Analogue modulation of back-propagating action potentials enables dendritic hybrid signalling

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We report that back-propagating action potentials (bAPs) are not simply digital feedback signals in dendrites but also carry analogue information about the overall state of neurons. Analogue information about the somatic membrane potential within a physiological range (from −78 to −64 mV) is retained by bAPs of dentate gyrus granule cells as different repolarization speeds in proximal dendrites and as different peak amplitudes in distal regions. These location-dependent waveform changes are reflected by local calcium influx, leading to proximal enhancement and distal attenuation during somatic hyperpolarization. The functional link between these retention and readout mechanisms of the analogue content of bAPs critically depends on high-voltage-activated, inactivating calcium channels. The hybrid bAP and calcium mechanisms report the phase of physiological somatic voltage fluctuations and modulate long-term synaptic plasticity in distal dendrites. Thus, bAPs are hybrid signals that relay somatic analogue information, which is detected by the dendrites in a location-dependent manner.
A
ction potentials (APs), the digital signals of neurons, provide essential functions by converting incoming inputs to neuronal outputs along the dendro–somatic–axonal axis. In addition to this straightforward direction, back-propagating action potentials (bAPs) carry a digital feedback to the synaptic input zone regarding the output activity of the neuron and participate in the integration, modulation and maintenance of synaptic inputs. In contrast to APs, the somatic membrane potential is an analogue signal that dynamically reflects the overall input activity. However, in addition to the conventional analogue–digital–analogue conversion, recent studies in mammalian central neurons have demonstrated that analogue signals directly modulate the function of axonal APs, which enables them to act as hybrid signals. Specifically, the axonal membrane potential modulates the efficacy of individual APs in evoking postsynaptic responses in an analogue manner. Thus, the primary digital information (that is, presynaptic activity) is preserved, but the analogue content (that is, membrane potential) modifies the weight of the hybrid signal allowing for more information to be contained in single APs compared with conventional digital signalling. However, it is not known whether bAPs in dendrites are also capable of hybrid signalling.

At dendrites, local voltage changes modulate the properties and impact of bAPs, for example, by affecting the relief of Mg2+ block from N-methyl-D-aspartate (NMDA) receptors, which enables the spatially precise long-term synaptic plasticity. However, these voltage changes do not spread into the entire 3. dendritic tree because of the restricted propagation along the long dendritic cables. In contrast, the actual somatic voltage, which reflects the summed activity of inputs onto the complete dendritic tree, globally contributes to the dendritic voltage because of the physical and electrotonic dominance of the somatic membrane over the dendrites. Such asymmetric propagation potentially enables general somato-dendritic hybrid signalling and it is pronounced in dentate asymmetric propagation potentially enables general somatic voltage, which reflects the summed activity of propagation along the long dendritic cables. In contrast, the spread into the entire dendritic tree because of the restricted membrane potentials. In contrast to proximal dendrites, the active components at distal locations were not sufficient to compensate for the initial membrane potential difference; thus, the bAPs from depolarized membrane potentials reached lower peak voltages than the bAPs from hyperpolarized membrane potentials. In contrast to proximal dendrites, the active components at distal locations were not sufficient to compensate for the initial membrane potential difference; thus, the bAPs from depolarized membrane potentials reached lower peak voltages than the bAPs from hyperpolarized membrane potentials.

**Results**

**Dual effects of somatic potential on dendritic bAP waveforms.** To determine whether bAPs retain analogue information about the somatic membrane potential, we simultaneously recorded single bAPs from the dendrites and soma of GCs. During these experiments, the thin dendrites were patched first under infrared differential interference contrast (IR-DIC) optics. The pipettes contained Alexa Fluor 594 dye for the visualization and patching the parent soma (Fig. 1c). Only the somatic pipette was used for current injection to control physiologically plausible depolarized (−64.6 ± 0.6 mV) or hyperpolarized (−77.2 ± 0.6 mV) membrane potentials and to evoke single action potentials. Because of the reliable voltage monitoring (Supplementary Fig. 1) and interleaved sampling of the two conditions these high series resistance recordings allowed the direct comparison of bAP shapes. First, we confirmed previous observations that somatic steady-state voltage spreads effectively into the dendrites of GCs, and that the peak amplitudes of the bAPs decrease toward the tips of the dendrites (Fig. 1b; Supplementary Fig. 2). To directly compare bAP waveforms at two physiologically relevant membrane potentials, the elicited bAP pairs were aligned to the maximum rate of rise (Fig. 1c). The apparent threshold, rising phase and absolute peak voltage of the bAPs in the proximal locations were unchanged regardless of the preceding membrane potential, which further confirms the reliability of the recording conditions. However, there was a transient voltage difference between the depolarized and hyperpolarized bAPs during their repolarization phase (Fig. 1c–e). The proximal bAPs repolarized faster when they were evoked from hyperpolarized membrane potentials. In contrast to proximal dendrites, the active components at distal locations were not sufficient to compensate for the initial membrane potential difference; thus, the bAPs from hyperpolarized membrane potentials reached lower peak voltages than the bAPs from depolarized membrane potentials.

**Bidirectional effects of somatic potential on calcium signals.** Do these bAP waveform changes have any functional consequence in the dendrites? One of the most important functions of bAPs is to evoke local calcium influx; the calcium ions that enter are crucial in various mechanisms, including synaptic plasticity, local excitability and cellular metabolism.

