

Supralinear dendritic integration in murine dendrite-targeting interneurons

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eLife Assessment

In this manuscript, Griesius et al analyze the dendritic integration properties of NDNF and OLM interneurons, and suggest that the supralinear NMDA receptor-dependent synaptic integration may be associated with dendritic calcium transients only in NDNF interneurons. These findings are **important** because they suggest there might be functional heterogeneities in the mechanisms underlying synaptic integration in different classes of interneurons of the mouse neocortex and hippocampus. The revised work remains **incomplete** due to remaining concerns about experimental methodology, cell health, and lack of dendritic Na-spikes which have been recorded in previous works.

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Abstract

Non-linear summation of synaptic inputs to the dendrites of pyramidal neurons has been proposed to increase the computation capacity of neurons through coincidence detection, signal amplification, and additional logic operations such as XOR. Supralinear dendritic integration has been documented extensively in principal neurons, mediated by several voltage-dependent conductances. It has also been reported in parvalbumin-positive hippocampal basket cells, in dendrites innervated by feedback excitatory synapses. Whether other interneurons, which support feed-forward or feedback inhibition of principal neuron dendrites, also exhibit local non-linear integration of synaptic excitation is not known. Here we use patch-clamp electrophysiology, and 2-photon calcium imaging and glutamate uncaging, to show that supralinear dendritic integration of near-synchronous spatially clustered glutamate-receptor mediated depolarization occurs in NDNF-positive neurogliaform cells and oriens-lacunosum moleculare interneurons in the mouse hippocampus. Supralinear summation was detected via recordings of somatic depolarizations elicited by uncaging of glutamate on dendritic fragments, and, in neurogliaform cells, by concurrent imaging of dendritic calcium transients. Supralinearity was abolished by blocking NMDA receptors (NMDARs) but resisted blockade of voltage-gated sodium channels. Blocking L-type calcium channels abolished supralinear calcium signalling but only had a minor effect on voltage supralinearity. Dendritic boosting of spatially clustered

synaptic signals argues for previously unappreciated computational complexity in dendrite-projecting inhibitory cells of the hippocampus.

Introduction

Accumulating evidence indicates that dendrites do more than merely summate excitatory and inhibitory synaptic inputs (1–6). Dendrites endow neurons with increased computational capacity (7) and the ability to act as multi-level hierarchical networks (8, 9) or even to reproduce some features of artificial and deep neural networks (10, 11). The underlying mechanisms include extensive dendritic arborisation, compartmentalisation, synaptic plasticity and expression of specific receptors and ion channels that in turn facilitate nonlinear input summation. Nonlinear dendritic integration of excitatory inputs in particular can potentially support signal amplification (12), coincidence detection (13), XOR logic gating (14, 15), and computing prediction errors (2, 11). One striking example of non-linear integration is local supralinear summation of signals that arrive at a dendritic fragment in a narrow time window, a phenomenon that can be investigated by exploiting the spatial and temporal precision of somatic depolarizations elicited by multi-photon glutamate uncaging (16–21). Several receptors and channels have been implicated in supralinear summation in principal neurons, including NMDARs (16–19, 21–27), calcium-permeable AMPARs (21, 25, 26), and voltage-gated sodium channels (20, 21, 24, 25, 27, 28), calcium channels (19, 21, 25, 27), and potassium channels (29, 30). Closely related to voltage supralinearity is the occurrence of dendritic calcium transients which can be detected with calcium-sensitive fluorescent indicators. Such calcium transients are implicated in synaptic plasticity and result not only from influx via voltage-dependent channels but also release from intracellular stores (25, 26, 31, 32).

Inhibitory interneurons have a crucial role in brain circuit and network excitability, rhythmicity, and computations (33–35). However, in comparison with principal cells, relatively little is known about non-linear dendritic integration in interneurons. Early evidence for NMDAR-dependent supralinear summation of EPSPs, and dendritic calcium transients, in hippocampal CA1 interneurons was not accompanied by molecular or genetic markers of interneuron type (18). Subsequent studies in parvalbumin-positive (PV+) interneurons reported supralinearly summing EPSPs and dendritic calcium transients (21, 25, 26). Using multiphoton glutamate uncaging for increased spatial precision, Cornford et al. also showed evidence for NMDAR-dependent supralinear EPSP summation in PV+ basket cells, although this was only seen in dendrites receiving feedback excitation in stratum oriens and not in dendrites receiving feed-forward excitation in stratum radiatum (16). Failure to detect supralinear summation in stratum radiatum dendrites of PV+ interneurons is consistent with evidence from dendritic recordings that somatic action potentials attenuate rapidly as they back-propagate, and that PV+ dendrites express abundant Kv3 family potassium channels and relatively few sodium channels (36). Indeed, compartmental modelling has been used to infer that synaptic inputs to PV+ interneuron dendrites summate sublinearly, and that this helps to ensure that they do not disrupt population spike synchrony during gamma oscillations (37). In contrast to the evidence for both supra- and sublinear summation in PV+ interneurons (likely to occur simultaneously in different dendrites) (16), it remains unknown how synaptic inputs are integrated in other forebrain interneurons. As for other brain regions, cerebellar cortex stellate cells have been shown to exhibit sublinear summation of uncaging-evoked EPSPs whilst simultaneously exhibiting supralinear summation of dendritic calcium transients (19).

Dysfunction of interneurons generally has been associated with a variety of psychiatric and neurological pathologies, including epilepsy (38–41), schizophrenia (42–44), autism spectrum disorder (44, 45) and Alzheimer's disease (46, 47), among others (48). There is emerging evidence suggesting that impairments in dendritic integration (20, 49–52), and

specifically in interneurons (20 [↗](#)), may be important contributors to pathological phenotypes in psychiatric and neurological pathologies. We therefore set out to characterize dendritic integration in two types of interneurons innervating the distal dendrites of CA1 pyramidal neurons: PV-negative neurogliaform interneurons, which support feed-forward inhibition, and oriens-lacunosum moleculare (OLM) cells, which additionally mediate feedback inhibition. Although their cell bodies reside in different layers, and their developmental origin and intrinsic properties are strikingly different (48 [↗](#), 53 [↗](#)–58 [↗](#)), their axons innervate the same targets, the apical tuft dendrites of pyramidal neurons in the SLM. Neurogliaform cells receive inputs from the entorhinal cortex and the nucleus reuniens (56 [↗](#)), with distinct plasticity rules at synapses made by these afferents (59 [↗](#)), and a subset can be identified in stratum lacunosum-moleculare (SLM) using knock-in mice expressing cre recombinase at the neuron-derived neurotrophic factor (NDNF) locus (56 [↗](#), 58 [↗](#)–60 [↗](#)). OLM cells, in contrast, have their cell bodies and dendrites in stratum oriens, are excited by axon collaterals of both CA1 and CA3 pyramidal neurons, and can be identified using mice expressing cre at the somatostatin locus (61 [↗](#)).

We quantified non-linear dendritic summation using 2-photon glutamate uncaging at multiple locations within a small dendritic fragment, by comparing the arithmetic sum of the uncaging-evoked EPSPs evoked individually to the result of near-synchronous glutamate uncaging (16 [↗](#)). We asked if neurogliaform and OLM interneurons exhibit non-linear summation and whether this depends on NMDARs or sodium or calcium channels. In parallel, we asked if non-linear summation could also be detected in these cell types by concurrent measurement of dendritic calcium-dependent fluorescence transients.

Results

Supralinear dendritic integration in a representative neurogliaform interneuron

NDNF-positive (NDNF+) neurogliaform interneurons were studied in the hippocampal CA1 stratum lacunosum/moleculare in acute slices from NDNF-cre mice injected with AAV2/9-mDLX-FLEX-mCherry virus (Fig. 1a [↗](#)). In order to probe the spatiotemporal dynamics of dendritic integration, glutamate was locally delivered by 2-photon uncaging of bath-perfused MNI-glutamate (3 mM) using an infrared (720nm) pulsed laser at points along dendrites, whilst recording from their somata with a patch pipette. The absence of dendritic spines prevented identification of the sites of excitatory synapses, and the uncaging locations were therefore spaced 1–2 μm apart, either side of the selected dendritic fragment (16 [↗](#)) (Fig. 1b [↗](#)). The locations were kept within a dendritic length of $\sim 15\text{ mm}$, consistent with the size of interneuron dendritic domains exhibiting correlated tuning of synaptic calcium transients in vivo (62 [↗](#)). Glutamate uncaging was achieved with 0.5 ms pulses separated by an interval of either 100.32 ms (asynchronously) or 0.32 ms, near-synchronously. The number of uncaging stimuli at neighbouring locations was cumulatively increased from 1 to 12 in the near-synchronous condition (Fig. 1c [↗](#)). In the illustrated example, the somatic uEPSP evoked by near-synchronous uncaging was substantially greater in amplitude and duration than predicted from the arithmetic sum of the individual somatic uEPSPs (Fig. 1d [↗](#)).

In parallel with measurement of uEPSPs, we measured dendritic calcium transients by monitoring the fluorescence of the calcium sensor Fluo-4, which was included in the pipette solution, using a second pulsed infrared scanning laser tuned to 810 nm (Fig 1e, f [↗](#)). The interval between glutamate uncaging pulses in the asynchronous condition was too short to allow the dendritic Fluo-4 signal to relax to baseline in between stimuli. Instead, we examined the peak amplitude of the dendritic calcium response, either in response to asynchronous uncaging at all 12 locations (Fig. 1e [↗](#)) or with an increasing number of near-synchronous stimuli (Fig. 1f [↗](#)). This revealed a

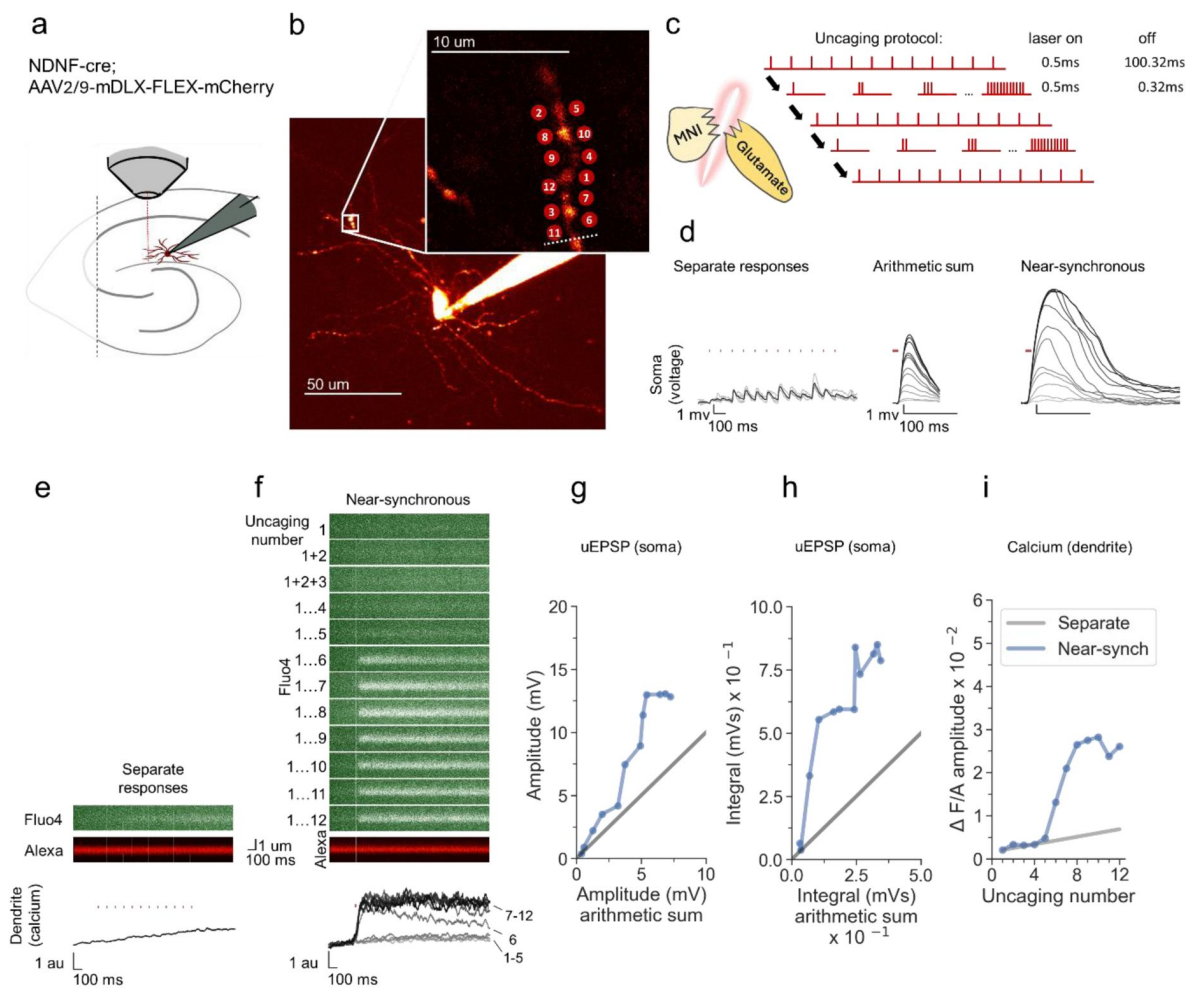


Fig 1.

Experimental overview and representative example showing supralinear dendritic integration in a neurogliaform interneuron.

- (a)** Summary depicting the mouse model, viral expression, and acute slice preparation with patch-clamp and imaging and uncaging.
- (b)** Alexa-594 fluorescence Z-stack of a representative neurogliaform NDNF+ interneuron, with uncaging locations and the position of the linescan used to measure dendritic calcium transients (dotted white line)(inset). The image was obtained after the experiment was concluded.
- (c)** Uncaging protocol for asynchronous (separate responses) and near-synchronous conditions.
- (d)** Representative somatic voltage traces across asynchronous separate response and near-synchronous conditions. Laser uncaging light stimuli are indicated by red dots above the traces. uEPSPs were elicited in the asynchronous condition with light pulses separated by an interval of 100.32 ms (left; grey traces: individual sweeps; black traces average). The arithmetic sum traces were calculated by aligning and summing an increasing number of asynchronously evoked responses (middle). Near-synchronous uEPSPs were elicited with light pulses separated by an interval of 0.32 ms (right). The families of traces for the arithmetic sum and near-synchronously evoked responses are shown as light to dark grey traces, indicating an increase in the number of uncaging loci from 1 to 12.
- (e)** Fluo-4 and Alexa-594 linescans during asynchronous sequential uncaging at 12 loci at times indicated by the vertical red dashes. The ratio of dendritic Fluo-4 to Alexa fluorescence is shown below (au: arbitrary unit).
- (f)** Linescans during near-synchronous uncaging at increasing numbers of locations (1 – 12, top to bottom).
- (g)** Peak amplitude of the recorded near-synchronous response plotted against the amplitude of the arithmetic sum as the number of uncaging loci was increased. The line of identity is represented by a grey line.
- (h)** Time integral of the recorded near-synchronous response plotted against the integral of the arithmetic sum. The line of identity is represented by a grey line.
- (i)** Fluo-4 fluorescence transient amplitude, normalised by Alexa-594 fluorescence, plotted against the number of locations uncaged near-synchronously. The grey line indicates the estimated separate response amplitude interpolated from the fluorescence measured between 1 and 12 locations uncaged asynchronously (see Methods).

discontinuity, where the amplitude of the dendritic calcium fluorescence transient increased abruptly with 6 or more near-synchronous uncaging locations, and exceeded the fluorescence observed with asynchronous uncaging at all 12 locations (**Fig. 1e, f** [↗](#)).

