letters to nature

Methods

Tree access

We accessed tree crowns by shooting arrows trailing filament over branches with a powerful bow. Rope was then hauled over the branches and climbed via mechanical ascenders. Access to the treetop was achieved by arborist-style techniques. Heights were measured by lowering weighted fibreglass measuring tapes from the treetop to average ground level.

Physiological measurements

Water potential of small branches ($\leq$15 cm length) located within 1 to 3 m of the main trunk was measured using a pressure chamber (PMS Instruments). Measurements of photosynthesis used a portable photosynthesis system (LI6400, LiCor) with a 2 cm $\times$ 3 cm chamber with red/blue LED light source. Photosynthesis was measured under controlled conditions: air temperature, 22 $\pm$ 1 °C; CO_2 concentration, 365 $\pm$ 10 p.p.m.; vapour pressure deficit, 1.2 $\pm$ 0.2 kPa; light, $\geq$1,400 μmol photons m^{-2} s^{-1}). Samples used for laboratory measurements of photosynthesis and pressure–volume relationships were cut from different heights, then re-cut immediately under water, allowed to re-hydrate overnight, and then measured. This produced high water potentials (-0.6 ± 0.3 MPa) and allowed comparisons of photosynthetic capacity without the influence of height-related variation in water potential. During these measurements, the C_i values did not differ significantly in foliage from different heights (239 $\pm$ 16 p.p.m., $P = 0.42$). Turgor was estimated by the pressure–volume method[29].

Morphological measurements

To determine LMA, projected surface areas of 10 second-year internodes from each sample height were measured using a digital surface-area meter (Delta T Instruments). Samples were oven-dried at 70 °C, weighed, and mean LMA calculated as g m^{-2}. Area and mass measurements included the entire foliated internode.

Stable carbon isotope composition

δ^{13}C of foliage samples was analysed at the Colorado Plateau Stable Isotope Laboratory (http://www4.nau.edu/cpsil/). In 2000, second-year internodes were collected at different heights, dried (70 °C), ground to 40 mesh, and then a subsample was pulverized, encapsulated in tin, and combusted (CE Instruments NC2100) at 1,000 °C. The resultant CO_2 was purified and its $^{13}CO_2/^{12}CO_2$ ratio was analysed by isotope-ratio mass spectrometry (Delta Plus XL, ThermoQuest Finnigan) in continuous-flow mode. The δ^{13}C values were expressed as the relative abundance of ^{13}C versus ^{12}C compared with a standard (Pee Dee Belemnite): δ^{13}C $= (R_{sam}/R_{std} - 1)1,000$‰, where R_{sam} and R_{std} are the ^{13}C/^{12}C ratios in sample and standard, respectively. The standard deviation of repeated measurements of secondary standard material was <0.1‰ (external precision).

Light environment

Hemispherical photographs were taken directly above leaf sample locations throughout tree crowns using a digital camera on a self-levelling mount. Photographs were analysed with WinSCANOPY (v.2002a, Régent Instruments Inc.) to calculate direct site factor, which is the average proportion of direct radiation received during the 12-month growing season.

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Perceived luminance depends on temporal context

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Brightness—the perception of an object's luminance—arises from complex and poorly understood interactions at several levels of processing[1]. It is well known that the brightness of an object depends on its spatial context[2], which can include perceptual organization[3], scene interpretation[4], three-dimensional interpretation[5], shadows[6], and other high-level percepts. Here we present a new class of illusion in which temporal relations with spatially neighbouring objects can modulate a target object's brightness. When compared with a nearby patch of constant luminance, a brief flash appears brighter with increasing onset asynchrony. Simultaneous contrast, retinal effects, masking, apparent motion and attentional effects cannot account for this illusory enhancement of brightness. This temporal context effect indicates that two parallel streams—one adapting and one non-adapting—encode brightness in the visual cortex.