Therefore, to determine whether and how somatic membrane potential is reflected by bAP-evoked local calcium influx, we used conventional confocal imaging (Supplementary Fig. 3) along dendrites with slightly depolarized (−64.0 ± 0.3 mV by 16.5 ± 3.1 pA) or hyperpolarized (−77.6 ± 0.3 mV by −13.4 ± 3.0 pA) states (Fig. 2a). Depolarization in this range did not induce sustained calcium influx, as indicated by the lack of a significant difference in the baseline green fluorescence (Fluo-5F; −0.8 ± 0.6%, P = 0.17, n = 54, t-test).

Somatic hyperpolarization had bidirectional effects on bAP-evoked dendritic calcium signals, which depended on the distance from the soma (Fig. 2b,c; Supplementary Fig. 4a). In the proximal region, bAPs evoked at hyperpolarized somatic membrane potentials resulted in larger average calcium signals (20–75 μm from the soma, 12.5 ± 1.5%, P = 2.5 × 10−9, n = 31 locations, t-test) compared with bAPs evoked at depolarized membrane potentials. However, the enhancement of bAP-evoked calcium signals was restricted to the proximal dendrites. In the distal dendritic region, somatic hyperpolarization had the opposite
effect: the bAP-evoked calcium signals were smaller during somatic hyperpolarization (beyond 120 µm, $-15.6 \pm 3.9\%$, $P = 0.0022$, $n = 12$, t-test). The location dependence could be clearly demonstrated by the linear correlation among individual data points ($R^2 = 0.604$, $P = 2.9 \times 10^{-12}$). Similar membrane potential- and location-dependent bAP-evoked calcium signalling was identified at physiological temperatures (Supplementary Fig. 4a). Importantly, in addition to reflecting the somatic membrane potential in a graded manner (Supplementary Fig. 5), dendritic calcium signals were also modulated by a physiological hyperpolarization elicited by G protein-coupled inwardly-rectifying potassium (GIRK) channels through mGlu2 receptor activation in the proximal dendrites$^{11}$ (Supplementary Fig. 6). Altogether, physiological analogue information about the somatic membrane potential location-dependently and bidirectionally modulated the bAP-evoked dendritic calcium signals in GCs. Therefore, dendritic calcium signalling is capable of extracting analogue information from hybrid bAP signals. Yet, the moderate influence of the analogue content on calcium signals enables the hybrid bAP signal to maintain its integrity and major function of transmitting the digital content, which, thus, fulfills an important criterion for analogue modulation.

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**Figure 1** | Location-dependent effects of somatic membrane potential on bAP waveforms. (a) Superimposed IR-DIC and confocal z stack images of a GC, which was simultaneously recorded at a somatic and a dendritic site. Spikes were evoked with short current injections at the soma and the membrane potential was also set at this location with steady-state current injections to slightly depolarized (red, $-64.6 \pm 0.6$ mV, $n = 29$) or hyperpolarized (blue, $-77.2 \pm 0.6$ mV) potentials. The second pipette was used for monitoring the undisturbed voltage either at a dendrite or at the soma. (b) Propagation of somatically set membrane potential into dendrites (left panel). Connected symbols indicate individual experiments. Arrows show the average vector of the membrane potential difference. The right panel shows the distance dependence of the absolute bAP peak voltage (see also ref. 26). (c) Average bAP waveforms at various monitored locations. Representative somatic traces are shown on the left. Spikes were aligned to their maximum rate of rise (vertical grey lines). Black traces below show the voltage difference between the bAPs evoked from hyperpolarized and depolarized potentials. (d) The same average bAPs as in c on different timescale. Numbers show the included experiment numbers. (e) Effects of hyperpolarization on the peak voltage and relative repolarization of bAPs. Grey symbols indicate individual experiments, whose running average is shown in blue (calculated from 9 neighboring data points). To quantify the differences during the repolarization relative to the loss of the peak voltage, the voltage difference between the two bAPs at 0.2-1.2 ms after the peak was compared with the voltage difference at the peak.
Small distal bAPs lead to reduced calcium influx. What mechanisms provide the functional link between the analogue state of the GCs and the bAP-evoked dendritic calcium signals? Changes in the AP waveform may affect local calcium signalling in various cellular compartments through multiple potential mechanisms. First, more hyperpolarized voltages during the repolarization phase (when majority of calcium channels are open) can promote larger peak calcium influx because of the larger driving force. Second, wider (depolarized) spikes may be more effective in opening calcium channels, as has been demonstrated for narrow axonal and dendritic APs, resulting in more calcium entry. Third, the smaller absolute peak amplitude of the bAPs may activate fewer calcium channels.

Before we directly addressed the underlying mechanisms of the effect of bAP waveforms, we established a model configuration that provided testable hypotheses based on the available calcium currents in GCs. For this aim, we recorded native calcium currents in nucleated patches (Supplementary Fig. 7a). The total calcium current (\(\text{Cd}^{2+}\)-sensitive) consisted of low-voltage-activated (LVA, sensitive to T-type channel blocker, more effective in opening calcium channels, as has been demonstrated for narrow axonal and dendritic APs, resulting in more calcium entry).
Faster bAP repolarization promotes increased calcium influx. Next, we wanted to understand the mechanisms of the more surprising aspect of the analogue modulation of bAP-evoked calcium signals: their proximal enhancement during hyperpolarization. The initial clue was the difference in the proximal bAP waveforms, which repolarized faster when evoked from hyperpolarized membrane potentials. To determine the isolated effects of the faster repolarization on calcium influx, we generated an experimental model that reproduced the observed membrane potential-dependent changes in the proximal bAP waveform without other variables (for example, preceding membrane potential). Using conductance clamp, we took advantage of a high-threshold-activated transient potassium conductance (\(g_{\text{KA}}\)), which was an ideal native tool for modulating the repolarization phase of APs (Supplementary Fig. 9). GCs were recorded with two somatic pipettes, including one pipette for monitoring the membrane potential made the majority of inactivating HVAs (hyperpolarized) bAP waveforms evoke increased calcium influx when HVA models had AP relevant fast inactivation. This effect was relatively independent of other current parameters, such as activation kinetics and channel type. Notably, N-type currents also became sensitive to the repolarization speed in the same way when they were supplemented with voltage-dependent inactivation. Thus, these simulations suggest that inactivation of HVA calcium currents is sufficient for the enhancement of calcium influx through faster repolarization.

