Attentional modulation in visual cortex depends on task timing

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Paying attention to a stimulus selectively increases the ability to process it. For example, when subjects attend to a specific region of a visual scene, their sensitivity to changes at that location increases. A large number of studies describe the behavioural consequences and neurophysiological correlates of attending to spatial locations[1–8]. There has, in contrast, been little study of the allocation of attention over time[9,10]. Because subjects can anticipate predictable events with great temporal precision[11–15], it seems probable that they might dynamically shift their attention when performing a familiar perceptual task whose constraints changed over time. We trained monkeys to respond to a stimulus change where the probability of occurrence changed over time. Recording from area V4 of the visual cortex in these animals, we found that the modulation of neuronal responses changed according to the probability of the change occurring at that instant. Thus, we show that the attentional modulation of sensory neurons reflects a subject's anticipation of the timing of behaviourally relevant events.

Two rhesus monkeys were trained to maintain fixation on a small dot and release a lever when a stimulus at a cued peripheral location changed orientation. The monkeys were required to ignore orientation changes of stimuli presented simultaneously at one to three other locations (Fig. 1a). The physiological effects of spatial attention were assessed by comparing responses from individual neurons between trials in which the visual stimulation was identical, but the cued location was different. Orientation changes occurred at random times after stimulus onset, but the probability of a change occurring as a function of time within each trial was fixed (Fig. 1b). The same probability distribution was used for changes at all stimulus locations. The behaviourally relevant probability for the monkeys was the probability that a change would occur at a given point in time, given that no change had occurred yet (Fig. 1c). This conditional probability is called the hazard function.

Data were collected from 80 V4 neurons. Figure 2 illustrates data from a typical neuron. Responses to visually identical stimuli are aligned to stimulus onset and plotted as a function of time for two cases: when attention was directed to a stimulus within the receptive field of the cell (Fig. 2a) and when attention was directed to a stimulus outside the receptive field (Fig. 2b). In some respects, the responses in these two cases were similar—there was little spontaneous activity (grey line) before stimulus onset and a transient response to stimulus onset was followed by a moderate sustained response rate. Although the transients differed little, the sustained response was much larger when attention was directed within the receptive field of the cell (Fig. 2, compare panels a and b). An attention index was calculated as a function of time using the responses shown in Fig. 2a and b. Attentional modulation (Fig. 2c) was not maximal at time of maximal response, which occurred shortly after stimulus onset, but grew with time, paralleling the increase in the hazard function (Fig. 2d). Unexpectedly, attentional modulation was negative before stimulus onset, indicating a relative suppression of activity at the cued location. The large change in attentional modulation over the course of the trial shows that the typical measure of attentional modulation—a time-averaged mean over the entire stimulus presentation—can obscure important aspects of the modulation.

On average the attentional modulation of neurons increased gradually during trials (Fig. 3a, solid line). Although this increase over time may result from the subjects' representations of trial statistics (Fig. 3a, dotted line), it is also possible that this correlation is coincidental, and that the observed dynamics reflect a fundamental characteristic of spatial attention after stimulus onset. One reason to believe that this time course is not fundamental is the fact that other reports have suggested different modulation dynamics[4,6,16]. To establish more conclusively the dependence of attentional modulation on task timing, we retrained the same monkeys for 3 weeks on the identical task, but with an altered hazard function (dashed line, Fig. 3b) and recorded from an additional 83 V4 neurons. Whereas the original task had an initial period of 500 ms in which there were no changes in orientation, in the modified task there was a high probability of change during this period and a period of zero probability in the subsequent 500 ms. Figure 3 illustrates the physiological effects of this change. With the modified timing, there was a sharp rise in attentional modulation shortly after stimulus onset that was not seen with the previous timing Fig. 3, compare b and a). Moreover, in contrast to the smoothly rising modulation seen in the first recording session and in most previous reports[4,6,7,16], attentional modulation did not rise monotonically with time. Instead, it decreased shortly after the hazard function went to zero. For both timings, the average eye position difference between attentional states never exceeded 0.01° at any time during the trials.

