Gene identification and annotation

We principally used two strategies for gene prediction and combined the results (see Supplementary Information for details). (1) Each read-pair of cDNA clones was mapped on the contigs using BLAST and putatively transcribed regions were determined by clustering the mapped pairs. (2) ORFs likely to encode a protein showing similarity to known proteins or having known motifs were identified respectively by the BLASTP program with GenBank nr database, or a HMMER program with a Pfam database. A functional classification was performed based on the NCBI KOG. The tRNA genes were detected using the tRNAscan-SE program with relaxed parameters (-X 15 -I -36).

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Correspondence and requests for materials should be addressed to T.K. (tsune@rikkyo.ne.jp) or M.M. (mzaki@biol.s.u-tokyo.ac.jp). Chromosome sequences were submitted to DDBJ with accession numbers AP006483–AP006502 (chromosome 1–20) and AP006600–AP006614 (unassigned contigs). Sequences and annotation are available at http://merolae.biol.s.u-tokyo.ac.jp/ or http://dolphin.lab.nig.ac.jp/publish/.

Long-lasting sensitization to a given colour after visual search

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Visual attention enables an observer to select specific visual information for processing. In an ambiguous motion task in which a coloured grating can be perceived as moving in either of two opposite directions depending on the relative salience of two colours in the display, attending to one of the colours influences the direction in which the grating appears to move[1]. Here, we use this secondary effect of attention in a motion task to measure the effect of attending to a specific colour in a search task. Observers performed a search task in which they searched for a target letter in a 4 × 4 coloured matrix. Each of the 16 squares within a matrix was assigned one of four colours, and observers knew that the target letter would appear on only one of these colours throughout the experiment. Observers performed the ambiguous motion task before and after the search task. Attending to a particular colour for a brief period in the search task profoundly influenced the perceived direction of motion. This effect lasted for up to one month and in some cases had to be reversed by practising searches for the complementary colour, indicating a much longer-persisting effect of attention than has been observed previously.

To investigate the consequences of attending to a particular colour, we designed a search task that requires observers to attend to a particular colour and to ignore all other colours. The task is to report the location of a target letter among other 'distracter' characters. Each trial consists of ten consecutive 4 × 4 matrices displayed rapidly (Fig. 1a). Each of the 16 squares of a matrix is randomly assigned one of four colours (red, green, yellow or blue). The initial frame rate was two matrices per second and it was increased as observers' performance improved to as high as 19 matrices s^{-1} for the fastest observer. At the average final speed of 10 frames s^{-1}, each colour display was shown for 50 ms and then the to-be-searched letters and numbers appeared superimposed on the coloured squares for an additional 50 ms. The purpose of advancing the colour matrix relative to the target was to induce the observers to use the colour to find the target. A target colour, either red or green, was assigned to each observer for the duration of the first phase of the experiment.

At the beginning of each trial, observers were shown a randomly

selected letter that was to be the target for that trial. During the search sequence, this letter appeared on the target colour square once and on non-target colour squares an average of 3.6 times. The task was to report the spatial location of the square on which the target occurred. The target letter was to be ignored when it occurred on non-target colour squares. If observers were able to use colour information optimally, they would need to search only one-quarter of the squares for the target. As observers practiced, whenever performance reached 95% correct, the frame rate was increased. One-hour sessions were conducted until observers' performance failed to improve in three consecutive runs, which took 4–7 h. At the average final rate of ten search frames s^{-1} the duration of the search sequence itself was 1 s (ten search frames presented consecutively over a duration of 1 s; that is, 10 frames s^{-1}). In a 1 h session of 300 trials, there would be only 5 min of actual viewing of the stimulus. Throughout the entire experiment, no observer was exposed to the search displays for more than 1 h.