To test whether the GCs’ HVA channels have AP relevant fast inactivation, we recorded currents in nucleated patches with long voltage steps (300 ms). The decay of isolated HVA currents (in the presence of sodium-, potassium- and T-type calcium channel blockers) were fitted with a double exponential \(\tau_{\text{fast}}: 8.3 \pm 1.3 \text{ ms}, \tau_{\text{slow}}: 46 \pm 11 \text{ ms, } n = 6 \text{ recordings; Fig. 4c},\) indicating that a substantial fraction (52.8 \(\pm 8.9\%\)) of the calcium currents in the GCs adhere to the inactivation criterion predicted by the above simulations.

Next, using the HN-HI model, we tested whether the calcium current inactivation was not only sufficient but also necessary for the enhancement of calcium influx induced by faster repolarization. In the commands, the voltage before the bAPs was set to \(-60 \text{ mV}\) for both the originally depolarized and originally hyperpolarized traces. Thus, as before, only the actual bAP waveforms were different, and the depolarized preceding membrane potential made the majority of inactivating HVAs unavailable. In these simulations, bAPs with faster repolarization
were no longer able to evoke larger calcium influx (Supplementary Fig. 11). Similarly, in nucleated patches, the same modified commands with $-60 \text{ mV}$ preceding voltage occluded the enhancing effect of the faster bAP repolarization ($-4.2 \pm 1.4\%$, $n = 8$ recordings). Thus, when only the difference in bAP shape was included, inactivation of the HVA calcium currents was both sufficient and necessary for the enhancement of calcium influx by the faster bAP waveform. However, the waveform was not independent of other parameters, such as the preceding membrane potential, which we addressed as described below.

**Inactivating HVAs are essential for hybrid bAP signalling.** The results described above predicted that fast-inactivating HVA calcium currents are sufficient and necessary for the hyperpolarization-induced enhancement of proximal bAP-evoked calcium signals. A likely candidate that meets these criteria is the R-type calcium current (primarily mediated by $\text{Ca}_{2,3}$, CACNA1E channels), which is present in GCs, it is activated at high voltages and inactivated at low voltages.$^{43,44}$ To experimentally dissect the contribution of different classes of calcium channels, we selectively inhibited specific subsets of calcium currents in nucleated patch recordings and during calcium imaging and compared the analogue modulation of the calcium influx. In these calcium current recordings (Fig. 5a), the voltage commands were the originally recorded bAP traces and included all components of the changes of the proximal bAPs (for example, the different membrane potential and repolarization). In the control conditions (without calcium channel blockade), the hyperpolarized bAP waveform evoked larger calcium currents;
the most prominent change was produced in the T-type component (which was likely to be overrepresented in nucleated patches38–40). When T-type channels were selectively blocked with NNC55-0396, the hyperpolarized bAP waveforms remained effective in eliciting larger calcium influx (9.2±1.7%, \( P=0.00073, n=9, t\)-test), confirming that calcium influx can be enhanced by the involvement of HVA channels only. The recordings with NNC55-0396 correspond to the HN:HI simulation scenarios (Fig. 5b). In contrast, when R-type currents were removed in addition to T-types by 500 \( \mu \text{M} \) NiCl\(_2\) (corresponds to the HN only simulations), the calcium influx evoked by the hyperpolarized proximal bAP waveforms was no longer increased compared with the calcium influx evoked by the depolarized bAPs (1.3±1.6%, \( P=0.44, n=6, t\)-test). These findings confirm that the inactivating HVA R-type currents are indeed sufficient and necessary for the larger calcium influx observed during hyperpolarized states.

Next, we investigated the contribution of various calcium channels in an even more intact situation—where only a specific subset of calcium currents is blocked and the AP propagation, native morphology and other channel functions were present—by imaging calcium signals in the proximal dendritic regions of GCs at two membrane potentials. Similar to the nucleated patch recordings, selective elimination of T-type currents by NNC55-0396 did not affect the modulation of bAP-evoked proximal calcium signals by the somatic membrane potential (Fig. 5c, control: 13.1±1.6%, \( P=2 \times 10^{-8}, n=26; \) NNC55-0396: 12.1±1.3%, \( P=2 \times 10^{-5}, n=9, t\)-test). However, in the presence of the R- and T-type blocker, NiCl\(_2\) (50 \( \mu \text{M} \)), the enhancing effect of hyperpolarization on local calcium influx was greatly reduced (2.1±1.4%, \( n=14; \) analysis of variance (ANOVA): \( P=6 \times 10^{-3}, \) Bonferroni Ni\(^{2+}\) versus NNC55-0396: \( P=0.0046 \)). Note that the SNX-482 toxin, which specifically inhibits R-type channels among different calcium channels, acts also on A-type potassium currents45; thus, it cannot be used for calcium channel identification using this experimental arrangement. Altogether, multiple lines of experimental evidence confirm the predictions of our simulations that the fast-activating, fast-inactivating HVA R-type channels are necessary and sufficient for the hyperpolarization-induced enhancement of proximal bAP-evoked calcium influx.