We report here a novel illusion in which temporal relationships affect brightness perception. Two flashes appeared on either side of a fixation point: one was brief (56 ms), the other long (278 ms; Fig. 1a). Observers reported which flash appeared brighter. When flashes of identical luminance had simultaneous onset, subjects

reported that the brief flash looked dimmer than the long flash (Fig. 1b). This was expected from the Broca–Sulzer effect, in which a flash of brief duration looks dimmer than a physically identical flash presented for a longer duration[7]. However, when the two flashes had simultaneous offset, the brief flash appeared brighter (Fig. 1b; $t = -5.32$, $P = 0.0009$). This surprising brightness enhancement averaged 30% and was seen by all observers tested. When the brief flash occurred at intermediate times (between onset and offset of the long flash), the brightness grew monotonically (Fig. 1c). We call this illusion the temporal context effect (TCE; see the online demonstration at http://nba.uth.tmc.edu/homepage/eagleman/TCE).

It could be that the brief flash looks the same in all conditions but is reported as brighter because it is compared with a dimming long flash. To address this possibility we tested whether the long flash dims perceptually. Observers were asked whether they detected a change in flashes that either physically ramped up or down in luminance or stayed constant. Observers reported that a physically
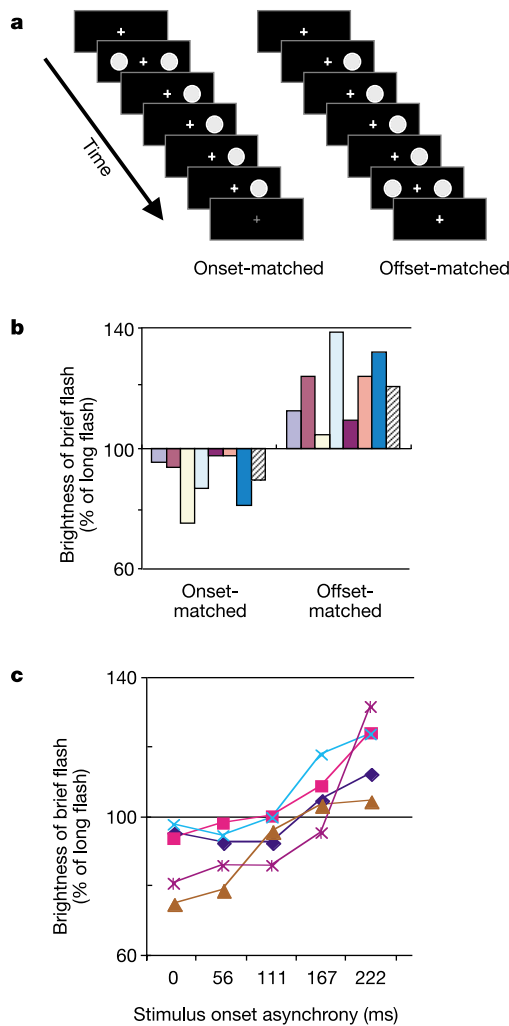
unchanging flash was perceived as unchanging; that is, the observers (at least in these conditions) were adaptation-blind (Fig. 2a). This suggests that relative timing actually changes the brightness of the brief flash. To test this, we presented the stimulus shown in Fig. 2b: a brief flash is onset-matched with two long flashes; a second brief flash is offset-matched with the long flashes. Observers directly compared the two brief flashes against each other. The offset-matched brief flash appeared brighter than the onset-matched brief flash. This effect depends on the presence of the long flashes: in a control experiment, in which only the brief flashes were presented, no brightness difference was seen between them (see Supplementary Information). In a second control, we found that in static displays of three patches, dimming the patches corresponding to the long ones in Fig. 2b did not induce simultaneous contrast effects that could account for the TCE (see Supplementary Information). Thus, the appearance of the brief flash itself changed, as a result of temporal context.