In order to characterize non-linear voltage integration measured at the soma, the amplitude of the observed uEPSP elicited by near-synchronous uncaging at an increasing number of locations was plotted against the arithmetic sum of the individual responses. Consistent with supralinear integration in principal neurons and at oriens dendrites in PV+ interneurons, as the number of uncaging sites was increased, the recorded near-synchronously evoked uEPSPs initially summated approximately linearly, and then deviated from the line of identity upon activation of multiple uncaging locations (**Fig. 1g** [↗](#)). The supralinearity was more marked when the uEPSP was quantified by integrating the somatic depolarization for 100ms (**Fig. 1h** [↗](#)), as expected from the recruitment of voltage-dependent conductances. As the number of uncaging locations was increased further, the peak amplitude and integral showed a tendency to reach a plateau, consistent with a decrease in driving force as the local dendritic membrane potential approached the synaptic reversal potential.

To estimate the supralinearity of the calcium transients, we interpolated the calcium response from 1 location uncaged to the maximal fluorescence observed with all 12 locations uncaged asynchronously, and compared the peak amplitude of the calcium transients as an increasing number of locations were uncaged near-synchronously (**Fig. 1i** [↗](#)). This revealed an abrupt deviation from the interpolated line at 6 locations uncaged, with a further increase as more locations were uncaged, before the observed response reached a plateau.

Supralinear dendritic integration in neurogliaform interneurons

We examined dendritic nonlinearity in a sample of neurogliaform NDNF+ interneurons studied in the same way. This revealed robust supralinear integration of uEPSP amplitude (**Fig. 2a** [↗](#)). The overall shape of the relationship between the observed and expected amplitude varied among cells, although its sigmoid shape persisted. We took as a measure of supralinearity in each cell the cumulative relative deviation of the observed amplitude from the expected value (see Methods). Including cells that were used for subsequent blockade of NMDARs (see below), the overall average amplitude supralinearity was $58 \pm 20\%$ (average $\pm$ SEM, $n = 11$, deviation from 0, $P < 0.001$; **Fig. 2b** [↗](#)). Consistent with the data from the sample neuron (**Fig. 1** [↗](#)) the degree of voltage nonlinearity was more marked for the voltage integral measured over 100 ms (integral nonlinearity $122 \pm 35\%$), implying the involvement of slow and/or regenerating conductances (**Fig. 2c, d** [↗](#)). As for dendritic calcium transients, 6 out of 7 interneurons where they were measured exhibited a greater than predicted Fluo-4 fluorescence transient, with a nonlinearity estimated at $37 \pm 10\%$ ($P = 0.0138$; **Fig. 2e, f** [↗](#)).

Previous studies on dendritic integration have generally examined either voltage or dendritic calcium signalling in isolation, with the exception of an investigation of cerebellar layer molecular interneurons, which reported a striking dissociation, where calcium signalling exhibited supralinear summation while voltage summation was sublinear ([19](#) [↗](#)), contrasting with the present results. The present findings suggest that dendritic regenerative currents are calcium-permeable, and that the calcium supralinearity is causally downstream from the voltage supralinearity. This principle was supported by the similar sigmoid shapes of the observed near-synchronous uEPSP amplitudes and calcium transients in the cell illustrated in **Fig. 1** [↗](#). Some cells exhibited a prominent plateau potential upon near-synchronous uncaging, with a peak calcium transient proportional to the uEPSP integral (Pearsons' correlation coefficient $r = 0.95$; **Fig. S1** [↗](#)). However, across the sample of 7 cells, the correlation coefficient relating calcium to voltage supralinearity varied substantially and was not systematically greater for voltage integral (0.44 ± 0.16 , average $\pm$ SEM) than for peak uEPSP amplitude (0.47 ± 0.18).

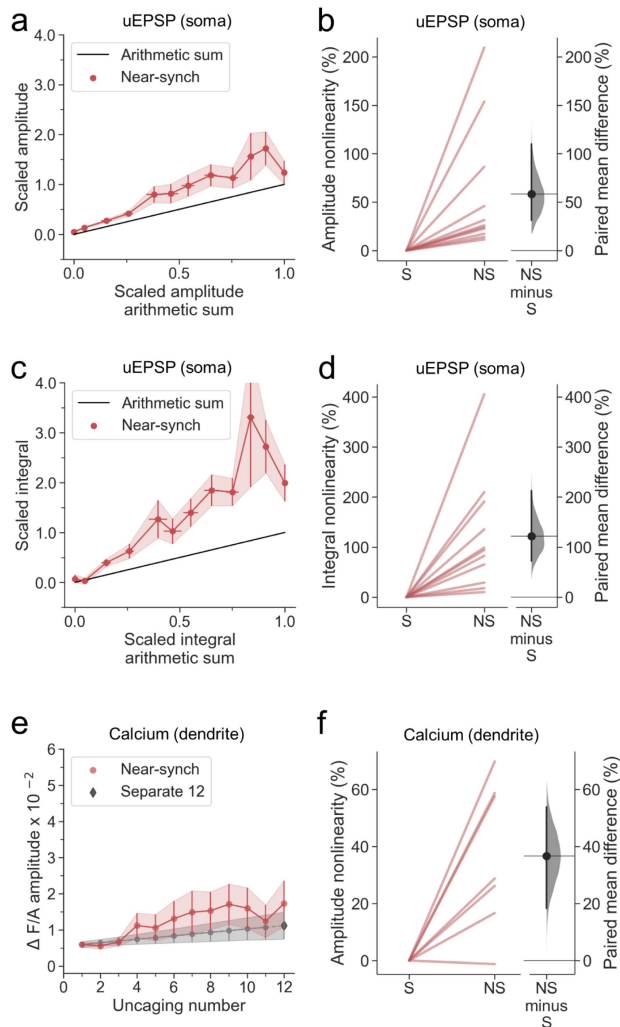


Fig. 2.

Summary of supralinear dendritic integration in neurogliaform interneurons.

(a) Average scaled amplitude of recorded near-synchronous uEPSPs plotted against scaled amplitude of the arithmetic sum of asynchronously evoked uEPSPs as the cumulative number of uncaging locations was increased. The scaled arithmetic sum overlaps with the line of identity. Error bars: SEM ($n=11$ dendrites in 11 cells from 9 animals).

(b) Amplitude nonlinearity quantified for data shown in (a). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 56% [95%CI 27%, 108%]. $P<0.001$ (two-sided permutation t-test compared to 0%).

(c) Average scaled near-synchronous uEPSP integral plotted against scaled arithmetic sum of asynchronous separate responses, for the same dataset. The scaled arithmetic sum overlaps with the line of identity.

(d) Integral nonlinearity corresponding to panel (c). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 122% [95%CI 73%, 213%]. $P<0.001$ (two-sided permutation t-test compared to 0%).

(e) Average Fluo-4/Alexa-594 fluorescence transients plotted against the cumulative number of uncaging locations. The grey line indicates the interpolated values used in the nonlinearity calculation. The interpolation was performed between the calcium transient amplitude measurement at 1 uncaging location from the near-synchronous condition and the 12th response from the asynchronous (separate responses) condition (see Methods). The dark grey diamond indicates the amplitude from the 12th asynchronous response. ($n = 7$ dendrites in 7 cells from 7 animals).

(f) Amplitude nonlinearity corresponding to panel (c) (dendritic calcium). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 37% [95%CI 18%, 54%]. $P=0.0138$ (two-sided permutation t-test compared to 0%).

For the slopegraphs and Gardner-Altman estimation plots, paired observations are connected by coloured lines. The paired mean differences between the near-synchronous (NS) and asynchronous separate response (S) groups are shown in Gardner-Altman estimation plots as bootstrap sampling distributions. The mean differences are depicted as black dots. The 95% confidence intervals are indicated by the ends of the vertical error bars.

Among possible sources of variability for voltage supralinearity, we did not observe a significant monotonic dependence on the average amplitude of individual uEPSPs, distance from the uncaging location along the dendrite to the soma, dendrite order, or dendrite diameter (Fig. S2).

Sublinear dendritic integration in neurogliaform interneurons with NMDARs blocked

Several conductances have been implicated in supralinear integration in principal neuron dendrites, including NMDARs (16–19, 21–27), sodium channels (20, 21, 24, 25, 27, 28) and calcium channels (19, 21, 25, 27). We asked whether they have similar roles in neurogliaform cells.

In a subset of cells reported in Fig. 2, we applied the NMDAR antagonist D-AP5 by bath perfusion. This robustly abolished voltage supralinearity, whether measured as the peak amplitude of uEPSPs ($P < 0.001$, $n = 6$; Fig. 3a, c, d) or uEPSP integral ($P < 0.001$; Fig. 3e, f). Summation of somatic uEPSPs became sublinear ($P < 0.001$, $P < 0.001$, for peak and integral respectively), consistent with the behaviour of a passive dendrite where the local synaptic driving force and impedance diminish as an increasing number of glutamate receptors open. Dendritic calcium fluorescence transients were also profoundly reduced (Fig. 3b, g, h).

Supralinear dendritic integration in neurogliaform interneurons does not require voltage-dependent sodium channels

We examined the role of voltage-gated sodium channels, which have been implicated in back-propagating action potentials in principal cells (63–65), by repeating the study in the continuous presence of tetrodotoxin (TTX). In a representative cell, robust supralinear integration was seen for both uEPSPs (Fig. 4a) and calcium fluorescence transients (Fig. 4b). Across all dendrites tested ($n = 7$), both the uEPSP and the dendritic calcium transient amplitudes summed supralinearly (Fig. 4c–h), with effect sizes closely resembling those observed in the drug-free condition (Fig. 2). The data argue against a contribution of voltage-gated sodium channels to supralinear dendritic integration in NDNF+ neurogliaform interneurons.

L-type voltage-dependent calcium channel blockade abolishes nonlinear dendritic calcium summation

In another group of neurogliaform cells, we examined dendritic integration in the presence of the L-type voltage-dependent calcium channel blocker nimodipine. Although the population average non-linearity was not different from 0, a subset of neurons continued to show supralinear summation for both peak and integral (Fig. 5a, c–f), contrasting with the effect of blocking NMDARs. Dendritic calcium transients were profoundly attenuated (Fig. 5b, g, h). We tentatively conclude that L-type calcium channels have a smaller contribution to voltage supralinearity than NMDARs, but contribute substantially to dendritic calcium influx.

SERCA inhibition abolishes nonlinear dendritic calcium summation

We next asked if release from intracellular stores also contributes to dendritic calcium transients, as has been reported in stratum oriens interneurons (26). We therefore examined the effect of depleting calcium stores with the SERCA inhibitor cyclopiazonic acid (CPA). Although voltage supralinearity persisted (Fig. 6a, c–f), dendritic calcium transients were abolished (Fig. 6b, g, h).

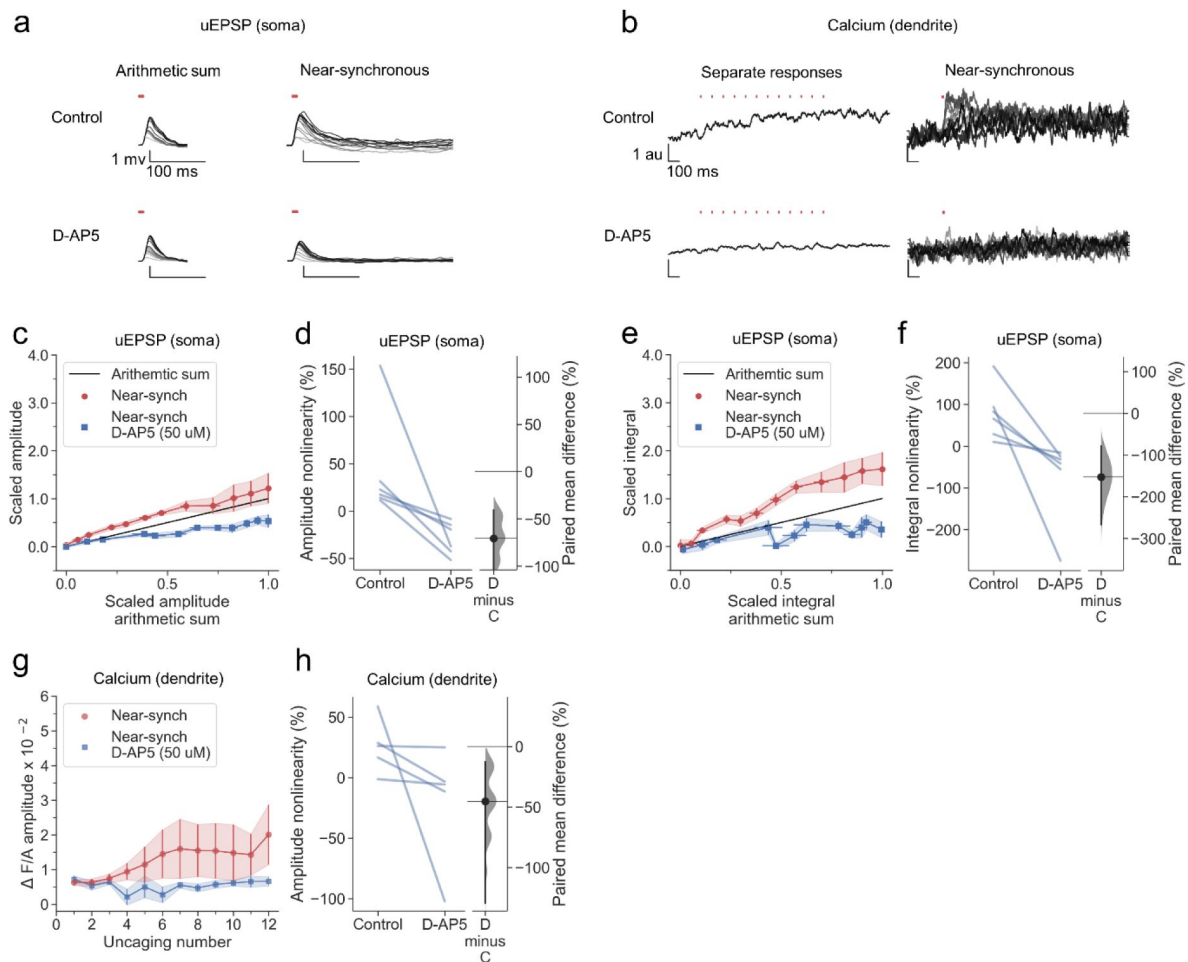


Fig. 3.

