circuit model, we expect the timing of input spikes to play a larger role. In particular, the late spiking of NDNF INs could introduce an additional temporal filter, further emphasizing their slow inhibitory action. Furthermore, we simplified the spatial diffusion of tonic GABA by modeling it as a global, slowly time-varying factor that uniformly affects all synapses within the local circuit. While a more detailed exploration of this is beyond the scope of the current study, future work should incorporate multiple coupled local subnetworks with spatially heterogeneous diffusion to investigate the spatiotemporal dynamics of GABA.

We modeled L1 cells as a homogeneous class of INs that express NDNF. While their characteristics are still an active area of research, several lines of evidence point to at least two electrophysiologically distinct subclasses of L1 NDNF INs (29, 30, 58). However, the exact delineation and whether subclasses can be identified by additional genetic markers (such as NPY) remains controversial and may depend on the brain area, model species, and developmental stages (30). Notwithstanding, an important question for future work is to determine whether potential subtypes of NDNF INs may either differentially recruit the presynaptic mechanism we describe here (e.g. by differences in GABA diffusion) or may in turn be differentially influenced by it (e.g. by different presynaptic $GABA_B$ receptor expression on their inputs).

To validate our central assumption regarding the impact of GABAergic volume transmission on SOM outputs, we conducted experiments in mouse auditory cortex slices. Employing the $GABA_B$ receptor agonist Baclofen to emulate the release of ambient GABA, we observed a marked reduction in SOM-to-NDNF synaptic transmission. The activation of presynaptic $GABA_B$ receptors was confirmed by decreased IPSC amplitudes and an increased paired-pulse ratio. This substantiates our hypothesis that SOM synapses onto NDNF INs express presynaptic $GABA_B$ receptors, inhibiting synaptic transmission upon activation. However, the experiment does not directly show that endogenous GABA released by local NDNF INs has the same effect. An experimental approach where a single

NDNF IN is recorded while multiple surrounding NDNF INs are stimulated for GABA release, and SOM INs are stimulated for investigating synaptic connectivity, would require spatially resolved dual channel optogenetic stimulation. This is beyond our current technical scope. Even if possible, the experimental approach would additionally be restricted by artificial in vitro conditions where GABA diffusion and uptake are altered by physiological conditions. Given our primary focus on investigating the functional consequences of NDNF and SOM IN interactions, we concentrated on verifying our core hypothesis—the modulation of SOM outputs by ambient GABA.

Similarly, our approach emphasized achieving a qualitative alignment between the model and experimental results, rather than a quantitative match. Quantitatively fitting the model to observed data would require tuning numerous unknown parameters whose biological equivalent is challenging to measure, such as the amount of GABA released by NDNF INs, its diffusion range, and the time it takes to reach nearby $GABA_B$ receptors or undergo reuptake. The challenge in measuring these biological equivalents underscores the need for innovative techniques. Recent advances in optogenetic targeting of neuromodulators and neurotransmitters, such as GABA, offer promising avenues for more accessible quantification (59–61). Optogenetic manipulations could be extended to genetically engineered G-protein-coupled receptors like $GABA_B$, mimicking their activation through endogenous GABA. Apart from serving as a tool for optogenetic inhibition, such manipulations hold the potential to directly explore the effects of presynaptic inhibition, including SOM outputs, in behaving animals.

We modeled a simplified prediction-error circuit with NDNF INs to demonstrate their potential role in modulating prediction error responses. However, further research is needed to fully understand the contribution of NDNF INs to predictive processing. In our current model, NDNF INs do not directly receive sensory input or top–down predictions but are instead driven by neuromodulatory inputs. It will be important to investigate how NDNF INs, when driven by sensory input or predictions, influence the dynamics of prediction-error circuits. Additionally, the current model assumes static synaptic connections, without considering the effects of synaptic plasticity. Future iterations could explore how NDNF IN connectivity might adapt in response to prediction errors, which would enhance the circuit's flexibility and its ability to respond to changing environments. Last, our simplified model focuses on negative prediction error neurons, overlooking the role of positive prediction error neurons. Incorporating both types of neurons in future models would provide a more comprehensive understanding of how NDNF INs influence the representation of prediction errors in cortical circuits.

## Materials and Methods

To study how NDNF interneurons control inhibition in L1, we took a combined experimental and theoretical approach. We used computational modeling to frame our hypothesis that NDNF INs presynaptically inhibit the outputs of SOM INs in L1 and explore its functional implications. In addition, we tested our core hypothesis by performing electrophysiological recordings in slices from the mouse auditory cortex in combination with optogenetic and pharmacological manipulations.