The TCE does not arise from low-level interactions in the retina or lateral geniculate nucleus, because presenting the brief flash to one eye and the long flash to the other produces the full effect (see Supplementary Information). This indicates that the comparison of luminance ratios takes place in primary visual cortex or later, where binocular information first converges. Further, the TCE appears unrelated to apparent motion or meta-contrast masking, because it is surprisingly insensitive to spatial distance (Fig. 2c). This insensitivity can easily be demonstrated by presenting the long and brief
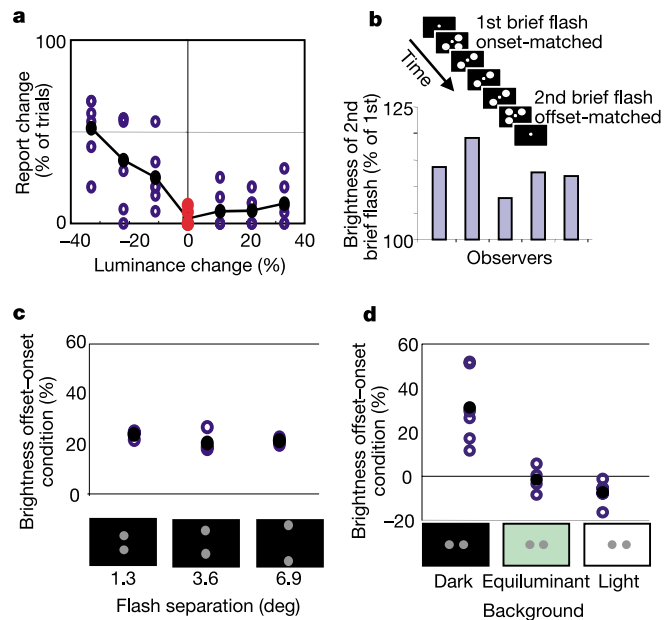

Figure 1 The temporal context effect. **a**, Time slices (56 ms) of two stimulus conditions. **b**, In a two-alternative forced-choice task, observers reported whether the brief flash was brighter or dimmer than the long flash. Brightness on the ordinate is determined by the point of perceived equivalence on a psychometric function, derived from the method of constant stimuli. The long flash had a luminance of 5.91 cd m^{-2}, the brief flash ranged from 3.55 to 8.27 cd m^{-2} and the background was less than 1 cd m^{-2}. $n = 7$. The average is shown with a hatched bar. **c**, Brightness of the brief flash at intermediate stimulus onset asynchronies.

Figure 2 Only the brief flash changes perceptually. **a**, Adaptation-blindness. The abscissa shows the total change of flash luminance during the 278-ms duration (linear ramping). Subjects reported whether the flash did or did not change in brightness during its appearance. Subjects are better at detecting decrements than increments[15], but the emphasis for this study is on the zero point of the x-axis: a physically unchanging flash is perceived as unchanging. Stimulus properties and starting luminance are identical to those of the long flashes in Fig. 1. $n = 6$. **b**, Observers compared the brightness of the diagonal brief flashes in a two-alternative forced-choice task. **c**, Insensitivity to distance. Stimuli were enlarged according to a cortical magnification factor[16] for easier judging. $n = 3$. **d**, Contrast polarity determines the direction of brightness change: the experiment shown in Fig. 1b was repeated with dark (less than 1 cd m^{-2}), equiluminant (grey flashes against a 5.91 cd m^{-2} green background), and light (11.82 cd m^{-2}) backgrounds. The luminance of the long flash was 5.91 cd m^{-2}; the luminance of the brief flash ranged between $\pm$ 40% of that of the long flash. The ordinate shows the percentage difference between the two conditions.

flashes at opposite edges of the computer screen (see Supplementary Information).

We also investigated the possibility that the offset-matched flash appears brighter (or perhaps more salient) because attention shifts to it. However, attention shifts are slow ($\sim$200–300 ms, ref. 8), whereas the brief flash appears and disappears within 56 ms. Nonetheless, we manipulated the contrast polarity of the background to test the attentional explanation. When the background was brighter than the flashes, the offset-matched brief flash appeared dimmer, not brighter, than the onset-matched flash (Fig. 2d; $t = -2.2$, $P = 0.026$). More importantly, when the background and flashes were made equiluminant, observers perceived no significant brightness illusion, arguing against a salience-enhancing attentional shift (Fig. 2d; $t = -0.58$, $P = 0.28$).

