C63

A co-operative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons

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In many neuronal types, backpropagating action potentials (APs) are critically involved in the induction of synaptic plasticity. We manipulated AP backpropagation and investigated its role in synaptic plasticity in L5 pyramidal neurons in acute neocortical brain slices taken from P14-21 Sprague-Dawley rats. Paired recordings were made at 34°C with presynaptic pyramidal neurons in L2/3 or in L5. The location of putative synaptic contacts, confirmed anatomically in some cases using 2-photon laser scanning microscopy of dye-filled cells and/or bright-field microscopy of fixed and histologically processed neurons, indicated that 20-80% EPSP rise time (RT) predicts input location accurately (r=0.89). L2/3-to-L5 synapses tended to be more distal than their L5-to-L5 counterparts, as indicated by RT: 3.0±0.4 versus 2.0±0.2 ms (mean±SEM, $p<0.05$; Student's t test). Pairing APs and EPSPs (5 at 50 Hz, +10 ms post-pre relative timing difference) reliably evoked typical Hebbian LTP at proximal inputs (RT<3 ms; after/before=136±5%, n=43), but surprisingly produced LTD at distal synapses (RT>3 ms; 80±5%, n=26; $p<0.001$). However, distal LTP was rescued by EPSPs larger than those produced by typical unitary inputs (>1.0 mV; 140±6%, n=14; $p<0.001$). Similarly, dendritic depolarizing current injection rescued LTP of weak distal EPSPs (<1.0 mV; 163±6.7%, n=5; $p<0.001$). Both conditions may evoke LTP because backpropagating APs are boosted by depolarization (Stuart & Häusser, 2001). In agreement, supralinear Ca^{2+} signals — measured with 2-photon laser scanning microscopy as the change in Fluo-5F signal normalized to Alexa 594 fluorescence — were generated throughout the distal dendritic tree by pairing APs with large EPSPs (percent of linear sum=200±30%, n=37, $p<0.001$), or with dendritic depolarizing current injection (368±68%, n=19, $p<0.001$), but not with small EPSPs (98±8%, n=9; 0.37±0.2mV). Ca^{2+} signal supralinearity increased with distance from the soma, ranging from 20% proximally to 500% at 900 μm from the soma, and the distance-dependence was described by a power law. Interestingly, for distal inputs synaptic stimulation alone, but not APs alone, resulted in LTD (70±6%, n=10 vs 102±6%, n=10; $p<0.01$). This type of LTD was abolished by the cannabinoid receptor CB1 blocker AM251 (0.9 μM; 99±3%, n=6; $p<0.01$). Our results indicate that long-term plasticity rules in L5 pyramidal neurons depend on dendritic location of the synapses, and that cooperative synaptic input or dendritic depolarization can switch plasticity between LTD and LTP by boosting backpropagation of action potentials. This activity-dependent switch provides a mechanism for associative learning across different neocortical layers that process distinct types of information.

Stuart GJ & Häusser M (2001). Nature Neurosci 4, 63-71.

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C64

Tonically active $GABA_A$ receptors at hippocampal mossy fibres

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Several ionotropic receptors activated by ambient neurotransmitters have been reported to modulate axonal excitability and synaptic transmission in the cortex. These include extrasynaptic and presynaptic nicotinic, $GABA_A$ (Ruiz et al. 2003), kainate, AMPA and NMDA receptors. We have examined the properties of axonal $GABA_A$ receptors in rat hippocampal mossy fibres by studying changes in the threshold for antidromic action potential activation, recorded via somatic voltage clamp. Antidromic action currents were elicited in dentate granule cells by positioning a stimulating electrode in stratum lucidum. Application of the endogenous neurosteroid tetrahydrodeoxycorticosterone (THDOC, 10 nM) increased the threshold for antidromic action potential activation ($n = 7$; $p < 0.01$, t test) arguing for a role of δ subunit-containing $GABA_A$ receptors at mossy fibres. Conversely, the $GABA_A$ receptor blocker bicuculline (10 μM) lowered the threshold. Furthermore, the GABA receptor agonist muscimol (10 μM) evoked single channel openings in outside-out patches taken from mossy fibre boutons directly demonstrating axonal $GABA_A$ receptors. The single channel conductance was estimated between 35 and 43 pS in different patches ($n = 3$). The THDOC sensitivity suggests that mossy fibres express tonically active $GABA_A$ receptors with δ containing subunits. However, γ subunit-containing receptors may coexist.

Ruiz A *et al.* (2003). *Neuron* **39**(**6**), 961-973.