Sublinear dendritic integration in neurogliaform interneurons with NMDARs blocked.

(a) Somatic uEPSPs evoked by uncaging glutamate in a representative neuron before (top) and after (bottom) D-AP5 (50 μ M) perfusion. Uncaging protocol and data representation as in Figs. 1 and 2.

(b) Dendritic Fluo-4 traces in the same neuron, in response to asynchronous and near-synchronous uncaging, before (left) and after NMDAR blockade (right).

(c) Scaled amplitude of the recorded near-synchronous uEPSP plotted against scaled amplitude of the arithmetic sum, as the number of uncaging locations was increased. The scaled arithmetic sum overlaps with the line of identity. Error bars: SEM ($n=6$ dendrites in 6 cells from 6 animals). The supralinear relationship became sublinear with NMDARs blocked.

(d) Amplitude nonlinearity for individual neurons shown in (c) before and after D-AP5 perfusion. The paired mean difference between the two conditions is -71% [95%CI -147%, -41%]. $P<0.001$ (two-sided permutation t-test).

(e) Scaled time-integral of uEPSPs plotted against scaled integral arithmetic sum of asynchronous separate responses, before and after NMDAR blockade. The scaled arithmetic sum overlaps with the line of identity.

(f) Integral nonlinearity corresponding to panel (e). The paired mean difference between baseline and D-AP5 conditions is -153% [95%CI -267%, -78%]. $P<0.001$ (two-sided permutation t-test).

(g) Fluo-4 fluorescence transients (normalised to Alexa-594) plotted against the cumulative number of uncaging locations, before and after NMDAR blockade. Data plotted as in Fig. 1.

(h) Amplitude nonlinearity corresponding to panel (g). The paired mean difference between baseline and D-AP5 conditions is -45% [95%CI -129%, -13%]. $P<0.001$ (two-sided permutation t-test).

Slopegraphs and Gardner-Altman estimation plots as in Fig. 2.

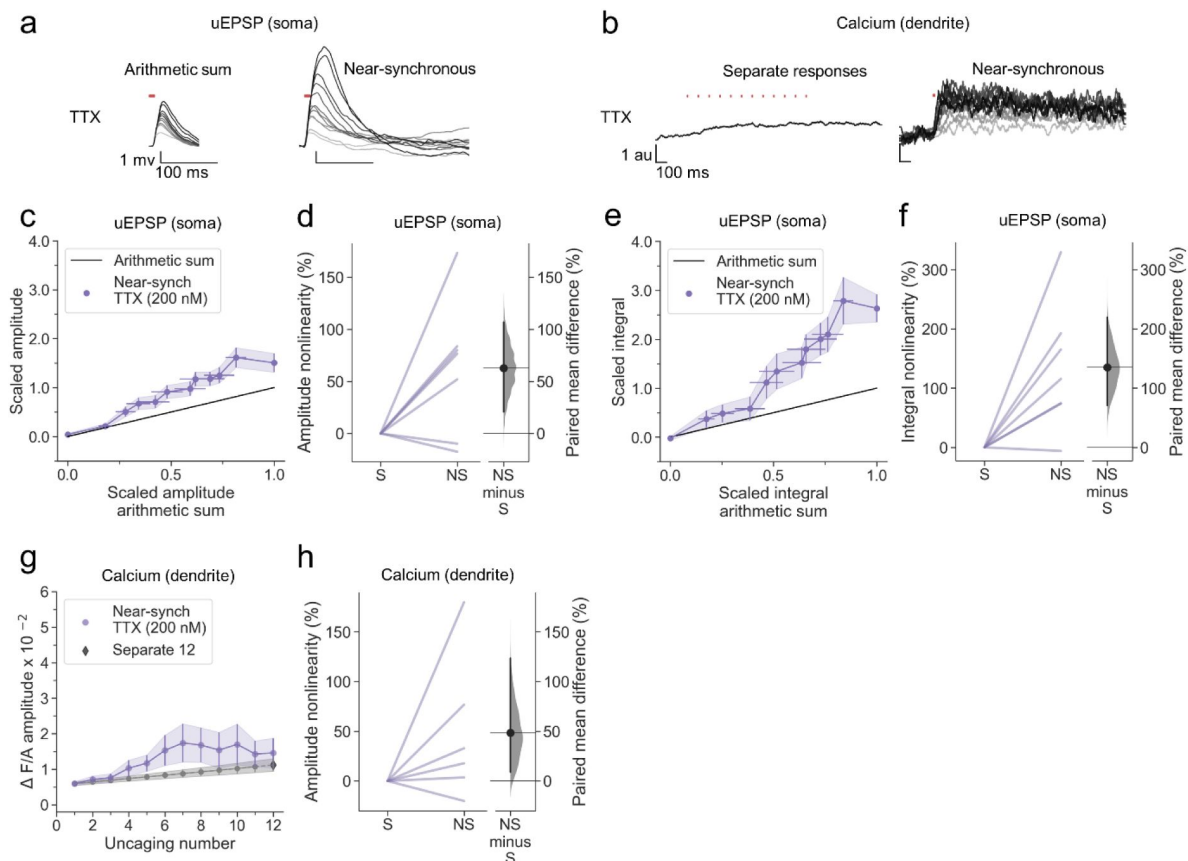


Fig. 4.

Supralinear dendritic integration in neurogliaform interneurons does not require voltage-dependent sodium channels.

(a) Somatic uEPSPs evoked by uncaging glutamate in a representative neuron in the presence of TTX (200 nM), showing supralinear summation.

(b) Dendritic Fluo-4 fluorescence responses in the same neuron, showing supralinear calcium signalling.

(c) Average scaled amplitude of recorded near-synchronous uEPSP plotted against scaled amplitude arithmetic sum with increasing numbers of cumulative uncaging locations. The scaled arithmetic sum overlaps with the line of identity. Error bars: SEM (n=7 dendrites in 5 cells from 5 animals).

(d) Amplitude nonlinearity corresponding to panel (c). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 63% [95%CI 21%, 107%]. $P=0.0382$ (two-sided permutation t-test compared to 0%).

(e) uEPSP amplitude nonlinearity quantified by integral, plotted in the same way as (c).

(f) Integral nonlinearity corresponding to (e). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 135% [95%CI 71%, 220%]. $P=0.011$ (two-sided permutation t-test compared to 0%).

(g) Fluo-4 fluorescence transient amplitude (normalised to Alexa-594) plotted against the cumulative number of uncaging locations. Data plotted as in [Fig. 1](#).

(h) Amplitude nonlinearity corresponding to panel (g). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 48% [95%CI 9%, 123%]. $P=0.088$ (two-sided permutation t-test compared to 0%).

Slopegraphs and Gardner-Altman estimation plots as in [Fig. 2](#).

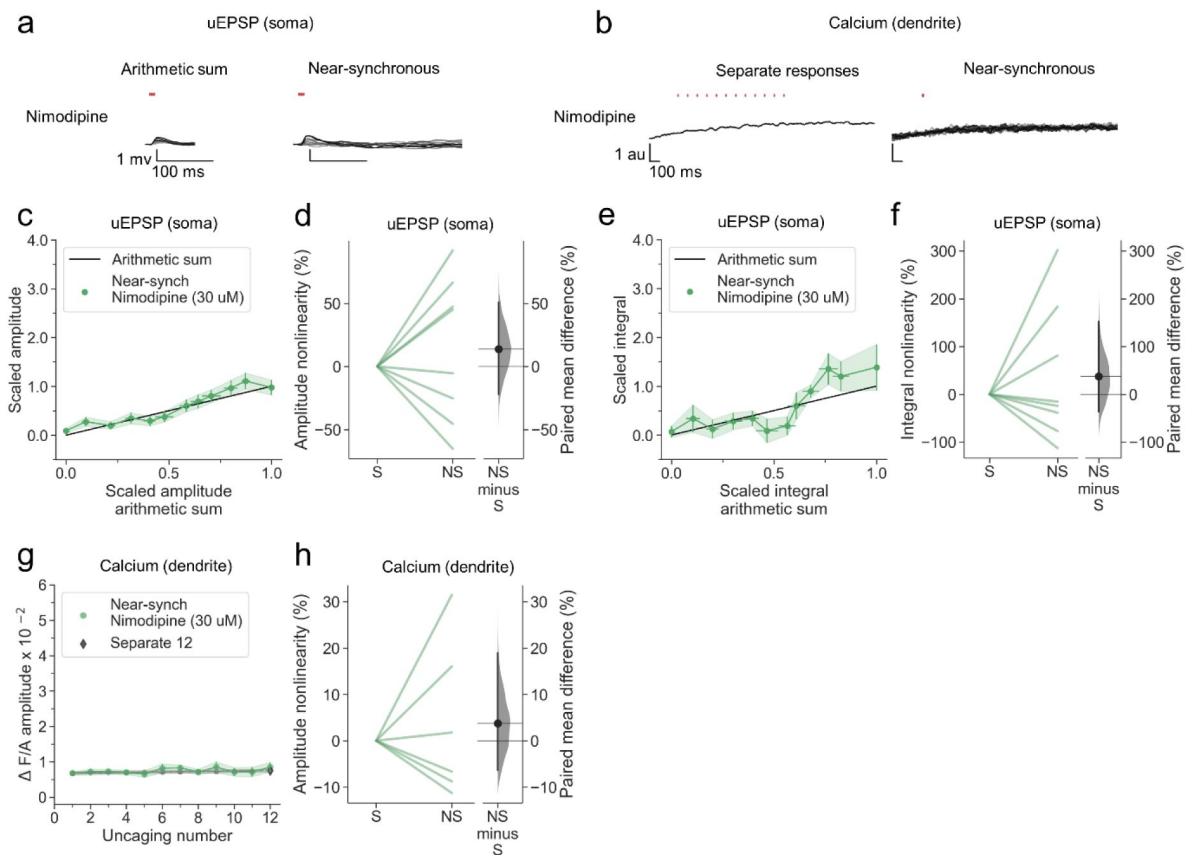


Fig. 5.

L-type voltage-dependent calcium channel blockade abolishes nonlinear dendritic calcium summation.

(a) Somatic uEPSPs evoked by glutamate uncaging in one neuron in the presence of nimodipine (30 μ M), showing supralinear uEPSP integration.

(b) Dendritic Fluo-4 fluorescence transients in the same neuron, showing absence of supralinear integration.

(c) Average scaled amplitude of the recorded near-synchronous uEPSP plotted against the arithmetic sum of the scaled asynchronous uEPSPs, as the cumulative number of uncaging locations was increased. The scaled arithmetic sum overlaps with the line of identity. Error bars: SEM ($n=8$ dendrites in 5 cells from 3 animals).

(d) Amplitude nonlinearity corresponding to (c). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 14% [95%CI -22%, 51%]. $P=0.505$ (two-sided permutation t-test compared to 0%).

(e) uEPSP amplitude nonlinearity quantified by integral, plotted in the same way as (c).

(f) Integral nonlinearity corresponding to (e). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 37% [95%CI -37%, 153%]. $P=0.497$ (two-sided permutation t-test compared to 0%).

(g) Fluo-4 fluorescence transient amplitude (normalised to Alexa-594) plotted against cumulative number of uncaging locations. Data plotted as in Fig. 1.

(h) Amplitude nonlinearity corresponding to (g). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 4% [95%CI -6%, 19%]. $P=0.561$ (two-sided permutation t-test compared to 0%).

Slopegraphs and Gardner-Altman estimation plots as in Fig. 2.

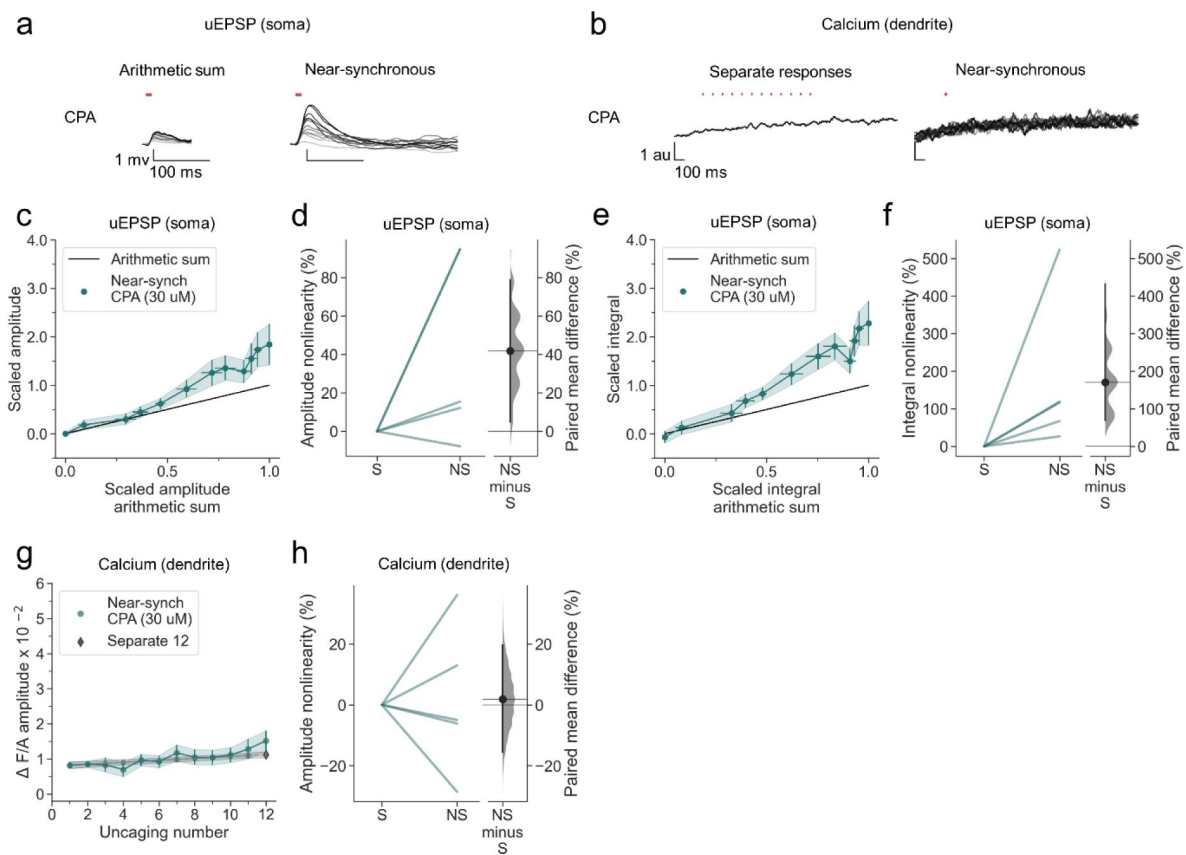


Fig. 6.

SERCA inhibition abolishes nonlinear dendritic calcium summation.

(a) Somatic uEPSPs evoked by glutamate uncaging in one neuron in the presence of CPA (30 uM), showing supralinear uEPSP integration.