Spatial interactions in brightness perception are well known[1,2] but provide an incomplete description; the TCE shows that brightness is influenced by temporal context as well. Our results further show a surprising juxtaposition of facts: first, that information encoded about the brightness of stimuli changes over time, such that the appearance of physically identical brief flashes compared to a persisting long flash varies as a function of stimulus onset asynchrony (Fig. 1c); and yet, second, the perceived brightness of a long flash remains constant over time (Fig. 2a). This indicates that brightness encoding might involve at least two neural populations: one with an adapting response that diminishes over time, and the other with a downstream response that assigns brightness labels to objects and does not adapt. We propose that the TCE arises from an interaction between these non-adapting and adapting encodings. In our model, activity in the non-adapting population remains constant—thereby encoding an unchanging label—even while its input from the adapting population diminishes (Supplementary Fig. S1; for an example of such hysteresis, see ref. 9). The hypothesis that some neurons maintain a brightness label is consistent with the idea that the brain strives to monitor the external world undistracted by predictable changes in its own physiology.

The activity of some neurons in primary visual cortex is correlated with brightness; that is, the firing rate varies as a function of surrounding luminance, even as the luminance in the receptive field remains unchanged[10–14]. The activity of these brightness-responsive neurons adapts with time: the firing rates peak after 100 ms and then drop off precipitously[14]. This decrease in firing is consistent with an adapting population, although in some circumstances the later part of the spike train may correspond to the response of a non-adapting population[14]. This raises the possibility that the two encodings could be multiplexed into the same population of cells in V1. The TCE separates the physical measure of luminance and the perceptual quality of brightness (which typically co-vary) and offers a new approach for examining the neural coding of brightness. □

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Integration of quanta in cerebellar granule cells during sensory processing

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To understand the computations performed by the input layers of cortical structures, it is essential to determine the relationship between sensory-evoked synaptic input and the resulting pattern of output spikes. In the cerebellum, granule cells constitute the input layer, translating mossy fibre signals into parallel fibre input to Purkinje cells[1]. Until now, their small size and dense packing[1,2] have precluded recordings from individual granule cells *in vivo*. Here we use whole-cell patch-clamp recordings to show the relationship between mossy fibre synaptic currents evoked by somatosensory stimulation and the resulting granule cell output patterns. Granule cells exhibited a low ongoing firing rate, due in part to dampening of excitability by a tonic inhibitory conductance mediated by $GABA_A$ (γ-aminobutyric acid type A) receptors. Sensory stimulation produced bursts of mossy fibre excitatory postsynaptic currents (EPSCs) that summate to trigger bursts of spikes. Notably, these spike bursts were evoked by only a few quantal EPSCs, and yet spontaneous mossy fibre inputs triggered spikes only when inhibition was reduced. Our results reveal that the input layer of the cerebellum balances exquisite sensitivity with a high signal-to-noise ratio. Granule cell bursts are optimally suited to trigger glutamate receptor activation[3–5] and plasticity[6–8] at parallel fibre synapses, providing a link between input representation and memory storage in the cerebellum.

Cerebellar granule cells are the smallest and most numerous neurons in the mammalian brain and have a simple morphology with an average of only four short dendrites[1,2], each dendrite receiving a single excitatory mossy fibre input. Each granule cell also receives inhibitory input from one or two Golgi cells[1,2], the major granular layer interneuron (Fig. 1a). The combination of such a small number of inputs to an electrotonically compact structure[9,10] provides an ideal model in which to study synaptic

Supplementary material
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A suggested model for the Temporal Context Effect