Although task timing changed the time course of attentional modulation, it does not seem to be the sole determinant of the time course change. For example, in both timing sets there are peri-stimulus periods when the hazard function was zero and attentional modulation was positive, and pre-stimulus periods when the hazard function was zero and attentional modulation was negative. In schedule 1, the attentional modulation of the population of cells started to rise before the rise in the hazard function (Fig. 3a), whereas in schedule 2, the initial rise in attentional modulation

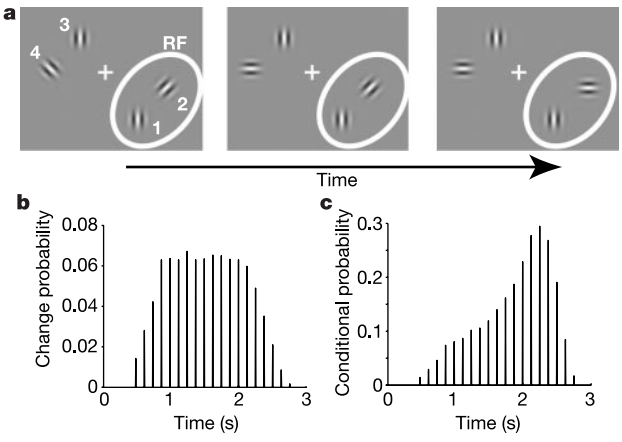

Figure 1 Orientation change detection task. **a**, Stimuli of a typical trial. Animals fixated on a central spot throughout the trial (cross). Stimuli were located at four different locations: two adjacent locations within the receptive field (oval, positions 1 and 2) and opposite the receptive field (positions 3 and 4). If the animal was cued to attend to position 2 (an 'attend in' location), it would have to release a lever when a change happened at this location (middle and right panels), while ignoring the change at position 4 (left and middle panels). Attentional modulation was measured by comparing responses for visually identical trials in which the animal attended to different locations. **b**, Orientation changes occurred at random times after stimulus onset according to the indicated probability distribution. **c**, The behaviourally relevant probability is the probability that a change will occur at a given point in time given that one has not occurred already (hazard function). The hazard function is zero for the first 500 ms (no changes occurred) and then rises to a maximum 2.25 s after stimulus presentation.

followed the rise in the hazard function by about 150 ms (Fig. 3b). To study the relationship between the hazard function and attentional modulation, correlation coefficients were computed after smoothing and shifting the two functions from the same schedule. Owing to the largely monotonic nature of both the hazard function and the attentional modulation in schedule 1, the schedule 1 functions were well correlated over a wide range of temporal shifts. On the other hand, schedule 2 correlations in both animals were maximal when the hazard function was delayed by 150 ms. It is therefore unclear whether this 150-ms delay between task timing and attentional modulation can differ between tasks, or if instead it is constant across tasks with different timings.

To quantify the relative contribution of the trained hazard functions towards attentional modulation, we applied a multiple regression model in which attentional modulation depended on the hazard functions of the two schedules. Regression coefficients were standardized to establish the relative importance of the two hazard functions in explaining attentional modulation (Table 1). For both animals and with both schedules, regression coefficients were significantly larger for the schedule type that preceded recording (Table 1, bold). This was true for a wide range of temporal offsets (100–450 ms) between the attentional modulation and the hazard functions. The attentional modulations of individual cells (Fig. 3c, d) were also correlated with the appropriate hazard function. For the first recording session, neuronal modulations were better correlated with schedule 1 than schedule 2 (above the diagonal line, Fig. 3c, paired-sample Wilcoxon $P \ll 0.01$); for the second recording session (after schedule 2 training), neuronal modulations were better correlated with schedule 2 (below the diagonal line, Fig. 3d; paired-sample Wilcoxon, $P < 0.01$). In both animals, the appropriate hazard function regression coefficient was poorer with the second schedule than the first. It is unclear whether this lower correlation is due to the more complicated temporal structure of the second schedule, or because the animals had received less training in the second schedule than in the first.

There are several reasons to doubt that this change in attentional modulation would have occurred had the animals not been exposed to schedule 2. First, the time course of the modulation after training schedule 2 is not simply a scaling or shifting of the time course seen after schedule 1. Instead, attentional modulation decreases in the schedule 2 population of cells during an interval over which it is increasing in the schedule 1 population (750–1,000 ms). Second, if the dynamics observed after schedule 2 were the inevitable result of training, then it should have been observed in other reports. However, almost all previous reports have indicated a monotonic rise in attentional modulation after stimulus presentation. Third, if this time course was a simple consequence of training, then we might expect differences between the animals because of the different time courses of their training: animal 2 was trained with schedule 2 two months after recording the data from schedule 1, whereas animal 1 performed no tasks for almost a year in between the two schedules. Moreover, no obvious changes were observed over a period of weeks: the modulations observed during the first few weeks of recording were similar to those observed in the final weeks for both schedules. If schedule 2 attentional modulation is an inevitable result of training then we would expect the regression coefficient associated with schedule 2 to increase over time and the coefficient associated with schedule 1 to decrease over time. Instead, comparing the first and second halves of the recording sessions, the schedule 2 coefficient remained near zero in recording session 1 (-0.07 compared with 0.06) and decreased in recording session 2 (0.54 compared with 0.23), and the schedule 1 coefficient increased slightly in both sessions (session 1, 0.68 compared with 0.76; session 2, 0.06 compared with 0.16). Finally, animal 2 was trained in a different threshold level detection task in which there was a flat hazard function subsequent to these experiments, and no modulations in reaction time or attentional modulation were observed (data not shown).