(b) Dendritic Fluo-4 fluorescence transients in the same neuron, showing absence of supralinear integration.

(c) Average scaled amplitude of the recorded near-synchronous uEPSP plotted against the arithmetic sum of the scaled asynchronous uEPSPs, as the cumulative number of uncaging locations was increased. The scaled arithmetic sum overlaps with the line of identity. Error bars: SEM (n=5 dendrites in 2 cells from 2 animals).

(d) Amplitude nonlinearity corresponding to (c). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 42% [95%CI 5%, 79%]. $P=0.067$ (two-sided permutation t-test compared to 0%).

(e) uEPSP amplitude nonlinearity quantified by integral, plotted in the same way as (c).

(f) Integral nonlinearity corresponding to (e). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 171% [95%CI 69%, 432%]. $P<0.001$ (two-sided permutation t-test compared to 0%).

(g) Fluo-4 fluorescence transient amplitude (normalised to Alexa-594) plotted against cumulative number of uncaging locations. Data plotted as in Fig. 1.

(h) Amplitude nonlinearity corresponding to (g). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 2% [95%CI -16%, 20%]. $P=0.754$ (two-sided permutation t-test compared to 0%).

Slopegraphs and Gardner-Altman estimation plots as in Fig. 2.

A parsimonious explanation for the dissociation of voltage supralinearity and calcium signalling is that NMDARs are the main driver of regenerative currents in the dendrites of neurogliaform cells, and that calcium influx via NMDARs and L-type calcium channels is amplified by calcium release from intracellular stores. Overall, the robust NMDAR-mediated supralinearity demonstrated in neurogliaform cells stands in contrast to the more subtle supralinearity observed in PV+ interneurons in response to an identical glutamate uncaging protocol, which was confined to dendrites in stratum oriens (16 [↗](#)).

Supralinear dendritic integration in OLM interneurons

Apical tuft dendrites of pyramidal neurons are innervated not only by neurogliaform cells but also by OLM interneurons, which exhibit numerous differences with respect to their developmental origin, morphology and intrinsic and synaptic properties. We asked whether the robust supralinear summation and calcium signalling exhibited by neurogliaform cells are also a feature of OLM cells, by repeating the experiments using multiphoton uncaging on dendrites in stratum oriens. OLM cells were identified in acute hippocampal slices from SOM-Cre x Ai9 mice, with dendrites running parallel to stratum pyramidale and an axon, where visualised, extending through stratum pyramidale and stratum radiatum (**Fig 7a, b** [↗](#)).

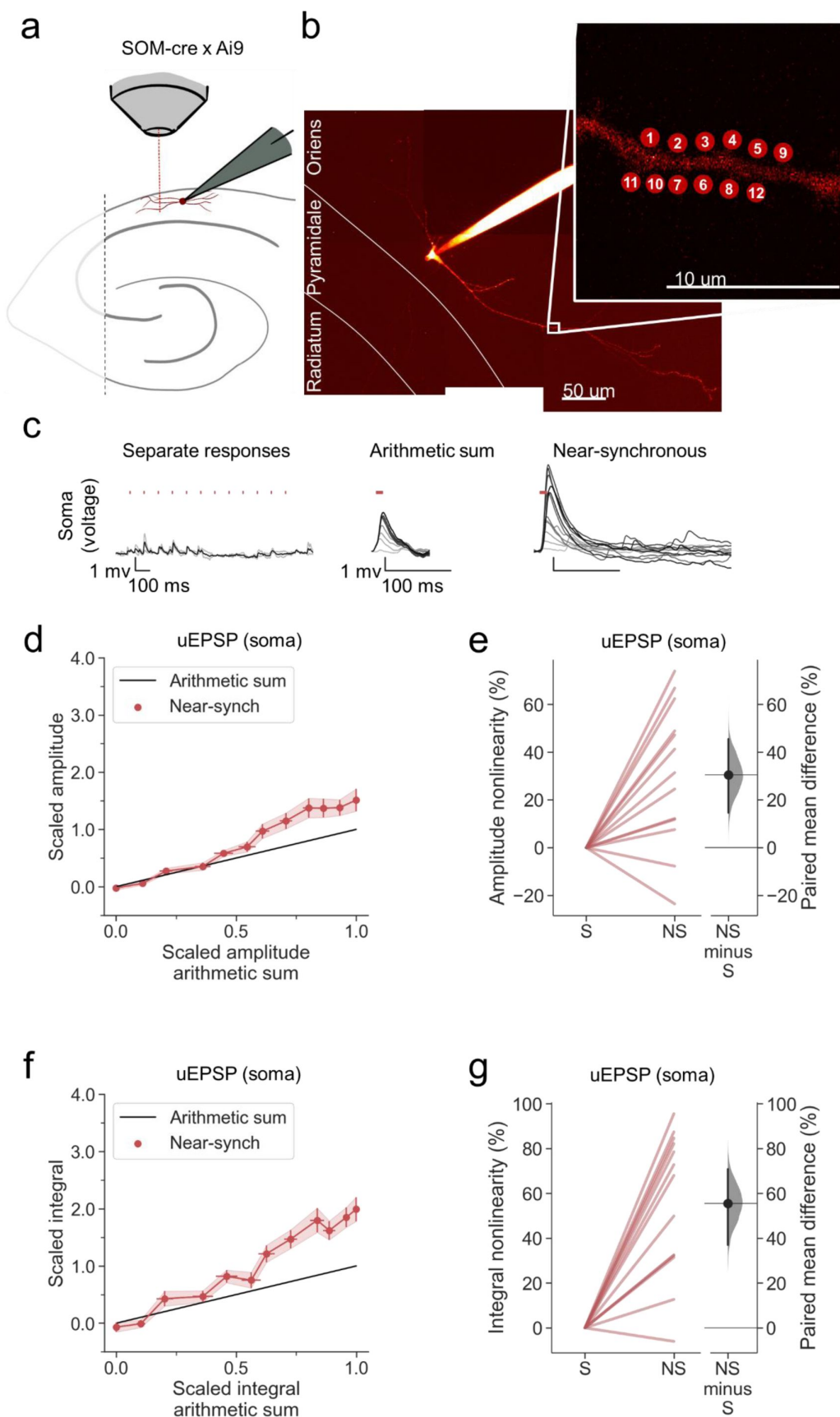


Fig. 7.

Supralinear dendritic integration in OLM interneurons.

(a) Summary depicting the mouse model and acute slice preparation with patch-clamp and imaging and uncaging. Uncaging protocol and data representation as in **Figs. 1** [↗](#) **2** [↗](#)

(b) Alexa-594 fluorescence Z-stack of a representative OLM SOM+ interneuron with uncaging locations (inset). The image was obtained after the experiment was concluded.

(c) Representative somatic voltage traces across asynchronous separate response and near-synchronous conditions, depicted as in **Fig. 1** [↗](#).

(d) Average scaled amplitude of the recorded near-synchronous uEPSP plotted against the arithmetic sum of the scaled asynchronous uEPSPs, as the cumulative number of uncaging locations was increased. The scaled arithmetic sum overlaps with the line of identity. Error bars: SEM (n=13 dendrites in 13 cells from 13 animals).

(e) Amplitude nonlinearity corresponding to (d). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 31% [95%CI 15%, 46%]. $P = 0.004$ (two-sided permutation t-test compared to 0%).

(f) uEPSP amplitude nonlinearity quantified by integral, plotted in the same way as (d).

(g) Integral nonlinearity corresponding to (f). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 56% [95%CI 31%, 71%]. $P < 0.001$ (two-sided permutation t-test compared to 0%).

Slopegraphs and Gardner-Altman estimation plots as in **Fig. 2** [↗](#).

Uncaging-evoked uEPSPs summated supralinearly in OLM cells, as detected by comparing either the peak amplitude or voltage integral of near-synchronously evoked responses to the arithmetic sum of the asynchronously evoked responses (**Fig. 7c-g** [↗](#)).

Linear dendritic integration in neurogliaform interneurons with NMDARs blocked

As for neurogliaform cells, there was no obvious dependence of the magnitude of supralinearity on the average size of the individual uEPSPs, dendritic distance, dendrite order, or dendrite diameter (**Fig. S3** [↗](#)). NMDAR blockade abolished supralinearity (**Fig. 8a-e** [↗](#)), further underlining the similarity with neurogliaform cells. However, sublinear summation was not observed in D-AP5. Another striking difference was that the uEPSPs evoked in OLM cells (with NMDARs intact) were sub-threshold for detectable dendritic calcium signals under our recording conditions.

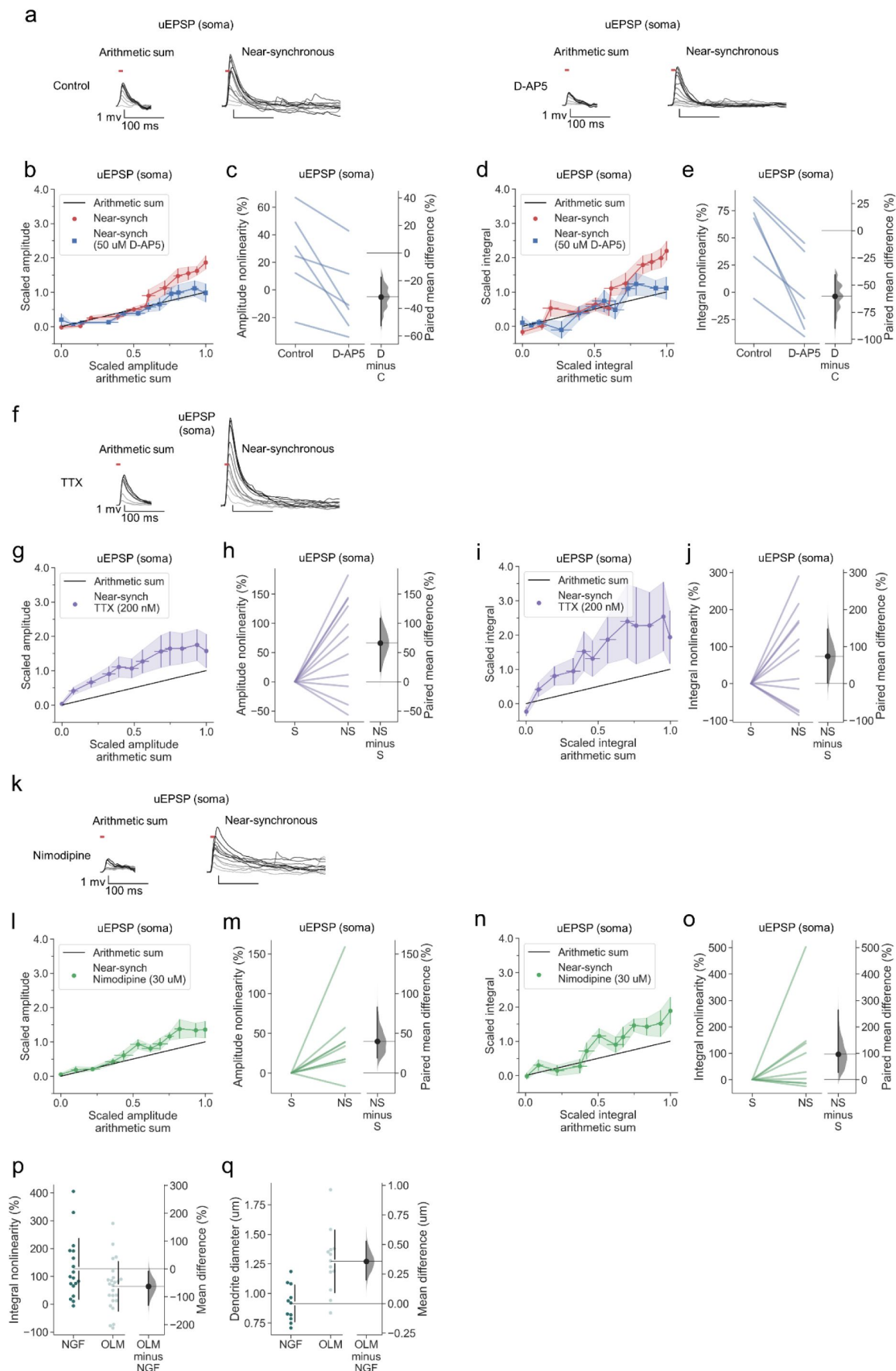


Fig. 8.

Linear dendritic integration in OLM interneurons with NMDARs blocked and no effect of voltage-dependent sodium channel nor of L-type voltage-dependent calcium channel blockade.

(a) Somatic uEPSPs evoked by uncaging glutamate in a representative neuron before (top) and after (bottom) D-AP5 (50 μ M) perfusion. Uncaging protocol and data representation as in **Figs. 1** [↗](#) **2** [↗](#).

(b) Scaled amplitude of the recorded near-synchronous uEPSP plotted against scaled amplitude of the arithmetic sum, as the number of uncaging locations was increased. The scaled arithmetic sum overlaps with the line of identity. Error bars: SEM (n=6 dendrites in 6 cells from 6 animals).

(c) Amplitude nonlinearity for individual neurons shown in (b) before and after D-AP5 perfusion. The paired mean difference between the two conditions is -32% [95%CI -53%, -18%]. $P < 0.001$ (two-sided permutation t-test).

(d) Scaled time-integral of uEPSPs plotted against scaled integral arithmetic sum of asynchronous separate responses, before and after NMDAR blockade.

(e) Integral nonlinearity corresponding to panel (d). The paired mean difference between baseline and D-AP5 conditions is -60% [95%CI -90%, -40%]. $P < 0.001$ (two-sided permutation t-test).

(f) Somatic uEPSPs evoked by glutamate uncaging in one neuron in the presence of TTX (200 nM), showing supralinear uEPSP integration.

(g) Average scaled amplitude of the recorded near-synchronous uEPSP plotted against the arithmetic sum of the scaled asynchronous uEPSPs, as the cumulative number of uncaging locations was increased. Error bars: SEM (n=11 dendrites in 7 cells from 6 animals).

(h) Amplitude nonlinearity corresponding to (g). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 66% [95%CI 17%, 102%]. $P = 0.029$ (two-sided permutation t-test compared to 0%).

(i) uEPSP amplitude nonlinearity quantified by integral, plotted in the same way as (g).

(j) Integral nonlinearity corresponding to (i). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 74% [95%CI 1%, 148%]. $P = 0.089$ (two-sided permutation t-test compared to 0%).

(k) Somatic uEPSPs evoked by glutamate uncaging in one neuron in the presence of nimodipine (30 μ M), showing supralinear uEPSP integration.

(l) uEPSP amplitude nonlinearity, plotted in the same way as (g). (n=8 dendrites in 7 cells from 7 animals).

(m) Amplitude nonlinearity corresponding to (l). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 40% [95%CI 19%, 83%]. $P = 0.011$ (two-sided permutation t-test compared to 0%).

(n) uEPSP amplitude nonlinearity quantified by integral, plotted in the same way as (g).

(o) Integral nonlinearity corresponding to (n). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 97% [95%CI 27%, 263%]. $P = 0.066$ (two-sided permutation t-test compared to 0%).