We suggest that a simple model (Fig S1) may explain our results: (1) the system commits to brightness values of objects in the scene, (2) these commitments hold even while neural responses adapt, (3) V1 neural activity encoding the brightness of two objects is compared, and the difference of activities produces a difference between assigned labels. Since the commitment to the first label is kept, that forces the second label to increase. Because the activities of neurons in primary visual cortex diminish, the TCE suggests that a different neuronal population (perhaps outside of V1) retains brightness labels. This second system could utilize, for example, the maintained level of activity in a downstream coalition of neurons. A coalition that wants to be adaptation-blind should stay high even as the input driving it adapts (hysteresis).
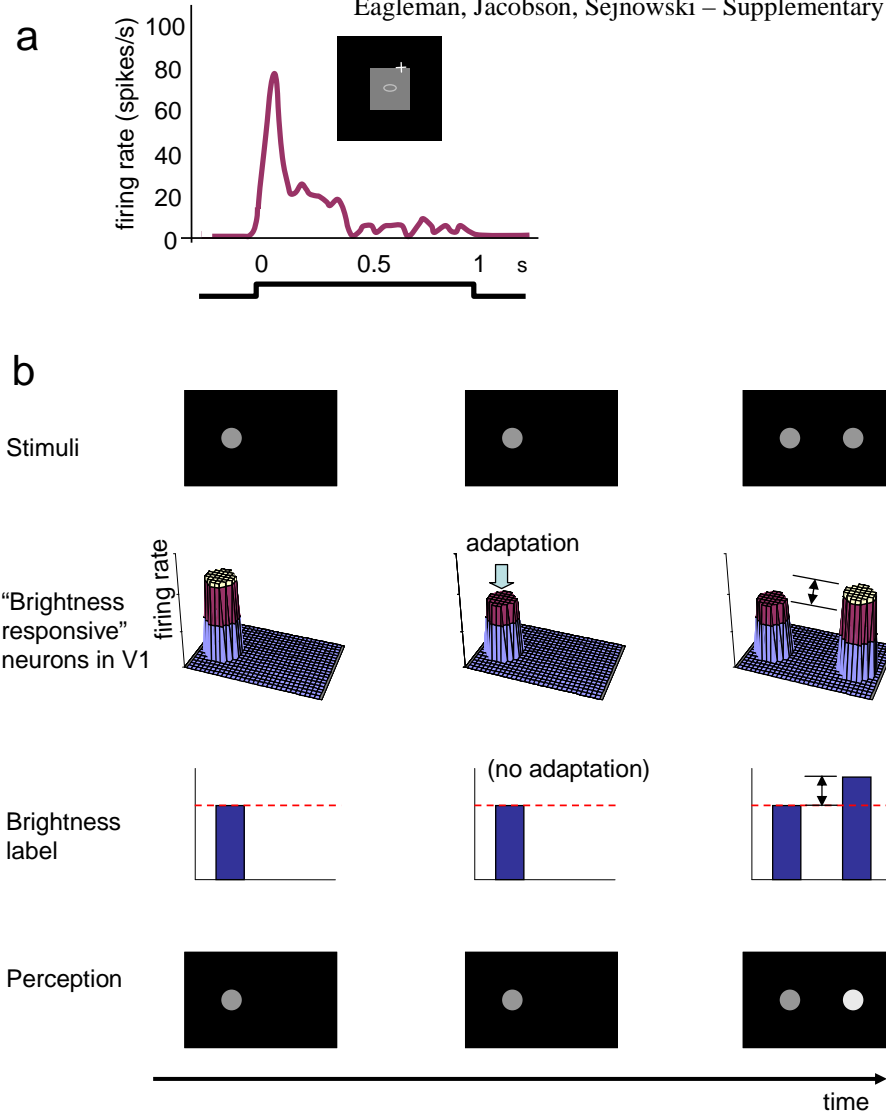
As a point of clarification: in our model, there is no *initial* V1 response difference for any of the flashes. The only thing that changes is the V1 response to the *long* flash, which decreases monotonically through time. (See arrow marked 'adaptation', Fig S1). The experimental justification for the adaptation of the V1 response to the long flash comes from Kinoshita & Komatsu (2001), who demonstrate the diminishing firing rate from the V1 brightness encoding cells when there is a dark background. (This diminishment is, of course, shown by essentially all neuron types). The activity generated by the brief flash (whether onset- or offset-aligned) will be compared against different activity levels of the long flash, depending on how long the latter has already been on.

As for *why* V1 activities are compared instead of the labels, we can only say that this hypothesis (comparison of V1 activities) gives us a parsimonious and consistent account of the data we have reported. The alternative hypothesis – a comparison between the labels – does not explain the brightness illusion. The illusion reported here is not, of course, the sole basis for the model. Instead, the observations leading us to the model are outlined in the first sentences of the abstract: understanding brightness encoding cells in the face of (1) neural adaptation, but (2) no perceptual adaptation. Combined with our results, we are led to propose that brightness labels are assigned to newly-appearing objects, but that brightness *comparisons* take place at a different level. The results of comparisons are combined with previous label commitments. Further accounting of the neural circuitry may help us to understand in the future why our proposed account matches the data. As a further point of clarification: while our hypothesis suggests that V1 activities are compared against one another, but this does not necessitate the comparison takes place *in* V1.