**Figure 5 | Hyperpolarization-induced enhancement of calcium influx requires inactivating HVA calcium currents.** (a) Average traces and changes in charge (0.35–1.35 ms after the peak of bAP) of the calcium currents in nucleated patches evoked by previously recorded proximal bAP pairs (24 \( \mu \text{m} \)) at two membrane potentials, in control conditions and in the presence of Cav3 blocker, NNC55-0396 (10 \( \mu \text{M} \)), or in the presence of Cav2.3 and Cav3 blocker, Ni\(^{2+}\) (500 \( \mu \text{M} \)). Crosses indicate the changes predicted by simulations (see b). (b) Simulated HVA calcium currents with inactivating and non-inactivating HVA or only non-inactivating HVA components corresponding to pharmacologically isolated currents in NNC55-0396 or Ni\(^{2+}\), respectively. (c) Average calcium imaging traces at two membrane potentials and the effect of somatic hyperpolarization in control conditions and during the blockade of Cav3 (10 \( \mu \text{M} \) NNC55-0396), or Cav2.3 and Cav3 channels (50 \( \mu \text{M} \) Ni\(^{2+}\), the lower concentration was needed to avoid interference with imaging). Numbers indicate numbers of experiments.

**Theta-dependent dendritic calcium signalling by hybrid bAPs.** What are the potential functional significances of the analogue modulation of bAPs? First, we tested whether the calcium signals were capable of reflecting physiologically relevant membrane potential fluctuations, such as theta oscillations. To accomplish this aim, we employed spinning-disk confocal imaging, which allows simultaneous sampling of large dendritic regions. Single APs were preceded by 5.2 Hz oscillations (between −62.2±0.7 and −83.9±0.3 mV) evoked via current injection (Fig. 6a,b). APs during different phases of the ongoing theta cycles resulted in different calcium signals (\( P=0.0066, n=5 \) cells, one-way repeated measure ANOVA, Greenhouse–Geisser correction for non-sphericity) in the distal dendritic region (100–175 \( \mu \text{m} \)). Between 40° and 160°, the signals were larger than the signals evoked without voltage fluctuations at rest (−73.3±0.3 mV); however, during the trough phase (219°–339°), the signals were smaller in the distal dendritic region. In contrast, the phase dependence was less pronounced in the proximal region (25–100 \( \mu \text{m} \); \( P=0.062, n=5 \) cells, one-way repeated measure ANOVA). Thus, the analogue modulation of bAPs enables theta-phase-dependent dendritic calcium signalling in the GCs, and this phenomenon also occurs at physiological temperatures (Supplementary Fig. 4b). Further investigation revealed the potential time frames, in which the two opposite effects of the hyperpolarization operated on dendritic calcium signals (Supplementary Fig. 12). Namely, the distal effect was instantaneously available, whereas the proximal enhancement required prolonged (>200 ms) hyperpolarization. The similarity of this time course to the recovery from inactivation of HVA currents in GCs (362±136 versus 422±291 ms) further supports the crucial role of the putative R-type calcium currents.

The analogue content of hybrid bAPs modify synaptic plasticity. Finally, we investigated whether the analogue content of bAPs

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**Note:** The text is a natural language representation of the document's content, focusing on the key points and avoiding excessive detail for clarity. The document discusses the role of calcium currents in neural signaling, particularly focusing on the significance of T-type and R-type currents in modulating calcium influx. It highlights the role of theta oscillations in dendritic calcium signalling and the potential implications for synaptic plasticity.
modulates plasticity at dendritic synapses as an attempt to find evidences for biological entities, which are potentially capable of detecting the relatively small contribution of the analogue content to the hybrid bAP signals. Given the complexity of the activity-dependent regulations of synaptic strength, we evoked long-term synaptic plasticity via the involvement of only the recorded postsynaptic cell and only a few synapses (smaller than 2 mm regions) on its dendrites activated by glutamate uncaging. Single postsynaptic APs were paired with distal dendritic glutamate uncaging stimulation of the same small regions (300 pairing at 1 Hz; the two events occurred nearly synchronously, within ±4 ms, 150–200 μm). This pairing protocol led to a larger net long-term potentiation when it was performed at depolarized somatic membrane potential (179.1 ± 15.1%; MP: —62.6 ± 0.5 mV, n = 8; Fig. 6c,e; Supplementary Fig. 13) compared with hyperpolarized pairing (130.2 ± 8.1%; —81.4 ± 0.5 mV, n = 8, P = 0.013, t-test, note that the relatively slow expression of changes is likely due to the weak induction protocols). Common voltage-dependent mechanisms may explain this observation.