To examine the specific aspects of attentional modulation that changed with the change in task timing, attentional modulation was quantified for each neuron in the two timing populations according to five different parameters (Table 2). Significant changes were seen only in those parameters related to timing. As expected, increases in

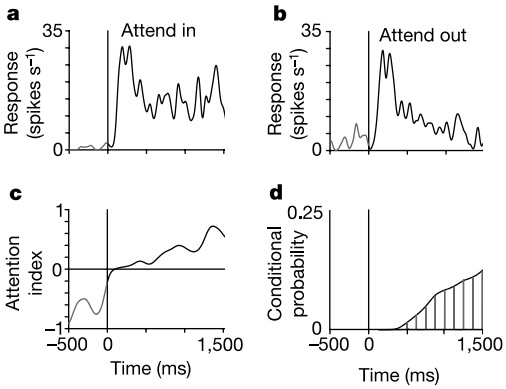

Figure 2 Dynamic attentional modulation in a V4 neuron. **a**, **b**, Averaged responses for trials in which a single stimulus was present in the receptive field, and a single stimulus was located in the quadrant diagonally opposite to the receptive field. For the trials included in **a**, the behaviourally relevant change occurred within the receptive field (attend in); in **b**, the change occurred in the opposite visual quadrant (attend out). **c**, Increasing effect of spatial attention with time: the attention index, based on the response difference between trials for **a** and **b**, increases during the course of a trial. **d**, The hazard function, which increases at intervals of 125 ms (unsmoothed, grey; interpolated, black) during the period over which responses were observed for this cell. All trials are aligned to stimulus onset.

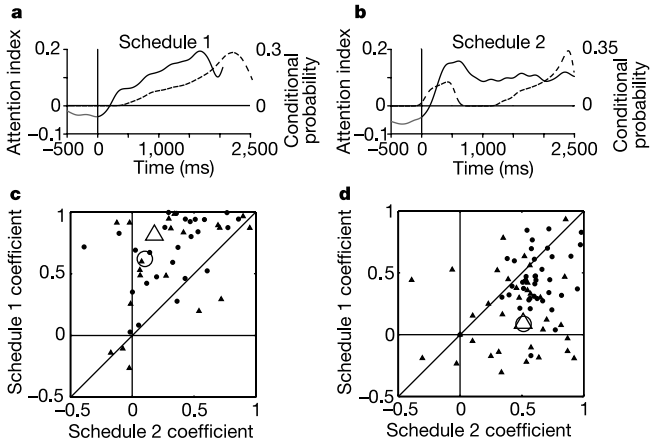

Figure 3 Attentional dynamics change according to task timing. **a**, **c**, Population attentional modulation averaged over all stimuli (schedule 1). **b**, **d**, Population attentional modulation for a sample of 83 neurons after retraining with a new task timing (schedule 2). Timing schedules (hazard functions) are shown as a dashed line (**a**, **b**) and attentional modulation as a solid line. All data have been smoothed by gaussian convolution (s.d. = 75 ms). In schedule 1, the attention index gradually rises to a maximum late in the trial (**a**). In schedule 2, the attention index rises more rapidly, reflecting an initially high change probability, and then slightly decreases during a zero change probability period starting at 500 ms. Standardized regression coefficients between the attentional modulation of individual cells and task timing show a similar change in both animals (**c**, **d**; circles, monkey 1; triangles, monkey 2). For the first recording session attentional modulation is significantly better correlated with schedule 1 than schedule 2 (**c**), whereas in the second recording session after schedule 2 training, the converse is true (**d**). The large symbols are the coefficients between the population responses in each animal and task timing (Table 1).