**What happens when the two flashes are not simultaneously present?**

We have also performed experiments in which there in no temporal overlap between the two flashes. When the SOA is -56 msec, for example, or 278 msec, the perceived

luminance of the brief flash moves toward equivalence with the long flash (i.e., the 100% mark; Fig S2). In other words, at negative or long SOAs, two flashes of equivalent luminance will appear equivalent. We observed some interesting results in these parameter ranges, including the fact that the variance of observers' reports rises steeply when the flashes are not simultaneously present. We cannot presently speculate on this variance difference except to note that a very different kind of comparison might be occurring when (a) there are two flashes present (main manuscript) versus (b) a comparison between a flash and the *memory* of a second flash (Fig S2). We will analyze these 'non-simultaneous flash' results in a separate manuscript, since they address a different testing condition – i.e., one that requires *memory* of the flash.

a

firing rate (spikes/s)

100
80
60
40
20
0

0   0.5   1   s

b

Stimuli

"Brightness responsive" neurons in V1

firing rate

adaptation

Brightness label

(no adaptation)

Perception

time

Supplementary Figure S1. (a) Response of a brightness-responsive neuron in awake macaque primary visual cortex, figure adapted from Kinoshita & Komatsu, Fig 7B (2001). In response to a gray stimulus on a black background, the response of this neuron grows quickly but begins to diminish after 100 ms. (b) Hypothesized model. The top row shows the sequence of stimuli in the offset-aligned condition; the long and brief flashes have identical luminance. The 2nd row shows a cartoon of the corresponding neural activity of brightness-responsive neurons in V1 (Rossi & Paradiso, 1996; Rossi et al, 1999; Kinoshita & Komatsu, 2001). As the long flash remains on the screen, the associated neural activity diminishes. The eventual appearance of the brief flash generates an unadapted neural response. 3rd row: In contrast to the brightness-responsive cell activity, the brightness label assigned to the long flash does not diminish. When the brief flash appears at the end of the sequence, a difference in neural

activities is translated into a difference in brightness labels. Since the system has already committed to labeling the long flash at a certain brightness value, the brief flash's label will be promoted to something higher than that value. The perceptual correlate is illustrated in the bottom row: the long flash does not appear to change in brightness, and the brief flash appears much brighter.
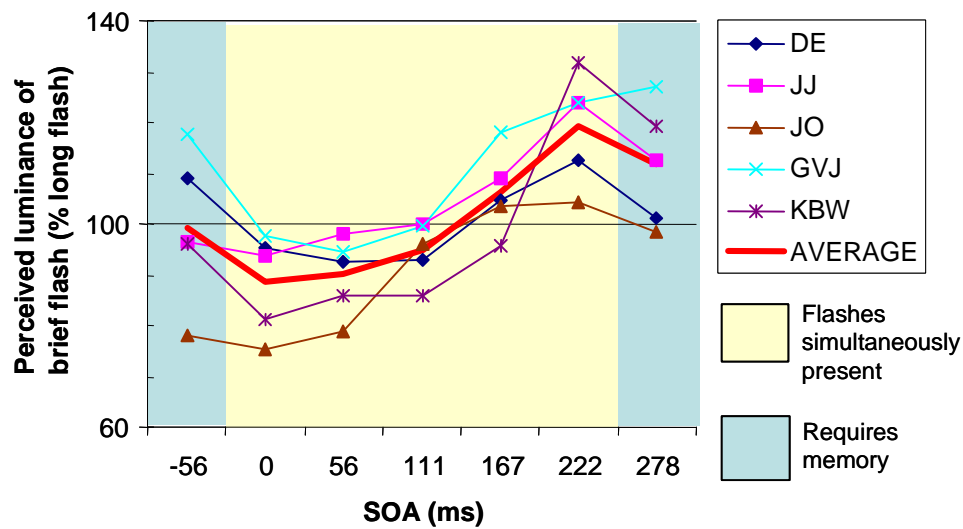
Supplementary Figure S2. Brightness of the brief flash, including the non-simultaneous regime. The experimental paradigm is the same as the one shown in Fig 1b, but additionally showing results when the two flashes have no temporal overlap (blue region).