Therefore, to directly prove their associative role, we uncoupled...
bAP and calcium influx by using inhibitors against all HVA calcium channels, which underlie the hyperpolarization-induced reduction of calcium influx in this region (Fig. 3), in the same experimental arrangement (Ni²⁺ against R- and T-types, ω-conotoxin GVIA against N-type, NNC55-0396 against T-type and nifedipine against L-type). In other control experiments, we also assessed the membrane potential dependence of the long-term plasticity induction without postsynaptic firing. In both conditions, the induction protocols at de- or hyperpolarized membrane potentials resulted in similar long-term plasticity (Fig. 6c,e; 170.0 ± 19.4% versus 169.7 ± 18.6%, P = 0.99, n = 8 and 8 cells; 164.7 ± 15.4% versus 184.4 ± 19.5%, P = 0.447, n = 6 and 6). Thus, these controls showed that the differences in plasticity depended only on voltage-gated calcium channels activated by bAPs and, thus, they excluded the possible involvement of other voltage-dependent mechanisms, such as the different functional availability of NMDA receptors. Even though the observed synaptic plasticity changes may not share each expression mechanisms, the necessity of bAP and bAP-associated calcium influx suggests that the mechanisms of the analogue modulation described above in this study enable synapses interpreting the analogue content of the hybrid bAPs.

Discussion

Here we demonstrated that bAPs in the dendrites of GCs are subject to analogue modulation by somatic membrane potentials. The first important step in this phenomenon is that analogue information is distance-dependently retained by the bAP waveform. Second, the graded changes in the bAP waveforms are reflected by local dendritic calcium signalling. Thus, as hybrid signals, bAPs carry both digital timing information about spiking activity and analogue information about the overall state of the cells. Notably, our findings highlighted that hybrid dendritic signalling is available during at least two physiological conditions; therefore, hybrid dendritic signalling increases the capacity of neurons and their synapses to code, relay and decode various types of neuronal information.

The moderate extent of the analogue modulation seems to be an important feature of the hybrid signalling. First, the low relative weight allows that the analogue content cannot override the primary digital timing information. Thus, each state-dependently modulated bAPs maintains its primary role to carry digital feedback signal to the dendrites about the output activity of the neurons. Although local dendritic membrane potential is capable for more effective interference with local bAP signalling and synaptic plasticity, these active boosting effects (such as NMDA receptor-mediated mechanisms) are spatially restricted and do not carry similar analogue information for neighboring dendrites. Second, the contribution of the analogue content to the weight of hybrid bAPs in the dendrites is similar to the axonal hybrid signalling; therefore, the moderate contribution of the analogue content seems to be a general and necessary feature of hybrid signalling. Third, the extent of the cell-autonomous analogue modulation is similar to the effect that can be achieved on bAP signals by localized activation of dendritic receptor pathways, such as the GABA, γ-aminobutyric acid) receptor-mediated downregulation of calcium channel functions, albeit the underlying mechanisms are fundamentally different. Forth, the sensitive mechanisms that we described here allow that physiological membrane potential differences are faithfully reflected by the dendritic hybrid signals, from which synapses can translate the analogue content as differential weight changes after plasticity induction. Voltage changes arise mostly from dendritic excitatory synapses, thus, the somatic membrane potential is not an independent modulator from the viewpoints of activated dendrites. However, somatic membrane potential reflects every dendritic branch and additional sources of voltage changes, such as the diverse perisomatic GABAergic inputs, supramamillary glutamatergic inputs and non-synaptic sources. Thus, the somatic membrane potential contains more information than the local dendritic potentials, which is continuously relayed back to each dendrite equally via the hybrid bAPs. A support for the biological relevance of the somato-dendritic signalling direction is our plasticity experiments, where synapses were activated on a single branch by uncaging, yet, the somatically introduced small, but physiological membrane potential difference was able to overcome the effects of the local voltage changes and impose a modulatory effect.

The morpho-physiological design of GC dendrites makes them ideal for hybrid bAP signalling because each dendrite independently collects inputs and the overall activity converges onto the soma. The exchange of analogue input signals between individual branches is weak as a result of the asymmetric voltage propagation. Thus, the dispersion of analogue information by bAPs is an effective way of notifying each dendrite about the ongoing overall subthreshold activity. Although GCs are well suited for hybrid dendritic signalling, the underlying mechanisms suggested here are present among other cell types. Thus, analogue information presumably modulates bAP signalling in other cells as well. Indeed, similar effects of the somatic membrane potential on single spike-evoked calcium signals have been observed in medium spiny neurons of the striatum.

Interestingly, the location dependence of dendritic hybrid AP-signalling matches the anatomical organization of the excitatory inputs to the GCs. Namely, the proximal third of the dendritic field (the average dendritic length in our sample was ~300 μm) is innervated by ipsilateral and contralateral mossy cells, whereas axons from the medial entorhinal cortex form synapses in the middle section of the dendrites. This input localization co-aligns with the opposing modulation of bAP-evoked calcium signals by the somatic membrane potential. These spatial profiles enable the differential regulation of active synapses depending on their origin and the analogue state of the postsynaptic cell when it fires APs. In this framework, the medial entorhinal cortical inputs will coincide with larger bAP-evoked calcium influx when the membrane potential is depolarized, whereas calcium influx is smaller at the mossy cell synapses. We provide evidences that changes in the analogue content of hybrid bAPs modulate the long-term synaptic plasticity of distal synapses, and that the contribution of the analogue modulation is fully available during ongoing somatic voltage fluctuations in the theta frequency. Therefore, medial entorhinal inputs that are preferentially active during different phases of the theta will be differentially modified by synaptic plasticity. It should be noted that our results provide only one example of the differential modulation of synaptic strength. Interference with synaptic plasticity, especially with the cooperativity rules of induction, by somatic membrane potential is not unprecedented. These observations raise the possibility that hybrid signalling is available in other principal cell types. Therefore, the contribution of the analogue content carrying hybrid bAPs to these observed effects should be clarified, in addition to the well-established voltage-dependent NMDA receptor functions (which were excluded in our experiments). Moreover, the contributions of each aspects of hybrid signalling to synaptic plasticity described in this study, such as its timing, location and state- dependence, must be resolved also in future studies.