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C65

A vesicular-dependent tonic $GABA_A$ receptor-mediated conductance modifies thalamic relay neuron burst firing in the mouse dLGN

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Many studies have suggested that low ambient GABA concentrations can persistently activate certain $GABA_A$ receptor subtypes, often remote from synapses, to generate a 'tonic' inhibition [1]. An as yet unidentified non-vesicular release mechanism is thought to be responsible for generating these low ambient GABA concentrations in cerebellar granule cells [2] calling into question the function of tonic inhibition in the dynamic control of neuronal excitability [2,3]. In this study we have examined the source of the GABA giving rise to a tonic inhibition which has recently been reported in thalamocortical neurons within the dorsal lateral geniculate nucleus (dLGN) in juvenile rats [4]. At physiological temperatures (37-38°C), whole-cell patch-clamp recording in an acute slice preparation from adult C57Bl/6J mice also shows a tonic $GABA_A$ conductance present within the mature dLGN. When normalised to cell capacitance, this conductance has a magnitude of 75.2 ± 20.3 pS/pF (mean ± sem; n=33). Reduction of the extracellular Ca^{2+} concentration from

2 to 1 mM, results in a decrease in the magnitude of this conductance to 6.7 ± 2.9 pS/pF (n=7). Furthermore, this conductance is also significantly reduced (unpaired t test, P<0.05) by the application of 500 nM tetrodotoxin (5.6 ± 2.3 pS/pF, n=7). Therefore, in thalamic relay neurons of the dLGN the tonic $GABA_A$ conductance does appear to be generated by the vesicular release of GABA.

Delta subunit containing $GABA_A$ receptors are often associated with this type of conductance. We observe strong delta mRNA labelling within the dLGN, with little evidence of this receptor population in the adjacent ventral LGN (vLGN); another retinorecipient nucleus. Consistent with the absence of delta subunits within the vLGN, no tonic $GABA_A$ conductance was detected in this nucleus (2.5 ± 5.5 pS/pF, n=26). In order to selectively activate these receptors the agonist 4,5,6,7-tetrahydroisothiazolo-[5,4-c]pyridin-3-ol (THIP; 1 μM) was bath applied to thalamic neurons. A clear THIP-activated conductance was elicited in dLGN (2.6 ± 1.0 nS, n=3) whereas, a markedly smaller conductance, with no clear peak associated with THIP application, was observed within the vLGN (0.1 ± 0.1 nS, n=3). Firing properties of LGN cells were then examined following the application of THIP. Hyperpolarising current steps generated high frequency rebound bursts of action potentials, analogous to the 'burst' firing mode of thalamic relay cells [5]. In dLGN, application of 1 μM THIP leads to an increase in the latency of these bursts from 29.6 ± 3.3 ms (n=4) to 41.7.± 4.6 ms, whereas there was no effect in vLGN (31.1 ± 8.5 versus 33.1 ± 8.9 ms, n = 6). Therefore, the tonic $GABA_A$ receptor-mediated conductance present in dLGN is capable of altering the timing of high frequency rebound bursts; a key component in the generation of thalamocortical oscillations.

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C66

Mapping olfactory circuits in *Drosophila*

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Olfaction appears to be a relatively shallow sense – it is likely to take rather fewer levels of neural processing to recognise a lemon by smell than by sight. We would like to understand the circuit basis of olfactory perception by combining both functional and neuroanatomical approaches. Our model system is *Drosophila*. The fruitfly olfactory system shares the organisation of vertebrates but is numerically much simpler. Furthermore, in contrast to vertebrates there are genetic methods to target specific classes of second and third order olfactory neuron down to the single neuron level.

In *Drosophila*, as in mice, olfactory information is spatially organised in the first olfactory relay of the CNS, the antennal lobe. Olfactory receptor neurons expressing the same odorant receptor molecule send axons to the same subunit or glomerulus within the antennal lobe. Indeed the molecular identity of the odorant receptor input to 37 of the 50 anatomically identifiable glomeruli of the antennal lobe has recently been described [1,2]. At the next level, we and others have shown that the axons of second order projection neurons (equivalent to vertebrate mitral cells) generate a new spatial map in the lateral horn, one of two higher olfactory centres [3,4].

We are most interested in the transformation that occurs between the second and third order neurons that meet in the lateral horn. As a prelude to electrophysiological investigation of identified lateral horn neurons, we have applied new image processing techniques to generate a 3D atlas of this higher olfactory centre.

We take 3D confocal images of individual brains each containing single fluorescently labelled neurons. Each brain is also stained with a synaptic marker; these images are registered with a reference brain using a high performance non-rigid intensity-based image registration algorithm, thus mapping every sample into a common coordinate system. 313/575 imaged brains registered sufficiently well to be included in our data set, which presently consists of 231 traced and identified second order projection neurons and 21 third order lateral horn neurons. 35 of an estimated 54 classes of olfactory projection neuron are present in our data set (median 5.5 samples per class, range 1-14). Four classes of projection neuron that originate from a pair of candidate pheromone-responsive glomeruli project to a distinctive region of the lateral horn, which is thus a candidate pheromone-processing centre. We have also used these data to investigate the spatial representations of different odours in the lateral horn.