**Interneuron Microcircuit Model.** Inhibitory neurons (NDNF, SOM, PV, and VIP interneurons) are point neurons described by a single activity value, whereas excitatory PCs consist of two compartments representing the dendrite and the soma. The activity of each neuron/compartment evolves according to a rectified, linear differential equation describing how the different cell types influence each other akin to

$$\tau \frac{\mathrm{d}\mathbf{r}}{\mathrm{d}t} = -\mathbf{r} + W\mathbf{r} + \mathbf{x}, \quad [1]$$

where r is the activity vector of all neurons, $\tau$ the time constant of the process, $\mathbf{x}$ the external input and $W$ the synaptic interaction strength between neurons in the circuit. All neurons in the microcircuit are randomly connected with connection strengths and connection probabilities that are consistent with electrophysiological recordings (14, 15). In addition, neurons receive constant, external background input that ensures nonzero baseline activity.

To model the effect of NDNF IN-mediated GABAergic volume transmission, we introduce the ambient GABA concentration $c_G$. The GABA concentration increases with the activity of all NDNF INs $r_j^N$ in the circuit with a time constant $\tau_G$:

$$\tau_G \dot{c}_G = -c_G + \sum_{j=1}^{N_N} r_j^N . \quad [2]$$

We include presynaptic inhibition by decreasing a release factor $p$ with increasing GABA concentration, thereby capturing the inhibition of synaptic transmission via presynaptic $GABA_B$. $p$ evolves according to

$$\tau_{pi} \dot{p} = -p + (1 - b\, c_G) \quad [3]$$

and is clipped between 0 and 1. The parameter $b$ describes the strength of presynaptic inhibition and $\tau_{pi}$ its timescale. We scale the influence of SOM INs on both the PC dendrites and the NDNF INs by the release factor $p$ such that the output synapses of SOM INs in L1 are modulated by presynaptic inhibition (Fig. 1*A*).

Detailed descriptions of the model, the simulation experiments as well as model parameters can be found in *SI Appendix*.

**Experimental Procedure.** All mouse lines used were maintained on a C57BL6J background. Mice were housed under a 12 h lightdark cycle and provided with food and water ad libitum. After the surgical procedure for virus injection, mice were individually housed. All animal procedures were executed in accordance with institutional guidelines and approved by the prescribed authorities (Regierungspräsidium Freiburg).

Mice of both sexes were anesthetized and fixed in a stereotaxic frame. Adeno-associated viral vectors were injected from glass pipettes connected to a pressure ejection system into the auditory cortex. After 6 to 8 wk of viral expression, mice were deeply anesthetized with isoflurane and decapitated into carbonated, ice-cold slicing solution. A vibratome was used to obtain 350 μm thick coronal slices from the auditory cortex.

Slices were held in a recording chamber and perfused with ACSF. Cells were visualized for patching using differential interference contrast microscopy or under epifluorescence for identification using an LED with a water immersion objective and a CCD camera. Cells were recorded in whole-cell patch clamp recordings using pipettes pulled from standard-wall borosilicate capillaries using a universal electrode puller. A Multiclamp 700B amplifier was used for whole-cell voltage-clamp recordings, together with a Digidata1550 for digitization. To study presynaptic $GABA_B$ receptor-mediated inhibition while blocking putative postsynaptic effects of $GABA_B$ receptor activation, NDNF INs were recorded with Cesium-based intracellular solution. In these experiments, cells were recorded at 0 mV in control conditions, after application of Baclofen and after addition of CGP55845. SOM IN inputs in L1 were optically stimulated with either 2 pulses of 0.5 ms at 10 or 20 Hz, or a naturalistic train of 10 pulses of 0.5 ms mimicking activity recorded from a L1 IN in vivo. To control for changes in SOM IN firing based on the $GABA_B$ receptor pharmacology, we recorded SOM INs at their spontaneous resting potential using potassium gluconate–based intracellular solution.

For microscopic analysis, the brain slices were incubated overnight following the acquisition. Slices were stained with DAPI and mounted on objective slides before being imaged with a microscope.

Detailed descriptions of the experimental procedure can be found in *SI Appendix*.

**Data, Materials, and Software Availability.** Electrophysiological recordings, source code for simulations, and data analysis have been deposited in GitHub (https://github.com/LNaumann/NDNF_control_inhibition_Naumann25) (62).

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