Sensitization to the target colour (either red or green) was measured by a third-order motion paradigm (Fig. 1b)[1]. The experiment was conducted in a different room and with a different apparatus than the search experiment. A trial of the motion paradigm is composed of five frames, with a 90° phase shift between consecutive frames. Odd frames contained horizontal isoluminant red–green sine wave gratings (the chromaticities of the red and green in the motion task were quite different from those used in the search task) (Fig. 1), whereas even frames contained thin stripes of high-contrast noise alternating with thick stripes of low-contrast noise. In both even and odd frames, the average luminance is constant throughout. When five frames are displayed successively, the perceived direction of motion depends on the relative salience of the red and green, as there are no traditional cues (for example, luminance or contrast) to motion perception. When red and green are equally salient, no consistent direction of motion is perceived.

Other things being equal, the salience of a coloured area is proportional to colour difference from the background. On our grey background, greater saturation means greater salience. Before the search task, observers performed the motion task with a range of colour gratings that varied in their red/green saturation ratios. Their responses are represented as a psychometric function depicting the percentage of trials in which motion was seen in a direction consistent with a particular colour (Fig. 2a, b, black line). This function was taken as the baseline. When training on the search task was concluded (after performance no longer improved), another set of motion measurements were made and a new psychometric function was recorded (Fig. 2a, b, coloured line). The lateral shift between 'before' and 'after' psychometric functions is equivalent to an increase in the saturation of the attended colour by an average of

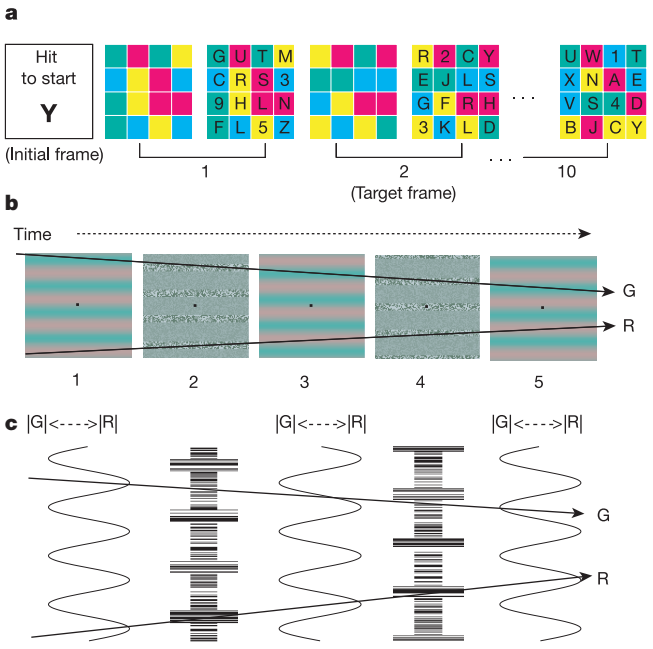

Figure 1 The visual search task and the third-order motion paradigm. **a**, The search task. A target colour (either red or green) is assigned to each observer (red in this example). An initial frame shows the observer the target letter for that particular trial (Y in this example). The search task contains ten search frames in which the coloured background is visible throughout but the letters only for the second half of the interval. The observer's task is to detect the target letter and report its location only when it appears on the target colour. Only one-half of trials contained the target item. In the other half, observers press the space bar to indicate there was no target item detected. **b**, The motion task. The third-order motion task is composed of five frames. The even frames contain a high-contrast thin stripe, which is perceived as figure against a low-contrast background. The odd frames contain a red–green grating modulated along the long–medium wavelength cardinal axis in colour space. Frames are arranged so that apparent motion can be perceived in either of two opposed directions or in neither direction. The perceived direction of apparent motion depends on whether the red (R) or green (G) of that display has higher salience. **c**, Schematic illustration of green–red and black–white contrast in the motion frames.