Finally we have applied the same technique to third order neurons of the lateral horn. We have investigated 21 individual neurons falling into three classes of lateral horn neuron and predicted which projection neurons are most likely to be their presynaptic partners. By comparing the input to these neurons with physiological data that we have recently begun to obtain, we hope to generate a detailed understanding of the transformation of odour representations that underlie olfactory perception in the fly.

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SA37

What do single neurons in the rat's brain contribute to movement and sensation

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The relationship between action potential (AP) discharge patterns of individual neurons and behavior is a core interest of neurobiology. Extracellular recording and stimulation techniques and have demonstrated that single neuron activity of neurons is closely associated with behavior in mammals, but both techniques are not suited to pinpoint the impact of single neuron activity on behavior. We addressed this issue by assessing effects of single cell stimulation in the vibrissal active touch system of mammals. Specifically we evoked APs in (i) the vibrissal motor cortex of lightly anesthetized rats, (ii) the vibrissal representation of facial nucleus of lightly anesthetized rats, (iii) in the vibrissa / barrel cortex of awake, behaving, head fixed rats.

In vibrissa motor cortex we find that AP initiation in individual cells causes long sequences of small and slow multi whisker movements. Intracellular stimulation in layer 5 evokes movements that are phase locked from trial to trial, whereas APs initiated in L6 cells evoke bursts of whisking without specifying the phase of the individual movement. Pyramidal cell and interneuron stimulation evoked movements of opposite directions confirming a functional antagonism of these cell types. AP number had only little effect on whisker movement amplitude but it strongly affected movement latency. AP frequency in contrast did not affect movement latency but determined movement amplitude and direction.

In the facial nucleus we find that AP initiation in individual cells causes mainly but not exclusively single whisker movements. Movements are brief and usually fast and each spike causes a very similar fixed latency movement. Thus, motor cortical and facial nucleus cells code movements in very different ways: Cortical APs affect movements on long time scales and APs are read as sequences or "words", such that the effect (movement latency and direction) of an AP depends on the AP context. In contrast, facial nucleus APs are translated spike by spike to movement twitches.

Finally, we investigated sensory effects of single cell stimulation in barrel cortex. To this end we electrically stimulated single neurons in primary somatosensory cortex of awake, behaving, head fixed rats. Animals were first trained to report trains of microstimulation pulses of the barrel cortex by breaking an infrared beam with the tongue. The animal's detection threshold for microstimulation decreased over a period of a few days to currents of 2-7 μA. Once the animal had reached asymptotic performance, microstimulation trials were randomly mixed with trials in which we evoked 5-40 action potentials (APs) in single cortical neurons using juxtacellular stimulation in the nA range. We found that animals responded significantly more often to single-cell stimulation than to randomly intermixed control trials. Results varied from cell to cell and the average size of the effect was modest: the average single-cell hit rate was 25.8% compared to a control hit rate of 19.9% (an increase of 30%). Additional control experiments showed that this sensory bias was dependent on the generation of APs by juxtacellular stimulation. Thus, subthreshold juxtacellular stimulation or injection of twice the stimulation current into the extracellular space did not affect the animal's behavior. The occurrences of sensory biases covaried with the animal's performance in the microstimulation task, as single-cell stimulation trials flanked by misses on microstimulation trials were not different from control trials.

We conclude: (i) the activity of individual neurons can affect behavior, (ii) single cell stimulation is a most powerful tool to decode the meaning spike trains, (iii) the capacity of an individual neuron to evoke behaviors is most astonishing given the large number of neurons in the rat brain. Thus, sparse AP activity may suffice control behavior and APs can be translated into action with the utmost precision.

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SA38

Dynamic coding of sound intensity

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Natural sound intensities can vary extensively over the long term. For short periods, and within a given environment, however, they more often fluctuate over a relatively limited range. An important function of hearing is to detect, and discriminate between, important acoustic signals against the background of interfering noise. Mammals can perform this task over a vast of sound intensities (120 decibels) with remarkable accuracy. Recently, using single neuron recordings, we have shown that neurons in the inferior colliculus of the guinea pig code sound intensity over this wide range by adjusting their firing rates to take account of the mean, variance, and more complex statistics of sound level distributions (Dean et al. 2005). These adjustments improve the accuracy of the neural population code close to the region of most commonly-occurring sound intensities, extending the range of sound levels that can be accurately encoded, fine-tuning hearing to the local acoustic environment. This adaptation to the stimulus statistics occurs rapidly; switching between different distributions of sound intensity reveals a time constant of approximately 100 ms. Currently we are exploring neural mechanisms that might account for this adaptive behaviour.

Dean et al. (2005) Nat Neurosci 8, 1684-1689.

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