One surprising aspect of our results on the underlying mechanisms was that the slight narrowing of dendritic bAPs was not necessarily associated with smaller calcium signals.
This is in contrast to axonal APs, where an increase of AP width leads to greater calcium influx in various cell types5–8 (but see ref. 59), including the axons of our subject cells34. This proximal action was unprecedented also in the dendrites, where local voltage has been shown to modulate bAPs similarly to what proximal action was unprecedented also in the dendrites, where local voltage has been shown to modulate bAPs similarly to what

**Methods**

Experimental procedures were made in accordance with the ethical guidelines of the Institute of Experimental Medicine Protection of Research Subjects Committee.

**Solutions and chemicals.** Our standard artificial cerebrospinal solution contained 126 mM NaCl, 2.5 mM KCl, 26 mM NaHCO3, 2 mM CaCl2, 2 mM MgCl2, 1.25 mM NaH2PO4, and 10 mM glucose (equilibrated with 95% O2 and 5% CO2 gas mixture). Slices were cut in a cutting solution consisting of 85 mM NaCl, 75 mM sucrose, 2.5 mM KCl, 25 mM glucose, 1.25 mM NaH2PO4, 4 mM MgCl2, 0.5 mM CaCl2, and 24 mM NaHCO3. External solutions were equilibrated with 95% O2, and 5% CO2.

**Slice preparation and microscopy.** Hippocampal slices (350 μm) were prepared from adolescent Wistar rats (P23–P36, both sexes) in ice-cold cutting solution in an orientation optimized to preserve the mossy fibre tract in the CA3 area58. After cutting, slices were kept at 32 °C for at least 30 min and then stored at room temperature until the recordings. With the exception of the data shown in Supplementary Fig. 4, experiments were performed at room temperatures (23–28 °C) using an upright microscope (Eclipse FN-1; Nikon) equipped with a high-numerical aperture objective (Nikon 1.4 NA, Apo LWD 25 × W or Nikon 0.8 NA Apo 16 × W), with a sCMOS or CCD camera (Andor Zyla 5.5 controlled by NIS Elements software or Hamamatsu C7500) for IR-DIC (900 nm) video microscopy and with a confocal system (see below).

**Dual somato-dendritic recordings.** To obtain dual current clamp recordings, we first established stable dendritic patch-clamp recordings with the aid of the IR-DIC optics. After a brief (no more than 10 min) dialysis of the cell, the position and morphology of fluorescently labelled parent soma was defined using the red channel of the confocal system and then patched under IR-DIC control. Recordings were terminated when substantial changes appeared in the dendritic electrical properties (that is, in the access resistance, in the input resistance or in the background noise) during the dialysis or during patching the soma. Patch pipettes were filled from thick-wall borosilicate glass tubes (inner diameter: 0.86–0.75 mm, outer diameter: 1.5 mm, Sutter or Hilgenberg) coated with dental wax to further reduce the pipette capacitance (mean pipette capacitance was 6.85 ± 0.12 pF, no correlation with the distance from soma, R2 = 0.04, P = 0.635, linear fit). Recordings had at least 2 Q2 seal resistance before the break-in. The average series resistance of the patch pipettes was 1187 ± 25 MΩ (weakly correlated with the distance from soma, R2 = 0.13, P = 0.04684), which was fully compensated and constantly monitored using fast large current steps within each recorded trace.

**Calcium imaging with spinning-disk confocal microscopy.** To image large areas of the dendritic field simultaneously, we employed spinning-disc confocal imaging (Andor Revolution XDv system including Yokogawa CSU-X1 Nipkow disc, Andor iXon 860 EMCCD Camera and Andor iQ2 software) using the same labelling and recording protocols, as above either at room temperatures (23–26 °C) or at near physiological temperatures (35–36 °C, Supplementary Fig. 4). Fluorescence was captured at 93.75 frames per second and 20 frames per second rates consecutively for the green (488 nm laser excitation, 125–230 μW) and red channels (561 nm, 32–58 μW). Fluorescence was quantified as the changes in the peak green signal (average of 6 or 7 frames after APs minus a 13–16 frame-long baseline period) relative to the steady red signal except for (Supplementary Fig. 5), where raw green fluorescence measurements (that is, no normalization to red signal and no baseline subtraction) are shown for detecting any potential change in baseline calcium levels. Theta oscillation was evoked by sinusoid current injection with 48 ± 6 pA in both polarities. Single APs were preceded by at least five full theta cycles (5.21 Hz) and each imaging trace was separated by at least 25 s.
Na-GTP and 10 mM phosphocreatine diosodium. The extracellular solution contained elevated calcium concentration (5 mM), 1 μM TTX and 5 mM 4-AP. All voltage protocols started with a 4-s-long baseline to allow calcium channels to reach their steady-state availability at the given baseline potential. Previously recorded AP waveforms were applied as voltage-clamp command to assess AP relevant macroscopic calcium currents. For constraining the model (Supplementary Fig. 7), we applied a 3 ms-long test pulse to 0 mV from −80 mV, and previously recorded bAP traces as voltage command. For the measurement of the inactivation of the native calcium currents (Fig. 4c), 300 ms-long test pulses (0 mV) were applied from −120 mV to maximize the contribution of inactivating calcium channels. For testing the completeness of the calcium current activation (Supplementary Fig. 8), two previously recorded bAP waveform pairs have been used as a template voltage command and these were extended with various lengths (0.75–5 ms) of steady voltage before they reached their peak. Thus, the repolarization phase, when the majority of bAP-activated calcium currents flow, remained as in the native waveform. Sweeps were collected with 12 or 25 s intervals and digitized at 50 or 80 kHz. Leak and capacitive current components were subtracted using P/rev testing the completeness of the calcium current activation (Supplementary Fig. 8), macroscopic calcium currents. For constraining the model (Supplementary Fig. 7), effects on the model calcium currents were quantified in the same way as described for the calcium current recordings except in case of simulations on Fig. 4b, where we measured current integrals in a larger time window (5 ms) to avoid potential variances introduced by the highly diverse time courses of the currents. We excluded those simulations, which resulted in unrealistic currents due to unlikely kinetics (that is, extremely fast inactivation and extremely slow activation rates. For details see Supplementary Fig. 14).