Table 1 **Standardized partial regression coefficients of attentional modulation**

Coefficient	Monkey 1		Monkey 2	
	Recording session 1	Recording session 2	Recording session 1	Recording session 2
Schedule 1	**0.62**	0.11	**0.81**	0.11
Schedule 2	0.10	**0.52**	0.18	**0.51**
Constant	0.25	0.33	0.01	0.32

To facilitate comparison all data were convolved with a gaussian (s.d. = 75 ms) and the hazard function was delayed to 150 ms before computing the correlation coefficient. This was the optimal delay seen in schedule 2 for both animals. Confidence intervals of 99% for all coefficients were between 0.03 and 0.04.

attentional modulation occurred significantly earlier with schedule 2 than with schedule 1. This task dependence underscores the difficulty in trying to use the latency of attentional effects as a guide to determining the physiological origin of attentional signals. On the other hand, the average magnitude of attentional modulation did not seem to vary between the two timing populations.

Previous reports have suggested, however, that attentional modulation depends on low-level factors such as the particular stimulus used to evoke responses. For example, attentional modulation can vary spatially within the receptive field[5] and depend on the orientation[17] and number of stimuli[1] within the receptive field. We studied the stimulus dependence of attentional modulation by grouping trials according to particular stimulus attributes: orientation, location and number of stimuli within the receptive field. We measured attentional modulation for single Gabor stimuli of two different orientations (preferred and null) and at two locations within the receptive field (preferred and non-preferred). To study the effect of orientation, both locations were considered, whereas to study the effect of location, both orientations were considered. The mean response difference between preferred and null orientation was 33%; between preferred and non-preferred location it was 20%. Both the time course and magnitude of attentional modulation were similar for preferred and non-preferred orientations (Fig. 4a, d). This is consistent with the finding that the magnitude of V4 attentional modulation is independent of the stimulus orientation[6]. Similarly, both the magnitude and the time course of attentional modulation were well matched at the preferred and non-preferred locations (Fig. 4b, e). Finally, the attentional modulation seen with two Gabor stimuli within the receptive field was similar in time course and magnitude to the attentional modulation seen when only one Gabor stimulus was present in the receptive field (Fig. 4c). Although contrary to some reports[1,4], these data are consistent with other reports showing significant attentional modulation when only a single stimulus is present within the receptive field[2,6,7,16,18]. In all of these cases, the relative contribution of stimulus variation, as measured by standardized regression coefficients, was significantly smaller than the contribution of the appropriate hazard function to attentional modulation. These results show that the attentional modulation of a neuronal population can be relatively unaffected by many variations in stimulus configuration, including the number of stimuli within a receptive field. Moreover, they demonstrate that such constancy can hold even when retraining causes large changes in the dynamics of attentional modulation. One unresolved issue is whether such consistency would be visible throughout visual cortex, even among neuronal signals not used in the task or with very different maximal attentional modulations.

One observation that has not been reported previously is that the average attentional modulation at a behaviourally relevant location can be negative for periods of time. This relative suppression of spontaneous activity at the attended location was seen across both timing sets and all stimuli (Fig. 4). Because the attentional modulation metric involves a subtraction between responses in which spatial attention has been shifted, it is unclear whether this negative value reflects a transient facilitation of responses at unattended locations, or a suppression of responses at the attended location. For example, suppression of visual responses to stimuli presented at previously attended locations has been observed in area 7a[19,20]. Whether it reflects spatially specific facilitation or suppression, the cause of the negative modulation is unclear. It is not a direct effect of cueing[21], because a cue stimulus was not presented at the beginning of the trials.

Our results show that subjects form an internal representation of task timing acquired during the course of training, and then use this representation to temporally modulate behaviourally relevant visual responses. Unlike the spatial cues, there was no explicit requirement for the animals to monitor task timing and modulate their attention accordingly. Moreover, task timing was changed without any explicit cue or instruction to alert the animals to the change. The consistency of results between the animals therefore suggests that temporal strategies are commonly adopted, and that these strategies reflect task timing. Thus, just as task difficulty can affect the magnitude of attentional modulations[17], so can task timing affect the timing of such modulations. Although the two monkeys were able to perform the required task at an accuracy level of 90% after only about an hour of training on the second timing, it remains to be established whether attentional modulations can change on a similar timescale.