(p) uEPSP amplitude nonlinearity quantified by integral in neurogliaform (NGF) and OLM interneurons (control and TTX experiments pooled). The mean difference between NGF and OLM integral nonlinearity is -63% [95%CI -132%, -8%]. $P=0.0424$ (two-sided permutation t-test).

(q) Dendritic diameter in NGF and OLM interneurons. The mean difference between NGF and OLM 0.36 [95%CI 0.20, 0.53]. $P<0.001$ (two-sided permutation t-test).

Slopegraphs and Gardner-Altman estimation plots as in [Fig. 2](#).

No effect of voltage-dependent sodium channel nor of L-type voltage-dependent calcium channel blockade

Neither sodium channel blockade ([Fig. 8f-j](#)), nor calcium channel blockade ([Fig. 8k-o](#)) prevented supralinear summation of uEPSPs. Depletion of intracellular calcium stores with CPA also had no effect ([Fig. S4](#)). The magnitude of supralinearity in the presence of either TTX or nimodipine was no different from the magnitude in the absence of blockers. The data thus point to a model where supralinearity is mediated exclusively by NMDARs, and occurs with uEPSPs that are subthreshold for dendritic calcium signalling.

The absence of sublinear summation in D-AP5 is reminiscent of the pattern observed in stratum oriens dendrites of PV+ interneurons ([16](#)), as is the relative magnitude of supralinearity. When neurogliaform and OLM data obtained in TTX were grouped together with the data without blockers, the supralinearity for voltage integral in OLM interneurons was significantly smaller than that in neurogliaform cells ([Fig. 8p](#)). Among possible explanations is that the local dendritic impedance is greater in neurogliaform cells, lowering the threshold for recruitment of regenerative currents. Consistent with such a pattern, the local dendrite diameter of neurogliaform interneurons was significantly lower than that of OLM cells ([Fig. 8q](#)). A morphological difference may thus contribute to the smaller voltage and calcium supralinearities in OLM than neurogliaform cells.

Discussion

The main results of the present study are that both hippocampal neurogliaform interneurons and OLM cells exhibit robust NMDAR-dependent supralinear integration of dendritic uEPSPs recorded at the soma. Supralinear NMDAR-dependent integration was also observed by measuring dendritic calcium fluorescence transients in neurogliaform cells, which were sensitive to blockade of L-type calcium channels or depletion of intracellular stores. However, near-synchronous glutamate uncaging that was sufficient to elicit NMDAR-dependent boosting of uEPSPs failed to recruit detectable dendritic calcium transients in OLM cells. In contrast to principal neurons, we observed no role for voltage-gated sodium channels in dendritic integration of uEPSPs in either cell type.

Taken together, the data are consistent with a model where clustered synaptic receptor activation leads to relief of voltage-dependent block of dendritic NMDARs by magnesium ions, accounting for supralinear summation of uEPSPs recorded at the soma. In neurogliaform cells (although apparently not in OLM cells) L-type voltage-gated calcium channels are also recruited by the local depolarization, and together with calcium influx via NMDARs, the resulting local elevation of intracellular calcium triggers further release from intracellular stores, thereby amplifying the dendritic calcium concentration transient. This account of the signalling cascade underlying local calcium signalling does not exclude additional roles for metabotropic glutamate receptors and calcium influx via rectifying calcium-permeable glutamate receptors, which have been implicated in dendritic calcium transients in oriens interneurons ([26](#)).

The dependence of supralinear dendritic summation on NMDARs but not voltage-gated sodium channels is reminiscent to the pattern observed at dendrites of parvalbumin-positive interneurons in stratum oriens (16 [↗](#)). NMDARs have previously been implicated in synaptic signalling in neurogliaform cells (56 [↗](#), 57 [↗](#), 66 [↗](#)) and also contribute to long-term potentiation (59 [↗](#)). Indeed, the ratio of NMDAR-to AMPAR-mediated signalling at glutamatergic synapses on neurogliaform cells has been shown to be higher than in other hippocampal interneuron subtypes (56 [↗](#)). This feature may explain why NMDAR-mediated supralinear integration is especially prominent in neurogliaform cells, although a further contribution may come from their unusually thin and short dendrites, which would be expected to represent a high impedance, thus facilitating their depolarization by glutamate receptor activation.

OLM cells exhibited several differences in comparison with neurogliaform cells. First, supralinear voltage integration was smaller. Second, blockade of NMDARs led to linear, as opposed to sublinear summation of uEPSPs. And third, uEPSPs evoked by glutamate uncaging, whether asynchronous or near-synchronous, were subthreshold for robust dendritic calcium transients. These differences can be explained by a relatively lower expression of NMDARs and/or lower dendritic impedance as expected from larger dendritic diameter and length. Indeed, we confirmed that the dendritic diameter at the uncaging locations was larger in OLM than neurogliaform cells. A further morphological difference is that the dendrites of OLM cells are equipped with sparse thin spines (67 [↗](#), 68 [↗](#)), although we did not visualize them. The density of both spines and NMDA receptors have also been reported to differ with distance from the soma in the dendrites of OLM cells (69 [↗](#), 70 [↗](#)). Whilst we saw no evidence suggesting sodium channel-dependent supralinear voltage integration in OLM cells, OLM cells have been reported to boost dendritic signals via active sodium conductances when increasing amounts of current were injected into the dendrites (67 [↗](#)). This difference may be due to the uncaging stimulation protocol remaining subthreshold for the initiation of sodium channel-dependent boosting, as current injection seemed to remain linear until EPSPs became greater than ~20 mV in amplitude (67 [↗](#)).

The present study highlights the ability of both neurogliaform and OLM neurons to perform local computations upstream of the site of axonal action potential initiation. The adaptive significance of this phenomenon remains to be determined. In principal neurons, synaptic inputs converging to common dendritic domains have been shown to exhibit correlated activity (71 [↗](#)–73 [↗](#)). Synaptically evoked calcium transients in dendritic domains of interneurons of the visual cortex have also been reported to exhibit orientation tuning that is not apparent in the global activity recorded at the soma (62 [↗](#)). The dendritic nonlinearities uncovered by the present study, together with different forms of synaptic plasticity described in interneurons (74 [↗](#)), are a candidate mechanism to achieve such an organization of information processing.

Material and methods

Animal and husbandry

Adult male and female mice of varying ages were throughout the study (ages 1–3 months). Transgenic mouse lines were maintained as heterozygotes. NDNF-cre^{+/+} or NDNF-cre^{+/-} mice (The Jackson Laboratory B6.Cg-Ndnf^{tm1.1(folA/cre)Hze}/J; Stock No: 028536; Bar Harbor, ME, USA), bred on a C57BL/6 background) were used to target neurogliaform interneurons (75 [↗](#)). SOM-cre^{+/+} (The Jackson Laboratory Sst^{tm2.1(cre)Zjh}/J; Stock No: 013044; Bar Harbor, ME, USA) crossed with Ai9^{+/+} mice (The Jackson Laboratory B6.Cg-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J; Stock No: 007909; Bar Harbor, ME, USA) were used to target OLM interneurons (61 [↗](#), 76 [↗](#)). Animals were group-housed under a normal 12 h light/dark cycle. Cages were enriched with paper bedding, cardboard tube, plastic tube, and wooden chewing blocks. Mice had had unlimited access to standard laboratory chow and water. All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Surgery for viral injection

Mice of at least 6 weeks of age were anaesthetised with isoflurane and placed into a stereotaxic frame, onto a heating pad to maintain body temperature. Mice were given Metacam (0.1 mg/kg) and buprenorphine (0.02 mg/kg) subcutaneously. Bilateral craniotomies were performed, positioned 3.1 mm caudal and ± 3.12 mm lateral of Bregma. Virus (AAV2/9-mDlx-FLEX-mCherry; titre $>10^{12}$ viral genomes/ml; VectorBuilder (Chicago, IL, USA)) was injected into the ventral CA1 region of both hippocampi using a Hamilton syringe 2.5 mm deep from the pia. 150 nL of virus was injected at each site at a rate of 100 nL/min. The needle was left in place for 5 min following injections before withdrawal. Mice were given 0.5 mL saline subcutaneously post-operatively to help with recovery and were monitored for 5 days following the procedure. Mice were sacrificed for experiments after a minimum of 3 weeks post-surgery.

Brain Slice preparation

Mice were sacrificed, the brains removed, and hippocampi dissected and sliced in ice-cold sucrose-based solution containing (in mM): 205 Sucrose, 10 Glucose, 26 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 5 MgSO₄, saturated with 95% O₂ and 5% CO₂. Transverse 400 μ m hippocampal slices were cut using a Leica VT1200S vibrating microtome. Slices were incubated in artificial cerebrospinal fluid (aCSF) at 35 °C for 30 min and then at room temperature for 30 min. aCSF contained (in mM): 124 NaCl, 3 KCl, 24 NaHCO₃, 1.25 NaH₂PO₄, 10 Glucose, 2.5 CaCl₂, 1.3 MgSO₄, saturated with 95% O₂ and 5% CO₂. The CA3 area was removed prior to slice transfer into the recording chamber to prevent recurrent activity.

Electrophysiology

The recording chamber was perfused with oxygenated aCSF maintained at 32 °C. Slices were first visualised using Dodt illumination on an Olympus FV1000 BX61 microscope. Fluorescent cells were identified either in the CA1 stratum lacunosum/moleculare (neurogliaform cells) or the stratum oriens (OLM cells) using Xcite epifluorescence. Borosilicate glass pipettes (pipette resistance of 4–5 M Ω) were pulled using a horizontal P2000 Sutter-instruments puller. The internal solution contained (in mM) 120 KMeSO₃, 8 NaCl, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 KCl, $\sim$ 295 mOsm, 7.4 pH. EGTA was not added to avoid introducing excess calcium buffering capacity. Internal solution aliquots were filtered on the day of experiment and small volumes of Alexa Fluor-594 (final concentration 50 μ M) and Fluo-4 (final concentration 400 μ M) solution aliquots were added. Recordings were obtained using a Multiclamp 700B Molecular Devices (USA) amplifier filtered at 10 kHz and digitized at 20 kHz (National Instruments PCI-6221, USA). WinWCP 5.5.6 (John Dempster, University of Strathclyde) software was used for data acquisition. After whole-cell break-in in V-clamp, the cells were switched to I=0 mode briefly to measure the resting membrane potential, before switching to I-clamp mode for experiments. All experiments were performed in I-clamp mode, with current continuously injected to maintain cell membrane between at $\sim$ –65 mV (0 to –50 pA). All data are presented without adjustment for the junction potential ($\sim$ –15 mV). Recordings were discarded if they had an access resistance >25 M Ω and if access or input resistance changed by $>20\%$ over the course of the experiment. All experiments were conducted in the presence of picrotoxin 50 μ M and CGP55845 1 μ M to block GABAergic transmission. D-AP5 (50 μ M), tetrodotoxin (TTX) (200 nM), nimodipine (30 μ M), cyclopiazonic acid (CPA) (30 μ M) were bath-applied to block NMDARs, voltage-activated sodium channels, L-type calcium channels, or to inhibit SERCA respectively. D-AP5 was applied over a period of 7 minutes following a control recording, whilst TTX, nimodipine, and CPA were present in the recording chamber throughout their respective experiments.

Two-photon imaging and uncaging

Simultaneous two-photon imaging and uncaging of MNI-caged glutamate were performed using two Ti-sapphire lasers tuned to 810 nm and 720 nm, for imaging and uncaging respectively (Mai-Tai, Spectra Physics, USA; Chameleon, Coherent, USA). MNI-caged-glutamate (3 mM; Hello Bio, UK) was added to the aCSF in a closed recirculating system upon patch-clamp break-in. 12 uncaging locations $\sim 2 \mu\text{m}$ apart and $< 1 \mu\text{m}$ away from the dendrite were chosen on either side of dendrites in pseudo-random patterns. 0.5 ms-long pulses of 720 nm light were used to uncage MNI-caged-glutamate. The uncaging light pulses were separated by either 100.32 ms or 0.32 ms in the control asynchronous (separate responses) or the near-synchronous protocols respectively. In the near-synchronous protocol, increasing numbers of uncaging locations were activated in a cumulative manner across sweeps 1 to 12. Inter-sweep interval was > 30 s, during which adjustments were made to account for any focal and x-y drift. Cycles of control asynchronous and near-synchronous stimulation protocols were done one after the other to produce mean responses, without changing the order of dendritic locations uncaged. Uncaging times and locations were controlled by scanning software (Fluoview 1000V) and a pulse generator (Berkeley Nucleonics, Ca, USA) with a Pockels cell. Calcium signals were acquired using 810 nm laser light, linescanning on the same dendritic branch as the uncaging and down-stream of the uncaging locations toward the soma. Cells were discarded upon observation of photodamage or upon change in resting membrane potential of > 10 mV. All z-stacks used for the analysis of morphology and example figures were captured at the end of experiments to maximise the amount of time for experiments before cell health deterioration. Photomultiplier tube filters used: 515–560 nm (Fluo-4); 590–650 nm (Alexa-594).

Quantification and statistical analyses

Amplitude and integral nonlinearity were regarded as the primary experimental outcomes.

Response nonlinearity was quantified using the following equation (16, 77).

$$\% \text{ nonlinearity} = \sum_{i=2}^n \frac{\frac{M_i}{A_i} - 1}{n - 1} * 100\%$$

Where M_i is the amplitude of the i th measured uEPSP (composed of i individual uncaging spots), A_i is the amplitude of the i th constructed arithmetically summed uEPSP, and n is the total number of uncaging locations.

Seeing as the calcium signal did not return to its pre-stimulation baseline in between flashes of uncaging laser light in the control asynchronous (separate responses) condition, calculating the arithmetic sum of individual responses was not possible. Therefore, to obtain an estimate of nonlinearity for calcium traces, values from linear interpolation were used. The first sweep of the test condition, where only a single location was stimulated using uncaging light, was treated as the first value of the control asynchronous (separate responses) condition for the purposes of interpolation. The second and final value used for interpolation was from the end of the control (separate responses) trace, so that the signal would encompass all 12 uncaging events.

Calcium signals were quantified using the following equation:

$$\Delta F / A$$

Where ΔF is the relative change in the Fluo-4 green channel fluorescence from baseline, and A is the Alexa-594 red channel fluorescence.

uEPSPs amplitude and integral measurements were scaled to facilitate visual comparisons across dendrites. This was performed by normalising the asynchronous (separate responses) measurements from 0 to 1 using the following equation:

$$z_i = \frac{x_i - \min(x)}{\max(x) - \min(x)}$$

Where x is the input array, and z_i is the normalised output element

The near-synchronous responses were scaled using the same formula and the minima and maxima from the corresponding asynchronous (separate responses) conditions.