**Computational modelling.** We performed single compartment computer simulations using the NEURON64 simulation environment (version 7.2, downloaded from http://neuron.yale.edu). Based on the assumption that the recorded macroscopic HVA calcium currents can be described by the combination of non-inactivating and inactivating components (Fig. 3), we constructed two model currents using Hodgkin–Huxley formalisms. Activation kinetics were constructed on the basis of previously published channel properties recorded from GCs41. Namely, N-type conductance served as template to construct a fast, non-inactivating (HN) HVA model, whereas we used the R-type current for the HVA, inactivating (HI) model. Equations describing the voltage-dependent activation and inactivation of the H1 current were:

\[
P_{HN}(t, V) = P_{max}(1 - \exp(-0.04(V - 42.5)))
\]

\[
P_{HI}(t, V) = \exp(0.0775(V - 34.5))
\]

Equations describing the HN current were:

\[
\frac{d m_{HN}}{dt} = \frac{a_{m_{HN}}(V)(1 - m_{HN}) - b_{m_{HN}}(V)m_{HN}}{1 + \exp(-0.04(V - 42.5))}
\]

\[
a_{m_{HN}}(V) = 4.9
\]

\[
b_{m_{HN}}(V) = 1.9
\]

\[
\frac{d n_{HI}}{dt} = \frac{\alpha_{n_{HI}}(V)(1 - n_{HI}) - \beta_{n_{HI}}(V)n_{HI}}{1 + \exp(0.0775(V - 34.5))}
\]

\[
a_{n_{HI}}(V) = 0.0003 \exp(-0.03(V + 10))
\]

\[
b_{n_{HI}}(V) = 0.003 \exp(0.06(V + 60))
\]

Equations describing the HI current were:

\[
\frac{d m_{HI}}{dt} = \frac{a_{m_{HI}}(V)(1 - m_{HI}) - b_{m_{HI}}(V)m_{HI}}{1 + \exp(-0.04(V - 42.5))}
\]

\[
a_{m_{HI}}(V) = 0.08 \times (V - 2) \exp(-0.08(V - 2))
\]

\[
b_{m_{HI}}(V) = \frac{13}{1 + \exp(0.095(V + 39.77))}
\]

Calcium current was calculated as follows:

\[
I_{Ca}(t, V) = P_{Ca}(t, V) \cdot GHK_{Ca}(t, V) \cdot A
\]

where \( P_{Ca} \) was the membrane permeability for calcium ion, \( A \) was an amplitude factor and \( GHK_{Ca} \) was a modified constant field equation6,66 in the form of:

\[
GHK_{Ca}(V) = V^{0.3933} \cdot \exp(-V/V_{m})
\]

\[
(1 - \exp(-V/V_{m}))
\]

where \( V \) was the modelled membrane potential. The net calcium permeability was the linear combination of individual model current activation:

\[
P_{Ca}(t, V) = P_{max}(t, V) + P_{Ca}(t, V)
\]

The total theoretically maximum permeability was fixed to 1 in all simulation conditions (HI only, HN only and HNHJ) to keep \( P_{Ca} \) in the range of 0 ≤ \( P_{Ca} \)(t, V) ≤ 1. The activation time constants were calculated using the formulation of:

\[
\tau(V) = \frac{1}{\alpha(V) + \beta(V)}
\]

Model was incorporated onto a single compartment with three segments (area = 100 μm², \( c_{m} = 1 \mu F cm^{-2}, R_{m} = 35.4 \Omega cm \)), where the membrane potential was forced to follow previously recorded AP waveforms or voltage steps. The elicited calcium currents were analysed in the middle of the compartment. Integration time steps were 2.5 or 12.5 μs for those experiments, which validated the models (Supplementary Fig. 7) and 0.5 μs for those simulations, where we tested the effects of AP shape differences on (Figs 3b, 4b and 5b; Supplementary Fig. 11). Effects on the model calcium currents were quantified in the same way as described for the calcium current recordings except in case of simulations on Fig. 4b, where we measured current integrals in a larger time window (5 ms) to avoid potential variances introduced by the highly diverse time courses of the currents. We excluded those simulations, which resulted in unrealistic currents due to unlikely kinetics (that is, extremely fast inactivation and extremely slow activation rates. For details see Supplementary Fig. 14).