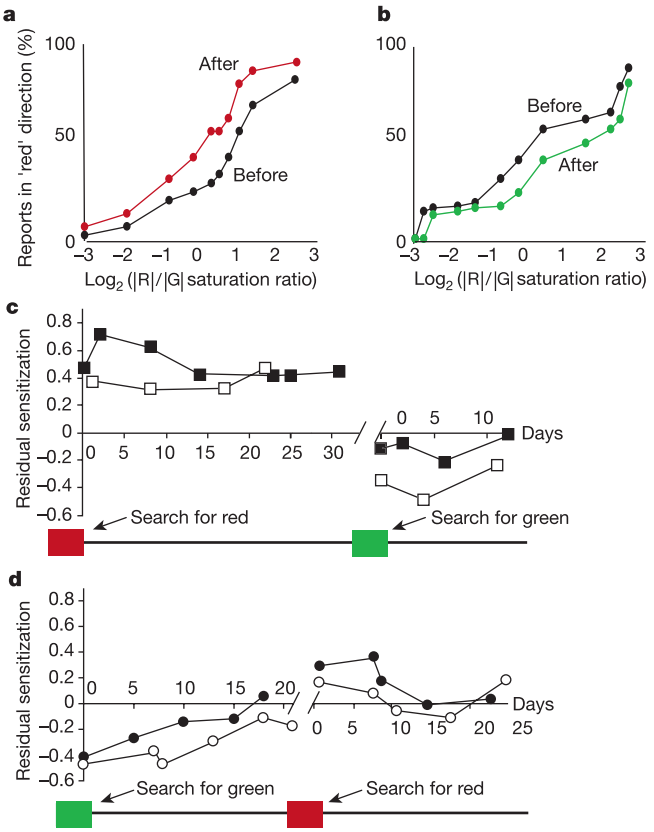

Figure 2 Results. **a**, Typical psychometric data from the third-order ambiguous motion paradigm before (black curve) and after (red curve) the search for red. The percentage of trials in which observers report motion in the red direction is shown as a function of the saturation of red divided by the saturation of green. The horizontal shift indicates that, after the search task, the searched-for colour behaves in the motion task as though it has become more saturated, that is, more salient. Data are for observer JYC. **b**, Typical psychometric data after search for green. Data are for observer CHT. **c**, Data from two observers (CCW, filled symbols; TSAI, open symbols) showing that the residual sensitization to red produced by searching for red lasts for weeks. Positive values indicate sensitization to red and negative values indicate sensitization to green. Positive values are obtained after observers search for red. The effect was reversed after observers searched for green. **d**, Data from observers (TWH, filled symbols; YCT, open symbols) who searched for green first, and then switched to searching for red.

30%. When observers were subsequently trained to search for a different colour, the shift in the psychometric function was reversed, and the observers become sensitized to the new search colour.

The third-order motion paradigm was used to measure how long the sensitization effect persisted. After finishing the search task, observers returned at regular intervals to perform the motion task, producing a new psychometric function at each visit. We take the sum of differences at all points between the new and baseline psychometric functions as an index of the magnitude of the sensitization effect. The data show that there is a long-term sensitization for all of our observers and that this sensitization persists for weeks, or even a month (Fig. 2c, d).

We explain how our third-order motion paradigm reflects observers' sensitization in a search task in terms of the hierarchical motion theory[2]. This theory proposes that the third-order motion system detects movements of salience. Salience is recorded in a salience map S (x, y, t) in which at time t, at location x, y, the areas indicated as 'figure' are marked with positive values whereas locations indicated as 'ground' or as unimportant are marked with zero or negative values. Some areas may be ambiguously classified as figure or ground, and attention can have a role in resolving this ambiguity. As a consequence of performing the search task, the relative importance of colours changes because one of them is the target colour and the others are distracter colours. This change in importance, which is understood at a high cognitive level, is used to influence how locations containing those attended and unattended colours are recorded in the salience map. The third-order motion system is assumed to use the salience values to compute motion direction just like the luminance motion system uses luminance values. By recording the performance change in the motion task, we are able to measure quantitatively the sensitization to colour produced by a completely different task.

It is surprising that the colour sensitization persists for weeks. The lifetime of most simple after-effects is measured in seconds. The most notable persisting after-effect is the McCollough effect[3] in which, after viewing a coloured grating for seconds or minutes, black–white stripes of a similar slant and spatial frequency appear to have a colour tint opposite to the adapting grating. The McCollough effect[3] (and several similar after-effects[4–7]) typically lasts for seconds or minutes, although subsequent studies have demonstrated that it can be made to last longer when the adapting procedure is very much longer[7–13]. Additionally, viewing through a coloured filter for several days can produce a weeks-long perceptual colour after-effect[14]. Again, the observer becomes relatively less sensitive to the colour passed by the filter, a kind of perceptual colour renormalization.