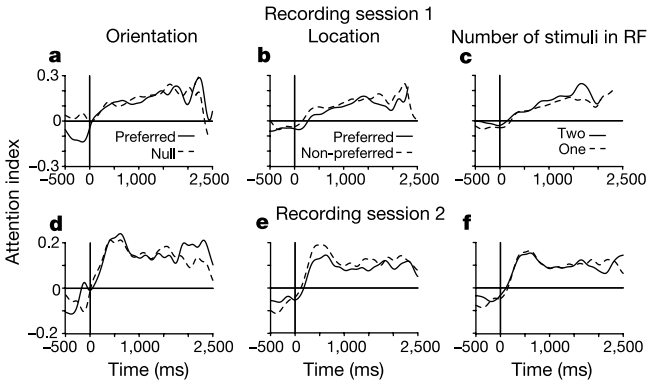

Figure 4 Population attentional modulation with different stimuli. **a**–**c**, Average attention index for V4 neurons from two animals after schedule 1 training. **d**–**f**, Index for the population of neurons studied after schedule 2 training. In **b** and **e**, modulation is examined according to whether or not the attended stimulus was at the preferred location within the receptive field; in **a** and **d**, according to the orientation of the stimuli; and in **c** and **f**, according to stimulus number within the receptive field (RF). Both the time course and the magnitude of attentional modulation in the neuronal population are similar for different stimuli (solid versus dashed lines). For all stimuli, the time course of attentional modulation is affected similarly by the change in task timing.

Table 2 **Median parameters of attentional modulation index among single neurons**

Parameter	Recording session 1	Recording session 2
Time of zero crossing (ms)*	225	168
Time of half-maximum (ms)†	415	254
Magnitude during pre-stimulus period	−0.06	−0.07
Maximum magnitude	0.23	0.22
Slope at zero crossing (s^{-1})	0.63	0.72

*Wilcoxon, $P < 0.02$.
†Wilcoxon, $P < 0.001$.

Whatever their origins, the existence of rapid response modulations shows that the effects of attention on individual cells or neuronal populations cannot be fully characterized by a single constant number that applies to all tasks. Such modulations pose a challenge to interpreting physiological data when behavioural timing is not strongly constrained. For example, when stimuli are presented for hundreds of milliseconds, the animal might attend only within a particular period of time such as the beginning or end of the presentation, or alternatively might randomly choose different periods from trial to trial in which to concentrate perceptual resources. When animals are free to use different temporal strategies, the neuronal modulations observed from trial to trial, or from cell to cell, might reflect a choice of strategy more than a fundamental characteristic of spatial attention. Such task-related changes in strategy can have a much larger effect on the attentional modulation of sensory responses than changes in sensory input itself (Fig. 4). Given that temporal strategies will probably arise in almost any task with which a subject becomes familiar, such effects should be visible across a broad range of sensory modalities and tasks. Our results highlight that extraretinal effects on sensory signals reflect not only immediately defined task requirements such as the location or content of a cue, but also strategies based on accumulated experience. □

Methods

Visual stimuli

The stimuli were Gabor stimuli of sinusoidally varying contrast (4 Hz, 100% peak contrast) truncated at a radius of twice the standard deviation of the gaussian envelope of the Gabor. Gabor stimuli were modulated around a mean luminance achromatic point in colour space that also defined the background. Single Gabor stimuli of varying chromatic modulation, size, spatial frequency, location and orientation were used to characterize receptive fields and specify the stimulus parameters used in the attention task. Spatial receptive fields were mapped quantitatively using single Gabor stimuli. We tested all well-isolated, visually responsive cells for attentional effects.

Task design

Two monkeys (*Macaca mulatta*) performed an orientation change detection task. Trials were presented in block mode (12 or 15 trials), in which the behaviourally relevant location was fixed within each block. Animals were required to fixate on a small dot (~0.1°) throughout each trial (fixation widow width = 1.0° to 1.4°). Attention was spatially cued using instruction trials at the beginning of each block, in which only a single stimulus was presented. Subsequent trials within the block, which were used in the data presented here, included stimuli at this cued location as well as at other locations. Stimuli were presented at two non-overlapping locations within the receptive field. These adjacent locations were always at the same eccentricity (between 2° and 5°) and offset symmetrically from the receptive field centre (positions 1 and 2 in Fig. 1a). In addition to these receptive field stimuli, stimuli were presented simultaneously at symmetrical locations in the quadrant diagonally opposite from the receptive field, so that during each trial there were either two or four Gabor stimuli present. Trials with two Gabor stimuli were randomly interleaved with trials in which four Gabor stimuli were presented. Cue location randomly switched between the four possible locations on block completion. Cells used in this study had at least eight blocks completed for each cued location.