**Two electrode dynamic-clamp experiments.** We employed a software based dynamic-clamp system to manipulate the AP repolarization phase. Current injections were calculated by the Stdp2012 software67 (downloaded from http://sourceforge.net/projects/stdpcl through a MIO-16E-4 analog/digital card (National Instruments Inc.) based on voltage signals of the measuring electrode and an A-type current. Using a Hodgkin–Huxley-type formalism for a native A-type inactivating potassium conductance (4–10 nS maximal conductance) at a clamp rate of 83 kHz or higher, we were able to interfere with action potentials. For the mock conductance, 4-A-sensitive (5 mM) outward currents were isolated in nucleated patches (1 μM TTX). A conductance \( G_{Ca} \) was calculated assuming anomalous channel behaviour and a potassium equilibrium potential of −90 mV. Times constants of activation and inactivation were measured by fitting the rising phase (from 0.25 ms after onset to the peak) and the decay phase (in a 100 ms-long time window after the peak) with monoexponential functions (Supplementary Fig. 9). The clamp current of A-type potassium conductance model was generated as follows:

\[
h_{k_{clamp}}(t, V) = \frac{g_{max}(t, V) \cdot h(t, V) \cdot (V_{rev} - V)}{\exp\left(-\frac{V_{rev} - V}{h_{rev}}\right)}
\]

where \( h_{k_{clamp}} \) was the calculated model current, \( g_{max} \) was the maximal conductance, \( m \) and \( h \) were gating variables, \( V_{rev} \) was the reversal potential of the model conductance and \( V \) was the measured membrane potential of the cell. Gating variables were modelled using the formalism of:

\[
\frac{dh}{dt} = \frac{m_{hi}(V) - m}{t_m} + \frac{h(V) - h}{t_h}
\]

\[
\frac{dm}{dt} = \frac{a_{m_{hi}}(V)(1 - h) - b_{m_{hi}}(V)h}{1 + \exp(0.0775(V - 34.5))}
\]

\[
a_{m_{hi}}(V) = 0.003 \exp(-0.03(V + 10))
\]

\[
b_{m_{hi}}(V) = 0.003 \exp(0.06(V + 60))
\]

For passive conductance measurements, we used the same Hodgkin–Huxley model, but the activation was instantaneous, whereas inactivation was extremely slow (10⁶ s⁻¹); therefore, conductance was always fully activated. Reversal potential of the model current was set to −70 mV to minimize the current flow at rest. Calcium imaging on dual-patch conductance clamped cells (membrane potential: −68.7 ± 0.6 mV) was performed as described above.

**Spike timing-dependent plasticity protocol.** To evoke plasticity at well-defined synaptic locations, we employed glutamate uncaging and the evoked responses were paired with individual APs. During the experiments, cells were patched using IR-DIC imaging and intracellularly filled with Alexa Fluor 594 for at least 15 min. Next, an intact and complete dendritic region was selected within 30–50 μm from the surface of the slice. During the long course of these experiments (at least 90 min), the imaging site was monitored and adjusted if necessary. Glutamate-EPSPs were evoked by 405 nm laser illumination51,58 (0.74 ms-long pulses repeated two to three times at 1 kHz) at a small dendritic spot (<2 μm) 150–200 μm from the soma (mean distance: 179.8 ± 22 μm, n = 44 cells) using the conventional confocal system described above. A concentration of 1 mM MNI-glutamate was supplied in the recirculated recording solution (10 ml). Before and after the pairing, EPSPs were tested every 20 s at −71.4 ± 0.1 mV. After a 20 min-long baseline period, the pairing protocol was applied, which consisted of 300 AP-EPSP pairings at 1 Hz (timing range: ±4 ms, mean: −0.82 ± 0.4 ms). During the pairing, the EPSPs were evoked by the same conditions as during the control and test periods except the stimulation frequency and membrane potential (depolarized: −62.2 ± 0.3 mV or hyperpolarized: −81.1 ± 0.3 mV). High resistance (>15 MΩ) recording pipettes were used to keep the cells intracellular milieu intact, and, thus,
to preserve the capability for synaptic plasticity. Data acquisition were stopped if the cell experienced lower access resistance than 50 MΩ. Access and input resistances were monitored in each trace by injecting a −10 pA 500 ms-long pulses. Calcium channel blockers were bath applied in the presence of 1 mg ml−1 bovine serum albumin. After the recordings, the morphology of the dendrites and the distance of the uncaging site from the soma were retrieved in three-dimensional using the confocal system in the red channel. EPSP amplitudes were estimated in a 5 ms-long window at the peak. Traces with spontaneous events were excluded from analysis. To test whether the plasticity was dependent on the membrane potential during the induction, the changes of the amplitude of the EPSPs were (that is, depolarized versus hyperpolarized induction) using Student’s t-test.

**Data analysis and statistics.** Data were analysed using Molecular Devices pClamp, OriginLab Origin, Microsoft Excel and Stata®. Voltage values are presented without correction for the liquid junction potential. Somatic distances were measured on confocal z stack images taken after the end of experiments using the Alexa Fluor 594 signal. Differences of the values from one or two different groups were tested using parametric, paired or unpaired, two-tailed Student’s t-tests (abbreviated as t-test in the text). Normality of the data was analysed with Shapiro–Wilks test. Data are presented as mean ± s.e.m. In case of the experiments on membrane potential effect in the presence of T-type channel blocker (Fig. 5c) Grubb’s test identified a significant outlier (P = 0.00027). Thus, this value (48.6%) was excluded from the further analysis. The removal modified the average from 15.8 ± 3.8% to 12.1 ± 3.3%, which does not change the conclusion of the experiment. The same analysis did not identify outliers in the control (P = 0.953) and in the Ni2+ (P = 0.19) data sets.

**Data availability.** The authors declare that all other relevant data supporting the findings of this study are available on request and from szabadsclab.koki.hu.

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Author contributions
Both J.B. and J.S. contributed to every phases of this work.

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