Traces including action potentials were excluded from analysis. Asynchronous separate uEPSPs were staggered by 0.82 ms for arithmetic summation to replicate the near-synchronous uncaging condition. Voltage and calcium traces were filtered using a Savitzky-Golay filter. Uncaging light artefacts were removed from linescans. Due to the substantial variability in responses across dendrites, dendrites were used as the experimental unit. The numbers of dendrites, cells, and animals are reported in figure legends. $\alpha = 0.05$ was applied for all statistical tests. The degrees of freedom, t , and p values are presented in the figure legends and discussed in text, as appropriate. Estimation statistics (78 [↗](#)) and the generation of slopegraphs and Gardner-Altman estimation plots were performed using code provided by Ho et al. (79 [↗](#)). The slopegraphs indicate the difference in nonlinearity between asynchronous (separate responses) and the near-synchronous conditions or the differences in nonlinearity between pre- and post-drug administration. The adjacent paired mean difference plots are the group summaries. 95% confidence intervals of the mean difference were calculated by bootstrap resampling with 5000 resamples. The confidence interval is bias-corrected and accelerated. P values are provided with estimation statistics for legacy purposes only. Data were processed using custom Python code (80 [↗](#), 81 [↗](#)), Microsoft Excel, and WinWCP 5.5.6 (John Dempster, University of Strathclyde). Figures were created using custom Python code, Microsoft PowerPoint, Procreate, ImageJ.

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Additional information

Funding

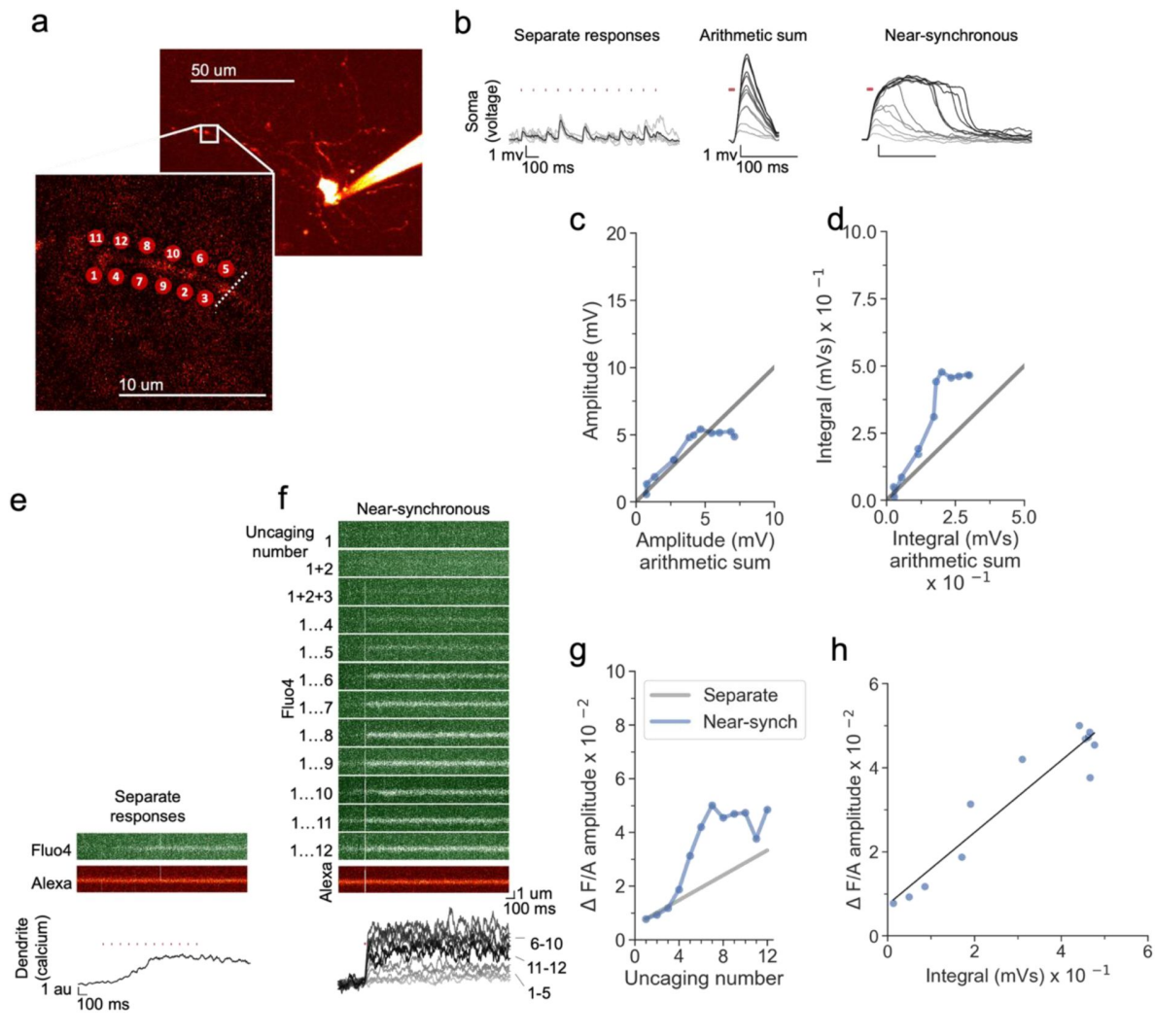
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Conflict of interest

The authors declare no competing interests.

Author contributions

Conceptualization, S.G and D.M.K; Methodology, S.G, A.R. and D.M.K; Software, S.G; Validation, S.G and D.M.K; Formal Analysis, S.G and D.M.K; Investigation, A.R. and S.G; Resources, D.M.K; Data Curation, S.G; Writing – Original Draft, S.G and D.M.K; Writing – Review & Editing, S.G, A.R. and D.M.K; Visualization, S.G and D.M.K; Supervision, D.M.K; Project Administration, S.G and D.M.K; Funding Acquisition, D.M.K.



Supplementary Fig. S1.

Comparison of uEPSP time-integral and dendritic calcium in a neurogliaform cell.

(a) Alexa-594 fluorescence Z-stack, with uncaging locations and linescan position (inset).

(b) Somatic voltage traces across asynchronous separate response and near-synchronous conditions, with times of uncaging stimuli indicated by red dots. In the asynchronous condition, individual traces are shown in grey, and the average shown in black. For the near-synchronous traces, light to dark traces indicate responses as the numbers of near-synchronous uncaging stimuli was increased from 1 to 12. The arithmetic sum traces are indicated in the same way.

(c) Peak amplitude of the recorded near-synchronous response plotted against the amplitude of the arithmetic sum for increasing numbers of uncaging locations.

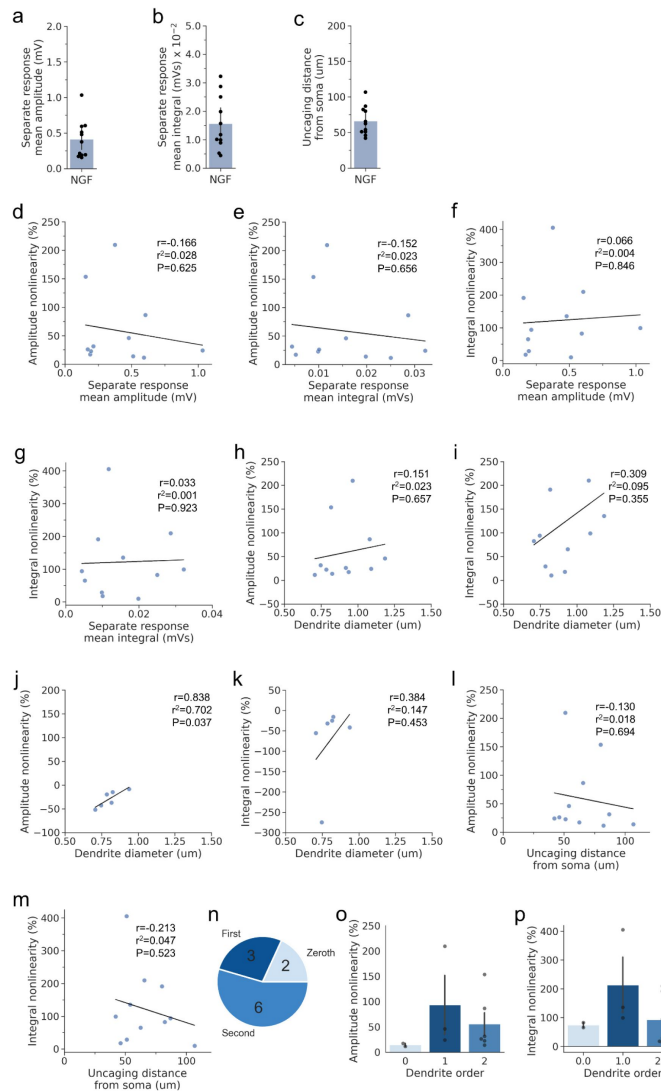
(d) Integral of the recorded near-synchronous response plotted against the integral of the arithmetic sum.

(e) Fluo-4 linescans in response to asynchronous stimulation. Locations 1 – 12 were sequentially stimulated at times indicated by red dots.

(f) Fluo-4 linescan in response to near-synchronous stimulation of increasing numbers of locations. The Fluo-4 fluorescence change from baseline was normalised to the fluorescence of the inert morphological dye Alexa-594 (the average linescan for Alexa-594 is shown beneath). Laser uncaging stimuli are indicated by red dots above the traces. Ratiometric calcium traces use arbitrary units (au).

(g) Fluo-4 fluorescence transient amplitude, normalised by Alexa-594 fluorescence, showing a deviation from the predicted response to asynchronous stimulation when more than 4 locations were uncaged near-synchronously. Grey line indicates the estimated asynchronous response amplitude using interpolated values (see methods).

(h) Fluo-4 fluorescence transient (normalised to Alexa-594 fluorescence) plotted against uEPSP integral.

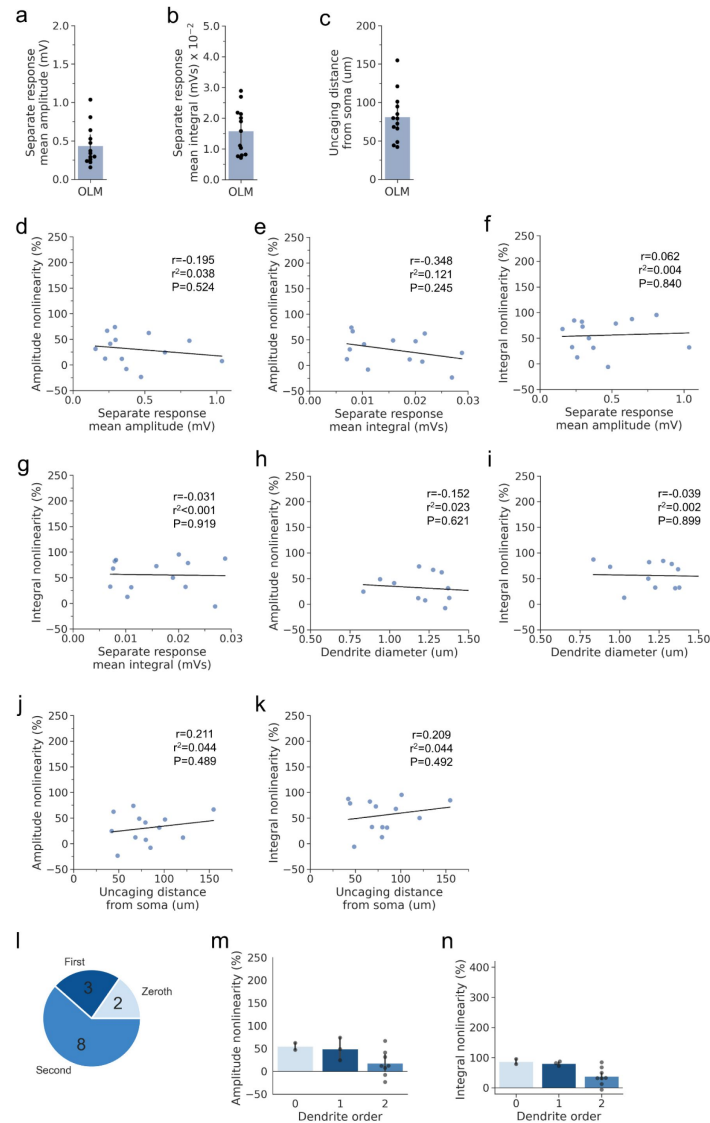


Supplementary Fig. S2.

Relationships among uncaging parameters and uEPSP nonlinearity (NGF).

- (a) Mean asynchronous separate response uEPSP amplitude.
- (b) Mean asynchronous separate response uEPSP integral.
- (c) Distance from the soma to the uncaging location along the dendrite.
- (d) Amplitude nonlinearity plotted against separate response mean amplitude.
- (e) Amplitude nonlinearity plotted against separate response mean integral.
- (f) Integral nonlinearity plotted against separate response mean amplitude.
- (g) Integral nonlinearity plotted against separate response mean integral.
- (h) Amplitude nonlinearity plotted against dendrite diameter.
- (i) Integral nonlinearity plotted against dendrite diameter.
- (j) Amplitude nonlinearity plotted against dendrite diameter (post D-AP5).
- (k) Integral nonlinearity plotted against dendrite diameter (post D-AP5).
- (l) Amplitude nonlinearity plotted against uncaging distance from soma.
- (m) Integral nonlinearity plotted against uncaging distance from soma.
- (n) Dendritic order for dendrites used for glutamate uncaging. The numbers in the pie chart represent the numbers of dendrites in the respective categories.
- (o) Amplitude nonlinearity split by dendrite order.
- (p) Integral nonlinearity split by dendrite order.

Linear regressions are shown as black lines. Pearson correlation parameters are given in the inset panels. Summary values are depicted as mean $\pm$ SEM. N = 11 dendrites in 11 cells from 9 animals.