The monkeys' task was to release a lever as soon as a change occurred at the cued location, while ignoring changes occurring at any other location. Orientation changes occurred only when the counterphasing Gabor stimuli reached zero contrast (every 125 ms, see Fig. 1c, d) and changes at different locations were forced to occur at different times. At each location no more than one orientation change occurred during the trial. Animals were rewarded with juice when they released a lever between 250 and 450 ms after a change at the cued location. Releases at any other time or eye movements outside the fixation window immediately terminated the trial without reward. About 10% of trials were 'catch' trials in which no change occurred at the cued location and the monkey was rewarded for keeping the lever depressed and maintaining fixation. The monkeys' performance, excluding fixation breaks, was greater than 90% for all data presented here and was independent of the time at which the behaviourally relevant change occurred.

Recording

Recordings were made from individual neurons in area V4 on the prelunate gyrus in daily sessions using transdural electrodes (0.5–1.5 MΩ at 1 kHz) and conventional extracellular recording techniques. Action potentials were recorded with a resolution of 1 ms, synchronized with the vertical retrace of the monitor. Eye position was monitored by scleral search coil. Eye position and lever releases were recorded with a resolution of 5 ms.

Data analysis

Attentional modulation was measured by comparing responses to identical stimulus configurations in different cue-location blocks. Modulation was quantified using an attention index $A(t) = (R_A(t) - R_B(t))/(R_A(t) + R_B(t))$, where R_A and R_B were the responses when attention was directed to locations A (within the receptive field) and B (opposite the receptive field), respectively. To avoid the attention index being dominated by optimal stimulus combinations, attentional modulation was computed for each stimulus combination separately before averaging. For most cells, the compared trials also had identical change times for all stimuli. Because trials were of variable length, averages were computed according to the number of trials contributing to each time point. All data shown are based on trials truncated at the first change that occurred within the receptive field, whether or not that change was at the cued location. The relevant probability within a change detection trials is the conditional probability that a change will occur at a given time in the trial given that a change has not already occurred. This is called the hazard function and is given by $H(t) = p(t)/(1 - \int_0^t p(x)\mathrm{d}x)$, where $p(t)$ is the change probability function. The hazard functions of this study go to zero because long trials are likely to be catch trials in which no orientation change takes place at the cued location. Because of the catch trials the total probability of change occurring within a trial is less than one. Correlation coefficients between task timing and physiological measurements were computed for a range of gaussian filters of varying standard deviation (1, 10, 25, 50, 75, 100, 150, 200 ms). Correlations were low for filter sizes for less than 50 ms across all temporal offsets but were about 0.7 for the optimal offset at a filter width of 75 ms and continued to rise for wider filters. For Figures 3 and 4 we used a filter width of 75 ms, which is similar to the fastest timescale over which spatial shifts in attention can be observed behaviourally[22–24]. To quantify the relationship between attentional modulation and the hazard functions, we computed multiple regression coefficients on the basis of a model in which attentional modulation was modelled as a linear sum of two hazard functions plus a constant: $A(t) = b_1H_1(t-\tau) + b_2H_2(t-\tau) + b_3$. Attentional modulation and hazard functions were convolved with gaussian filters of 75 ms s.d. and shifted before regression. The optimal temporal shift τ of the hazard functions was evaluated by miminimzing root mean square (r.m.s.) error as a function of τ and the b coefficients using the downhill simplex method. The maximum allowable shift was 150 ms in accordance with the optimal schedule 2 correlations. To evaluate the relative importance of the two coefficients (Fig. 3 and Table 1), they were normalized according to the standard deviations (σ) over time of the hazard and attentional modulation functions to compute standardized partial regression coefficients ($\beta_i = b_i\sigma_{H_i}/\sigma_A$). Stimulus effects were evaluated by incorporating additional terms into the attentional modulation model: $A(t) = b_1H_1(t) + b_2H_2(t) + b_3H_1(t)S + b_4H_2(t)S + b_5S + b_6$ where S was a dummy variable coding for the examined stimulus variation (orientation, location or stimulus number) with values of zero and one.

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1. Moran, J. & Desimone, R. Selective attention gates visual processing in the extrastriate cortex. *Science* **229,** 782–784 (1985).
2. Motter, B. Focal attention produces spatially selective processing in visual cortical areas V1, V2, and V4 in the presence of competing stimuli. *J. Neurophysiol.* **70,** 909–919 (1993).
3. Desimone, R. & Duncan, J. Neural mechanisms of selective visual attention. *Annu. Rev. Neurosci.* **18,** 193–222 (1995).
4. Luck, S., Chelazzi, L., Hillyard, S. & Desimone, R. Neural mechanisms of spatial selective attention in areas V1, V2, and V4 of macaque visual cortex. *J. Neurophysiol.* **77,** 24–42 (1997).
5. Connor, C., Preddie, D., Gallant, J. & Van Essen, D. Spatial attention effects in macaque area V4. *J. Neurosci.* **17,** 3201–3214 (1997).
6. McAdams, C. & Maunsell, J. Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. *J. Neurosci.* **19,** 431–441 (1999).
7. Treue, S. & Maunsell, J. Effects of attention on the processing of motion in macaque middle temporal and medial superior temporal visual cortical areas. *J. Neurosci.* **19,** 7591–7602 (1999).
8. Colby, C. & Goldberg, M. Space and attention in parietal cortex. *Annu. Rev. Neurosci.* **22,** 319–349 (1999).
9. Coull, J. T., Frith, C. D., Buchel, C. & Nobre, A. C. Orienting attention in time: behavioural and neuroanatomical distinction between exogenous and endogenous shifts. *Neuropsychologia* **38,** 808–819 (2000).
10. Nobre, A. C. Orienting attention to instants in time. *Neuropsychologia* **39,** 1317–1328 (2001).
11. Treisman, M., Faulkner, A., Naish, P. & Brogan, D. The internal clock: evidence for a temporal oscillator underlying time perception with some estimates of its characteristic frequency. *Perception* **19,** 705–743 (1990).
12. Zakay, D. & Block, R. The role of attention in time estimation processes in *Time, Internal Clocks and Movement* (eds Pastor, M. A. & Artieda, J.) 143–164 (Elsevier Science, New York, 1996).
13. Harrington, D., Haaland, K. & Knight, R. Cortical networks underlying mechanisms of time perception. *J. Neurosci.* **18,** 1085–1095 (1998).
14. Westheimer, G. Visual signals used in time-interval discrimination. *Vis. Neurosci.* **17,** 551–556 (2000).
15. Migliore, M., Messineo, L., Cardaci, M. & Ayala, G. Quantitative modeling of perception and production of time intervals. *J. Neurophysiol.* **86,** 2754–2760 (2001).
16. Seidemann, E. & Newsome, W. Effect of spatial attention on the responses of area MT neurons. *J. Neurophysiol.* **81,** 1783–1794 (1999).
17. Spitzer, H., Desimone, R. & Moran, J. Increased attention enhances both behavioural and neuronal performance. *Science* **240,** 338–340 (1988).
18. Colby, C., Duhamel, J. & Goldberg, M. Visual, presaccadic, and cognitive activation of single neurons in monkey lateral intraparietal area. *J. Neurophysiol.* **76,** 2841–2852 (1996).
19. Steinmetz, M., Connor, C., Constantinidis, C. & McLaughlin, J. Covert attention suppresses neuronal responses in area 7a of the posterior parietal cortex. *J. Neurophysiol.* **72,** 1020–1023 (1994).
20. Constantinidis, C. & Steinmetz, M. Neuronal responses in area 7a to multiple stimulus displays: ii. responses are suppressed at the cued location. *Cereb. Cortex* **11,** 592–597 (2001).
21. Robinson, D., Bowman, E. & Kertzman, C. Covert orienting of attention in macaques. II. Contributions of parietal cortex. *J. Neurophysiol.* **74,** 698–712 (1995).
22. Krose, B. & Julesz, B. The control and speed of shifts of attention. *Vision Res.* **29,** 1607–1619 (1989).
23. Saarinen, J. & Julesz, B. The speed of attentional shifts in the visual field. *Proc. Natl Acad. Sci. USA* **88,** 1812–1814 (1991).
24. Verstraten, F., Cavanagh, P. & Labianca, A. Limits of attentive tracking reveal temporal properties of attention. *Vision Res.* **40,** 3651–3664 (2000).

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FGFR-related gene *nou-darake* restricts brain tissues to the head region of planarians

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The study of planarian regeneration may help us to understand how we can rebuild organs and tissues after injury, disease or ageing[1]. The robust regenerative abilities of planarians are based upon a population of totipotent stem cells (neoblasts)[2–4], and among the organs regenerated by these animals is a well-organized central nervous system[5,6]. In recent years, methodologies such as whole-mount *in situ* hybridizations and double-stranded RNA have been extended to planarians with the aim of unravelling the molecular basis of their regenerative capacities[7–11]. Here we report the identification and characterization of *nou-darake* (*ndk*), a gene encoding a fibroblast growth factor receptor (FGFR)-like molecule specifically expressed in the head region of the planarian *Dugesia japonica*. Loss of function of *ndk* by RNA interference results in the induction of ectopic brain tissues throughout the body. This ectopic brain formation was suppressed by inhibition of two planarian FGFR homologues (*FGFR1* and *FGFR2*). Additionally, *ndk* inhibits FGF signalling in *Xenopus* embryos. The data suggest that *ndk* may modulate FGF signalling in stem cells to restrict brain tissues to the head region of planarians.