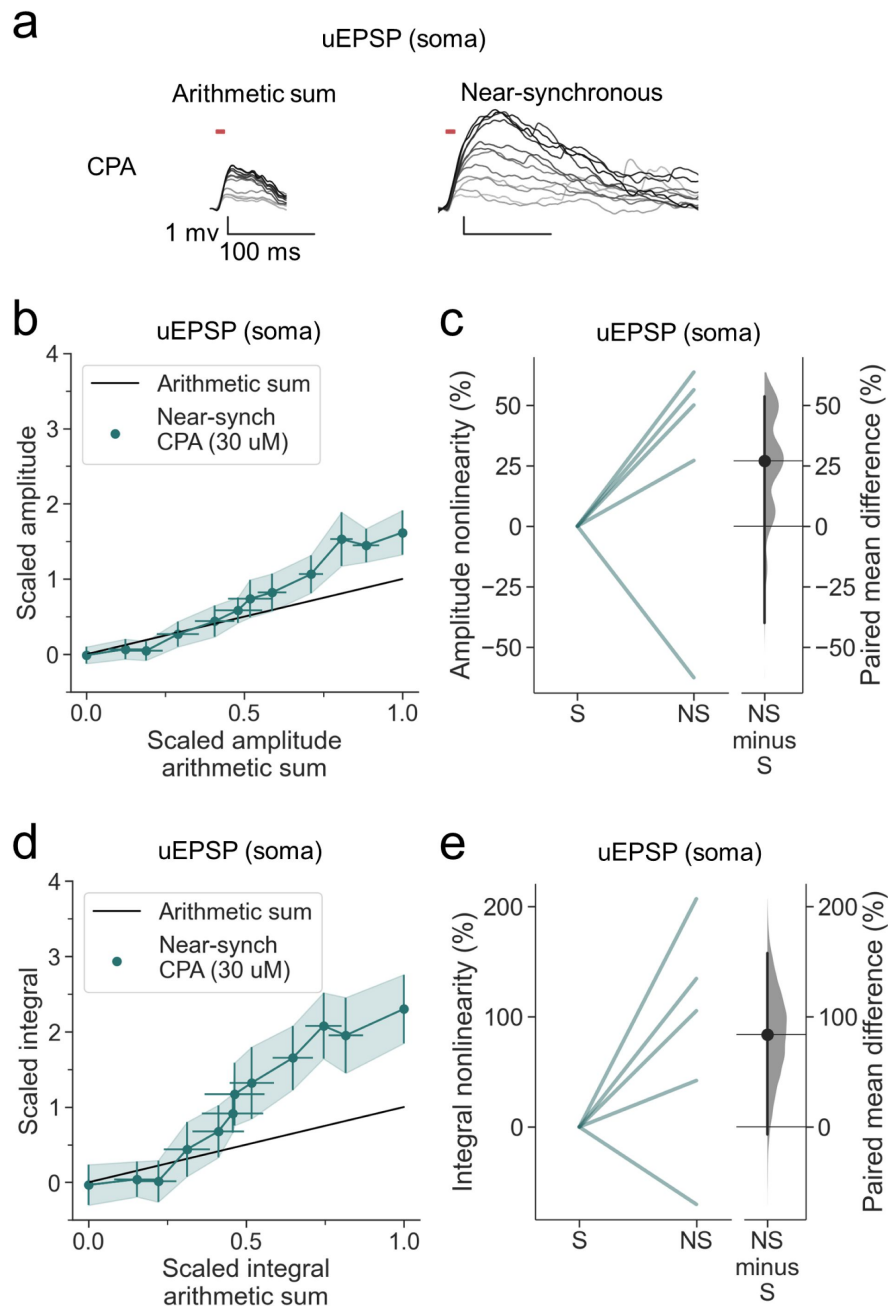


Supplementary Fig. S3.

Relationships among uncaging parameters and uEPSP nonlinearity (OLM).

- (a) Mean asynchronous separate response uEPSP amplitude.
- (b) Mean asynchronous separate response uEPSP integral.
- (c) Distance from the soma to the uncaging location along the dendrite.
- (d) Amplitude nonlinearity plotted against separate response mean amplitude.
- (e) Amplitude nonlinearity plotted against separate response mean integral.
- (f) Integral nonlinearity plotted against separate response mean amplitude.
- (g) Integral nonlinearity plotted against separate response mean integral.
- (h) Amplitude nonlinearity plotted against dendrite diameter.
- (i) Integral nonlinearity plotted against dendrite diameter.
- (j) Amplitude nonlinearity plotted against uncaging distance from soma.
- (k) Integral nonlinearity plotted against uncaging distance from soma.
- (l) Dendritic order for dendrites used for glutamate uncaging. The numbers in the pie chart represent the numbers of dendrites in the respective categories.
- (m) Amplitude nonlinearity split by dendrite order.
- (n) Integral nonlinearity split by dendrite order.

Linear regressions are shown as black lines. Pearson correlation parameters are given in the inset panels. Summary values are given as mean $\pm$ SEM. N = 13 dendrites, in 13 cells, from 13 animals.



Supplementary Fig. S4.

SERCA inhibition does not affect supralinear dendritic summation in OLM interneurons.

(a) Somatic uEPSPs evoked by glutamate uncaging in one neuron in the presence of CPA (30 uM), showing supralinear uEPSP integration.

(b) Average scaled amplitude of the recorded near-synchronous uEPSP plotted against the arithmetic sum of the scaled asynchronous uEPSPs, as the cumulative number of uncaging locations was increased. Error bars show SEM (n=5 dendrites in 4 cells from 4 animals).

(c) Amplitude nonlinearity corresponding to (b). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 27% [95%CI -40%, 54%]. $P = 0.239$ (two-sided permutation t-test compared to 0%).

(d) uEPSP amplitude nonlinearity quantified by integral, plotted in the same way as (b).

(e) Integral nonlinearity corresponding to (g). The paired mean difference between near-synchronous (NS) and asynchronous separate responses (S) is 84% [95%CI -7%, 158%]. $P = 0.123$ (two-sided permutation t-test compared to 0%).

Slopegraphs and Gardner-Altman estimation plots as in **Fig. 2** [↗](#)

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Reviewer #2 (Public review):

Summary:

Griesius et al. investigate the dendritic integration properties of two types of inhibitory interneurons in the hippocampus: those that express NDNF+ and those that express somatostatin. They found that both neurons showed supralinear synaptic integration in the dendrites, blocked by NMDA receptor blockers but not by blockers of Na⁺ channels. These experiments are critically overdue and very important because knowing how inhibitory neurons are engaged by excitatory synaptic input has important implications for all theories involving these inhibitory neurons.

Comments on revisions:

The authors have addressed the reviewers' comments, but haven't resolved most of the key issues.

Specifically, performing only a single uncaging experiment at a single dendritic location per cell prevents a detailed biophysical analysis of NDNF and OLM cell integration properties. A more extended exploration would have potentially addressed several of the reviewers' questions. It is particularly worrying that the authors cite cell health, dendritic blebbing, and changes in input resistance as the reason for terminating experiments after a single uncaging event. This suggests that the uncaging laser may be damaging the dendrite, potentially affecting the membrane potential directly, and overall cell health, beyond simply uncaging glutamate.

While the authors' qualitative conclusions about supra-linear integration and NMDA receptor dependency seem plausible, the limited data and potential methodological issues weaken any quantitative interpretations and comparisons between the two cell types.

Similarly, the absence of dendritic Na-spikes remains unexplained, despite reports of strong dendritic Na-currents in these cells.

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Reviewer #3 (Public review):

Summary:

The authors study temporal summation of caged EPSPs in dendrite-targeting hippocampal CA1 interneurons. The data indicate non-linear summation, which is larger in dendrites of NDNF-expressing neurogliaform cells versus OLM cells. However, the underlying mechanisms are largely unclear.

Strengths:

Synaptic integration in dendrites of cortical GABAergic interneurons is important and still poorly investigated. Focal 2-photon uncaging of glutamate is a nice and detailed method to study temporal summation of small potentials in dendritic segments. 2P calcium imaging is a powerful method to potentially disentangle dendritic signal processing in interneuron dendrites.

Weaknesses:

Due to several experimental limitations of the study including a relatively low number of recorded dendrites, lack of voltage-clamp recordings, lack of NMDA-dependent calcium signals in OLM cells and lack of wash-out during pharmacological experiments (AP5-application), the mechanistic insights are limited.

(1) NMDA-receptor signalling in NDNF-IN. The authors nicely show that temporal summation in dendrites of NDNF-INs is to a certain extent non-linear. Pharmacology with AP5 hints towards contribution of NMDA receptors. However, the authors report that the non-linearity is not significantly dependent on EPSP amplitude (Fig. S2), which should be the case if NMDA-receptors are involved. Unfortunately, there are no voltage-clamp data showing NMDA and AMPA currents, potentially providing a mechanistic explanation for the non-linear summation.

(2) Recovery of drug effect. Pharmacological application of AP5 is the only argument for the involvement of NMDA receptors. However, as long-lasting experiments were apparently difficult to obtain, there is no washout-data presented - only drug effect versus baseline. For all the other drugs (TTX, Nimodipine, CPA) recordings were even shorter, lacking a baseline recording. Thus, it remains open to what extent the AP5-effect might be affected by rundown of receptors or channels during whole-cell recordings or beginning phototoxicity.

(3) Nonlinear EPSP summation in OLM-IN. The authors do similar experiments in dendrite-targeting OLM-INs and show that the non-linear summation is smaller than in NDNF cells. The reason for this remains unclear. The diameter of proximal dendrites in OLM cells is larger than the diameter in NDNF cells. However, there is probably also an important role of synapse density and glutamate receptor density, which was shown to be very low in proximal dendrites of OLM cells and strongly increase with distance (Guirado et al. 2014, Cerebral Cortex 24:3014-24, Gramuntell et al. 2021, Front Aging Neurosci 13:782737). Therefore, it would have been helpful to see experiments quantifying synapse density (counting spines, PSD95-puncta, ...) and show how this density compares with non-linearity in the analyzed NDNF and OLM dendrites.

(4) NMDA in OLM-IN. Similar to the NDNF cells, the authors argue for an involvement of NMDA receptors in OLM cells, based on bath-application of AP5 (Fig. 8). Again, there seems to be no significant dependence on EPSP amplitude (Fig. S3). Even more remarkable, the authors claim that there is no dendritic calcium increase after activation of NMDA receptors without showing data. Therefore, it remains unclear whether the calcium signals are just below detection threshold, or whether the non-linearity depends on other calcium-

impermeable channels and receptors. To understand this phenomenon different calcium sensors, different $\text{Ca}^{2+}/\text{Mg}^{2+}$ concentrations or voltage-clamp data would have helped.

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Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public Review):

The manuscript by Griesius et al. addresses the dendritic integration of synaptic input in cortical GABAergic interneurons (INs). Dendritic properties, passive and active, of principal cells have been extensively characterized, but much less is known about the dendrites of INs. The limited information is particularly relevant in view of the high morphological and physiological diversity of IN types. The few studies that investigated IN dendrites focused on parvalbumin-expressing INs. In fact, in a previous study, the authors examined dendritic properties of PV INs, and found supralinear dendritic integration in basal, but not in apical dendrites (Cornford et al., 2019 eLife).

In the present study, complementary to the prior work, the authors investigate whether dendrite-targeting IN types, NDNF-expressing neurogliaform cells, and somatostatin(SOM)-expressing O-LM neurons, display similar active integrative properties by combining clustered glutamate-uncaging and pharmacological manipulations with electrophysiological recording and calcium imaging from genetically identified IN types in mouse acute hippocampal slices.

The main findings are that NDNF IN dendrites show strong supralinear summation of spatially- and temporally-clustered EPSPs, which is changed into sublinear behavior by bath application of NMDA receptor antagonists, but not by Na^{+} -channel blockers. L-type calcium channel blockers abolished the supralinear behavior associated calcium transients but had no or only weak effect on EPSP summation. SOM IN dendrites showed similar, albeit weaker NMDA-dependent supralinear summation, but no supralinear calcium transients were detected in these INs. In summary, the study demonstrates that different IN types are endowed with active dendritic integrative mechanisms, but show qualitative and quantitative divergence in these mechanisms.

While the research is conceptionally not novel, it constitutes an important incremental gain in our understanding of the functional diversity of GABAergic INs. In view of the central roles of IN types in network dynamics and information processing in the cortex, results and conclusions are of interest to the broader neuroscience community.

The experiments are well designed, and closely follow the approach from the previous publication in parts, enabling direct comparison of the results obtained from the different IN types. The data is convincing and the conclusions are well-supported, and the manuscript is very well-written.

I see only a few open questions and some inconsistencies in the presentation of the data in the figures (see details below).

We thank the reviewer for the evaluation and address the detailed points below.

Reviewer #2 (Public review):

Summary:

Griesius et al. investigate the dendritic integration properties of two types of inhibitory interneurons in the hippocampus: those that express NDNF+ and those that express somatostatin. They found that both neurons showed supralinear synaptic integration in the dendrites, blocked by NMDA receptor blockers but not by blockers of Na⁺ channels. These experiments are critically overdue and very important because knowing how inhibitory neurons are engaged by excitatory synaptic input has important implications for all theories involving these inhibitory neurons.

Strengths:

(1) Determined the dendritic integration properties of two fundamental types of inhibitory interneurons.

(2) Convincing demonstration that supra-threshold integration in both cell types depends on NMDA receptors but not on Na⁺ channels.

Weaknesses:

It is unknown whether highly clustered synaptic input, as used in this study (and several previous studies), occurs physiologically.

We are grateful to the reviewer for the critique. Indeed, the degree to which clustered inputs belonging to a functional neuronal assembly occur on interneuron dendrites is an open question. However, Chen et al (2013, Nature 499:295-300) reported that dendritic domains of PV-positive interneurons in visual cortex, unlike their somata, exhibit calcium transients in vivo which are highly tuned to stimulus orientation. This suggests that clustered inputs to dendritic segments may well belong to functional assemblies, much as in principal cells (e.g. Wilson et al, 2016, Nature Neuroscience 19:1003–1009; Iacaruso et al, 2017, Nature 547:449–452). In our earlier work reporting NMDAR-dependent supralinear summation of glutamate uncaging-evoked responses at a subset of dendrites on PV-positive interneurons, we demonstrated how this arrangement in an oscillating feedback circuit could be exploited to stabilise neuronal assemblies.

Reviewer #3 (Public review):

Summary:

The authors study the temporal summation of caged EPSPs in dendrite-targeting hippocampal CA1 interneurons. There are some descriptive data presented, indicating non-linear summation, which seems to be larger in dendrites of NDNF expressing neurogliaform cells versus OLM cells. However, the underlying mechanisms are largely unclear.

Strengths:

Focal 2-photon uncaging of glutamate is a nice and detailed method to study temporal summation of small potentials in dendritic segments.

Weaknesses:

(1) NMDA-receptor signaling in NDNF-IN. The authors nicely show that temporal summation in dendrites of NDNF-INS is to a certain extent non-linear. However, this non-linearity varies massively from cell to cell (or dendrite to dendrite) from 0% up to 400%

(Figure S2). The reason for this variability is totally unclear. Pharmacology with AP5 hints towards a contribution of NMDA receptors. However, the authors claim that the non-linearity is not dependent on EPSP amplitude (Figure S2), which should be the case if NMDA-receptors are involved. Unfortunately, there are no voltage-clamp data of NMDA currents similar to the previous study. This would help to see whether NMDA-receptor contribution varies from synapse to synapse to generate the observed variability? Furthermore, the NMDA- and AMPA-currents would help to compare NDNF with the previously characterized PV cells and would help to contribute to our understanding of interneuron function.

We thank the reviewer for the helpful comments.