Planarians have a well-organized and molecularly complex[12,13] central nervous system (CNS), which, in *D. japonica*, consists of two lobes that connect at their most anterior ends to form an inverted U-shaped brain. Each lobe is connected to a ventral nerve cord that traverses the animal along its anterior–posterior axis, and 9 lateral branches project away from each lobe towards the periphery of the head[5]. In order to identify and characterize molecules involved in the process of brain regeneration in planarians, we prepared microarrays containing 1,640 non-redundant transcripts derived from a head complementary DNA library (M.N. and K.M., unpublished results). Here we present the characterization of one such gene, which we have named "*nou-darake*" ("brains everywhere", in Japanese); the phenotype was observed in specimens of *D. japonica* that had been injected with double-stranded RNA (dsRNA).

The gene *ndk* is highly and specifically expressed in the head in both brain and non-brain tissues (Fig. 1a). During regeneration, *ndk* expression is first detected 24 h after amputation only in anterior blastemal cells, including the new brain primordium[6]. Sequence analyses reveal that *ndk* codes for a putative transmembrane protein with two extracellular immunoglobulin-like domains related to FGF receptors (Figs 1b and 2), but significantly divergent from the IgG domains found in two planarian FGF-receptor homologues recently isolated[14]. But NDK lacks the cytoplasmic kinase domains characteristic of this receptor family (Fig. 1b). Instead, the cytoplasmic domain of NDK is short, rich in serine residues, and has no significant homology to known sequences. When the entire deduced amino-acid sequence of *ndk* is considered, the highest similarity is found to the human *FGF receptor-like 1* (*FGFRL1*) gene[15].

We analysed the function of *ndk* in planarians by RNA interference (RNAi)[10,16]. Animals were injected with *ndk* dsRNA and subsequently amputated (see Methods). Silencing of *ndk* expression in dsRNA-injected animals was confirmed by whole-mount *in situ* hybridizations (Fig. 3a–d). Seven days after amputation, ectopic eyes began to differentiate in dorso-posterior regions of the body. After 15 d of regeneration, 94% of the injected animals (108/115) formed ectopic eyes (Fig. 3h), consisting of both pigment (Fig. 3h, arrows) and visual cells with projecting axons (Fig. 3i, j, arrowheads). In all of the injected animals (115/115), we also found ectopic brain tissues differentiating in posterior regions of the planarian body (Fig. 3j, l, n, p; arrows). Ectopic brain cells express specific genes of both lateral branches (Fig. 3n) and the central region (Fig. 3p) of the brain. In trunk pieces allowed to regenerate

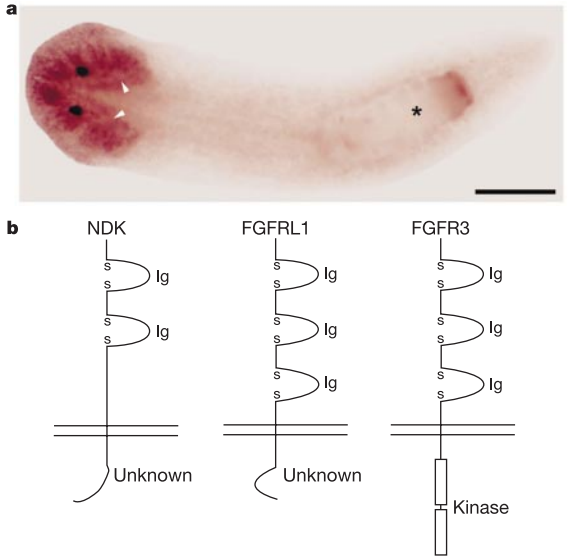

Figure 1 Expression pattern of *ndk* and predicted structure. **a**, *ndk* is expressed in the head region in both the inverted U-shaped brain (arrowheads) and non-brain cells. Signal detected in the pharynx (asterisk) is probably a non-specific signal due to trapped probe. Anterior to the left. Scale bar, 1 mm. **b**, Two extracellular IgG domains are predicted in NDK.