We did not actually claim that EPSP amplitude has no role in determining the magnitude of non-linearity: “Among possible sources of variability for voltage supralinearity, we did not observe a systematic dependence on the average amplitude of individual uEPSPs [...] (Fig. S2)”. Whilst we fully agree that, at first sight, a positive dependence of supralinearity on uEPSP amplitude might be expected simply from the voltage-dependent kinetics of NMDARs, there are two main reasons why this could have been obscured. First, the expected relationship is non-monotonic, because with large local depolarizations the driving force collapses, as seen in the overall sigmoid shape of the average relationship between the scaled observed response and arithmetic sum (e.g. Figs 2a & c; 4c & e). Therefore, we would arguably expect a parabolic relationship rather than a simple positive slope relating the degree of supralinearity to the average amplitude of individual uEPSPs. Second, given that the uncaging distance varied substantially, the average amplitudes of the individual uEPSPs recorded at the soma would have undergone different degrees of electrotonic attenuation and further distortion by active conductances before they were measured. Ultimately, the plots in Fig. S2 show too much scatter to be able to exclude a positive or parabolic relationship of nonlinearity to uEPSP amplitude. To avoid misunderstanding, we have changed the sentence in the Results that refers to Fig. S2 to: “Among possible sources of variability for voltage supralinearity, we did not observe a significant monotonic dependence on the average amplitude of individual uEPSPs, distance from the uncaging location along the dendrite to the soma, [or] the dendrite order (Fig. S2)”.

As for the relative contributions of NMDARs and AMPARs, voltage clamp recordings from both neurogliaform and OLM interneurons have already been reported, with the conclusion that neurogliaform cells exhibit relatively larger NMDAR-mediated currents (e.g. Chittajallu et al. 2017; Booker et al. 2021; Mercier et al. 2022), entirely in keeping with the conclusions of our study. Repeating these measurements would add little to the study. Furthermore, because the mean baseline uEPSP amplitude was <0.5 mV (Fig S2), it would be difficult to obtain reliable measurements of isolated NMDAR-mediated uEPSCs.

Turning to the high variability of supralinearity, indeed, the 95% confidence interval for the data in Fig. 2d is 73%, 213%. This degree of variability is consistent with the wide range of NMDAR/AMPA ratios reported by Chittajallu et al. 2017 (their Fig. 1g), compounded by the expected non-monotonic relationship alluded to above.

(2) Sublinear summation in NDNF-INS. In the presence of AP5, the temporal summation of caged EPSPs is sublinear. That is potentially interesting. The authors claim that this might be dependent on the diameter of dendrites. Many voltage-gated channels can mediate such things as well. To conclude the contribution of dendritic diameter, it would be helpful to at least plot the extent of sublinearity in single NDNF dendrites versus the dendritic diameter. Otherwise, this statement should be deleted.

We have plotted the degree of nonlinearity against dendritic diameter for neurogliaform cells (under baseline conditions and in D-AP5) in Fig S2h-k. We did not observe any significant

linear correlations, other than between amplitude nonlinearity and dendrite diameter post D-AP5. This does not negate the possibility that the significant difference in average dendritic diameters between neurogliaform and OLM cells contributes to differences in impedance (which we have rephrased as “Among possible explanations is that the local dendritic impedance is greater in neurogliaform cells, lowering the threshold for recruitment of regenerative currents”).

(3) Nonlinear EPSP summation in OLM-IN. The authors do similar experiments in dendrite-targeting OLM-INs and show that the non-linear summation is smaller than in NDNF cells. The reason for this remains unclear. The authors claim that this is due to the larger dendritic diameter in OLM cells. However, there is no analysis. The minimum would be to correlate non-linearity with dendritic diameter in OLM-cells. Very likely there is an important role of synapse density and glutamate receptor density, which was shown to be very low in proximal dendrites of OLM cells and strongly increase with distance (Guirado et al. 2014, Cerebral Cortex 24:3014-24, Gramuntell et al. 2021, Front Aging Neurosci 13:782737). Therefore, the authors should perform a set of experiments in more distal dendrites of OLM cells with diameters similar to the diameters of the NDNF cells. Even better would be if the authors would quantify synapse density by counting spines and show how this density compares with non-linearity in the analyzed NDNF and OLM dendrites.

The difference in average dendritic diameters between OLM and neurogliaform cells is highly significant (Fig. 8q, $P < 0.001$). We do not claim that dendritic diameter (and by implication local impedance) is the only determinant of the degree of non-linearity. The suggestion that a gradient of glutamate receptor density contributes is interesting. However, the results of uncaging experiments targeting more distal OLM dendrites of similar diameter as neurogliaform dendrites would be subject to numerous confounds, not least the very different electrotonic attenuation, likely differences in various active conductances, and the presence of spines in OLM dendrites (which are generally sparse and were not reliably imaged in our experiments). Moreover, the cell would have to remain patched for longer in order for the fluorescent dyes to invade the distal dendrites. This alone could potentially result in systematic biases among groups. We now cite Guirado et al (2014) and Gramuntell et al (2021) to highlight that factors other than dendritic diameter per se, such as inhomogeneity in spine and NMDA receptor density may also contribute to the heterogeneity of nonlinear summation in OLM cells.

(4) NMDA in OLM. Similar to the NDNF cells, the authors claim the involvement of NMDA receptors in OLM cells. Again there seems to be no dependence on EPSP amplitude, which is not understandable at this point (Figure S3). Even more remarkable is the fact that the authors claim that there is no dendritic calcium increase after activation of NMDA receptors. Similar to NDNF-cell analysis there are no NMDA currents in OLMs. Unfortunately, even no calcium imaging experiments were shown. Why? Are there calcium-impermeable NMDA receptors in OLM cells? To understand this phenomenon the minimum is to show some physiological signature of NMDA-receptors, for example, voltage-clamp currents. Furthermore, it would be helpful to systematically vary stimulus intensity to see some calcium signals with larger stimulation. In case there is still no calcium signal, it would be helpful to measure reversal potentials with different ion compositions to characterize the potentially 'Ca²⁺ impermeable' voltage-dependent NMDA receptors in OLM cells.

The same response to point 1) above applies to OLM cells. As with neurogliaform cells, mean OLM baseline asynchronous (separate response) amplitudes were < 0.5 mV, making it very difficult to record an isolated NMDAR-mediated uEPSC. Having said that, NMDARs do contribute to EPSCs elicited by stimulation of multiple afferents (e.g. Booker et al, 2021). We

do not claim that dendritic calcium transients cannot be elicited following activation of NMDARs in OLM cells. We simply reported that the evoked uEPSPs, designed to approximate individual synaptic signals, were sub-threshold for detectable dendritic calcium signals under conditions that were suprathreshold in neurogliaform cells. The statement has been amended to specify that there were no detectable signals under our recording conditions. There is no evidence presented in the manuscript to suggest that OLM NMDARs are calcium impermeable and indeed no such claim was made.

Recommendations for the authors:

Reviewer #1 (Recommendations for the authors):

There is a large variability in the observed dendritic nonlinearity, in NDNF IN dendrites e.g. the uEPSP amplitude nonlinearity measure varies from as low as 10-20% to over 200%. As only single dendrites were recorded from each IN, it is unclear if this variability is among the cells or between individual dendrites. While the authors analyzed some potential factors, such as distance along the dendrites, branch order, or response magnitude (amplitude and integral), they did not find any substantial correlation. It remains open if different dendrites of NDNF INs, located in the str. moleculare vs. those in or projecting towards str. radiatum, have divergent properties. Similarly, for SOM INs an important question is if axon-carrying dendrites show distinct properties.

In this context, it would be interesting to see not only values for the mean nonlinearity but also the maximal nonlinearity and its distribution.

Nonlinearity as defined in the manuscript is a cumulative measurement. The final value per dendritic segment is therefore the sum of nonlinearities at 1 to 12 near-synchronous uncaging locations. The data for the individual dendritic segments are shown in the slopegraphs as in Fig 2b, with their distribution visible. The averages referred to in the results correspond to the paired mean difference plots, which are the group summaries. The method section has been amended to clarify the analysis method. We did not address specifically whether dendrites projecting in different directions behaved differently. This is an interesting question beyond the scope of this study. Nor did we compare axon-carrying OLM dendrites to other dendrites.

Figures:

Figure 1: The gray line in plots g and h is not explained. While it looks like an identity line, the legend in plot i ("asynchronous") interferes.

In plots g and h the gray line is the line of identity. In plot i it is an estimate of the linear summation. In plot i it is not the line of identity as it does not start at the origin with a slope of 1. The figure legend has been amended to clarify.

In the same panels (Figure 1g,h, and subsequent figures) consider changing the title from "soma (voltage)" to uEPSP.

The titles have been amended.

In panel Figure 1i note the missing "(" in the title.

Title amended.

In panel Figure 1h: Shouldn't the X-axis label and legend text read "Arithmetic sum of (EPSP) integrals" instead of "Integral of arithmetic sum".

The wording more accurately reflects the analytical operations. The asynchronous (separate) responses were summed arithmetically first, and then the integral was taken of each cumulative sum. We have therefore left the axis title and legend unchanged.

Figure 2a,c: Could you please describe how the scaling was performed for the two axes?

Method section amended.

In the same panels (Figure 2a,c, and subsequent figures), the legend seems to be misleading: the plot is NS Amplitude/Integral vs Arithmetic sum, and the black line is the identity line (or scaled interpolation of the arithmetic sum, which is essentially the same).

The scaled arithmetic sums (uEPSP amplitude, integral) represent linear summation and so overlap with the line of identity. The interpolation estimate of the asynchronous (separate) calcium transient response does not overlap with the line of identity as this estimate does not start at the origin with a slope of 1. The legends throughout the manuscript have been amended to clarify this.

Figure 2b,d,f (and subsequent figures) slope plots: Please indicate that this is the average amplitude supralinearity for the individual recorded dendrites. Note here that the Results text mentions only the average amplitude supralinearity, but not the slope plots, paired mean difference, or Gardner-Altman estimation, illustrated in the figures.

Nonlinearity as defined in the manuscript is a cumulative measurement. The final value per dendritic segment is therefore the sum of nonlinearities at 1 to 12 near-synchronous uncaging locations. The data for the individual dendritic segments are shown in the slopegraphs as in fig 2b, with their distribution visible. The averages referred to in the results correspond to the paired mean difference plots, which are the group summaries. The method section has been amended to clarify.

Fig 2e: The legend (both text and figure, also in the following figures) is confusing, as the gray line and diamonds are defined as separate 12(?) responses, but it seems to represent a linear interpolation of the scaled arithmetic sums (ultimately nothing else but an identity line).

The grey line shows the linear interpolation output between the calcium transient measurements at 1 uncaging location and at 12 uncaging locations. The 12th uncaging location is indicated in the key as “separate 12”. The linear interpolation in these plots does represent linear summation but is not the line of identity as it does not begin at the origin and does not have a slope of 1.

Reviewer #2 (Recommendations for the authors):

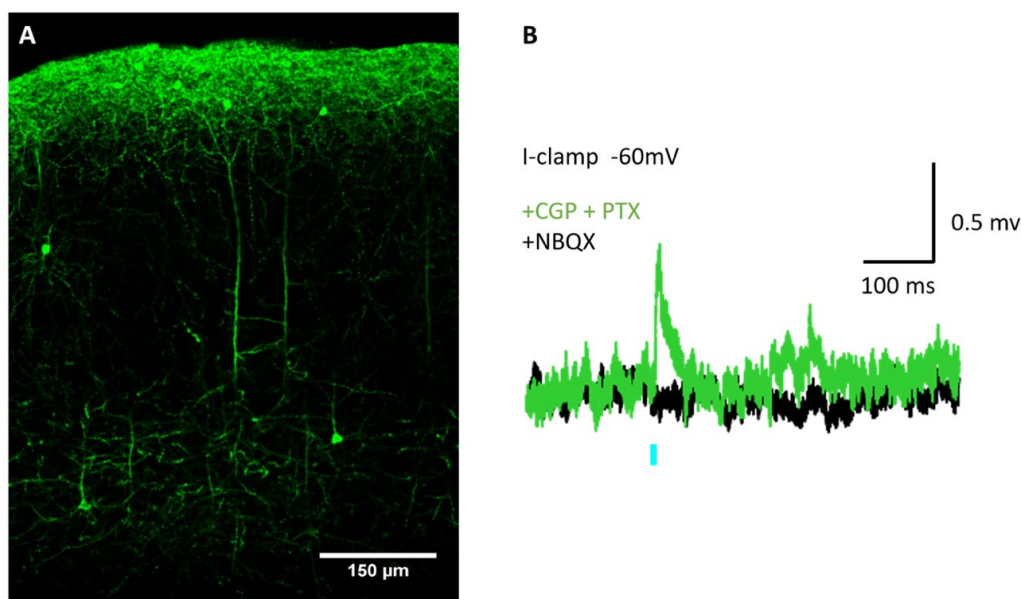
This study is well-developed and technically executed. I only have minor comments for the authors:

(1) To target NDNF+ neurons, the authors use the NDNF-Cre mouse line and a Cre-dependent AAV using the mDLX promotor. Why the mDLX promotor? Would it have been sufficient to use any Cre-dependent fluorophore?

Pilot experiments revealed leaky expression when a virus driving flexed ChR2 under a non-specific promoter (EF1a) was injected in the neocortex of Ndnf-Cre mice (Author response image 1). In our hands, and in line with Dimidschtein et al (2016), the use of the mDLX enhancer reduced off-target expression.

Author response image 1.

A. AAV2/5-EF1a-DIO-hChR2(H134R)-mCherry injected into superficial neocortex of Ndnf-Cre mice led to expression in a few pyramidal neurons in addition to layer 1 neurogliaform cells. B. Patch-clamp recording from a non-labelled pyramidal cell showed that an optogenetically evoked glutamatergic current remained after blockade of GABAA and GABAB receptors, further confirming limited specificity of expression of ChR2. (Data from M Muller, M Mercier and V Magloire, Kullmann lab.)



(2) The distance of the uncaging sites from the soma plays a key role. The authors should indicate the mean distance of the cluster and its variance.

Uncaging distance from soma is indicated for both NGF and OLM interneurons in the supplementary figures S2 and S3 respectively.

(3) Martina et al., in Science 2000, showed high levels of Na⁺ channels in the dendrites of OLM cells and hinted that spikes could occur in them. The authors should discuss this possible discrepancy.

Discussion amended.

(4) Looking at Figure 1d, the EPSPs look exceptionally long-lasting, longer than those observed by stimulating axonal inputs. Could this indicate spill-over excitation? If so, how could this affect the outcome of this study?

The asynchronous (separate responses) decay to baseline within 100 ms, similar to the neurogliaform EPSPs evoked by electrical stimulation of axons in the SLM in Mercier et al. 2022. We observed clear plateau potentials in a minority of cells (e.g. Fig. S1b). Such plateau potentials can be generated by dendritic calcium channels and we do not consider that glutamate spillover needs to be invoked to account for them.

(5) In the legend of Figure 2: "n=11 dendrites in 11 cells from 9 animals". Why do the authors only study 11 dendrites from 11 cells? Isn't it possible to repeatedly stimulate clusters of synaptic inputs onto the same cells? In principle, could one test many dendrites of the same cell at different distances from the soma? It is also remarkable that there were very few cells per animal.

The goal always was to record from as many dendrites as possible from the same cells whilst maintaining high standards of cell health. When cell health indicators such as blebbing, input resistance change or resting voltage change were detected, no further dendritic location could be tested with reasonable confidence. In a given 400 μm slice there would be relatively few healthy candidate cells at a suitable depth to attempt to patch-clamp.

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