Previously reported long-lasting after-effects, such as the McCollough[8], Neitz[14] and others[7–13], differ from the present attentional effect in several important ways. (1) These after-effects are opposite to the original stimulus, for example, exposure to red desensitizes red relative to green, exactly the opposite of our observed sensitization; (2) the initial adaptation stimuli and subsequent test stimuli are quite similar, whereas our search adapting and motion testing procedure differ in task, spatial structure and precise colour coordinates; (3) not surprisingly therefore, the typical test procedure, being similar to the adapting procedure, interferes with the persistence of the after-effect[8]. Our testing procedure (the third-order motion task) involved a great many motion trials on each test occasion but seemed to produce no diminution of attentional sensitization for at least some of the subjects for at least during the first month of repeated tests.

In perceptual learning an observer performs a discrimination task with a very specific stimulus. Over the course of many trials, and with interposed intervals of sleep, performance can improve markedly[15]; however, enhanced performance is restricted to the specific location in the visual field where the stimulus normally appears[16], to the specific stimulus itself[17–20], it frequently fails to generalize to the unexposed eye[21,22], and it can persist for years[15]. In all of these respects, it differs from the attention-produced enhanced salience of a specific colour observed here. In our study, observers' sensitization to the target colour can transfer between two unrelated tasks, demonstrating that the sensitization must be registered in a common area that both tasks can access. We propose a salience map to conceptualize the dynamic process that instantiates the sensitization to colour.

The third-order motion display is a good measure of attention to colour whether attention is manipulated directly by instruction or by other methods, such as by a search task. We found that searching for less than 1 h (distributed over 4–7 h) increased the salience of the searched-for colour by an amount that was equivalent to making the colour 1.3 times more saturated. The sensitization persisted for weeks. It is a long-term perceptual modification that is very different from adaptation, contingent after-effects and perceptual learning. □

Methods

Visual search task

Observers reported the location of a target letter among other distracters in ten consecutive 4 × 4 matrices displayed rapidly (Fig. 1a). The target letter varied in each trial, and observers were informed of its identity before search. A target colour, either red or green, was assigned to each observer for the duration of the search phase of the experiment. If present, the target letter occurred only once on the target colour in each trial. The target letter was to be regarded as a distracter if it occurred on non-target colour squares. We randomly assigned red, green, yellow or blue to be painted in each square of the 4 × 4 matrix. Initially, each colour display was shown for 267 ms and then to-be-searched letters and numbers appeared superimposed on the coloured squares for an additional 267 ms. If observers could fully utilize the colour information, they would only have to search one-quarter of the squares for the target, greatly simplifying the detection task. Whenever an observer's performance reached 95% correct, the display was speeded up and the duration of each frame was decreased. One-hour sessions were conducted every day until the performance of observers failed to improve in three consecutive runs.

Third-order motion paradigm

Observers reported the direction of motion in the third-order motion paradigm (Fig. 1b). Each trial contained five frames, with a 90° phase shift between consecutive frames. Even frames contained thin stripes of high-contrast noise alternating with thick stripes of low-contrast noise, whereas odd frames contained horizontal isoluminant red and green sine wave gratings (Fig. 1c). Red–green gratings were generated by modulating along the long–medium wavelength cardinal axis in Derrington–Krauskopf–Lennie colour space[23], and were calibrated carefully to eliminate the residual luminance. The first- and second-order motion systems are driven by stimuli (luminance and contrast/texture) that are very easy for our visual system to detect. Without careful calibration, the motion perception may be attributed to inputs from other stimulus components that are not in our interest to investigate, so it is important to ensure there is no contamination from those systems[24,25]. However, even if some first- or second-order motion contamination remained, it would only make it more difficult to measure an effect of attention on motion, because contamination cannot produce a spurious attention effect.

In both even and odd frames, the mean luminance is constant and equal to the grey background. When the five frames are displayed successively, the two colours produce opposite, thus ambiguous, motion perception. The final perceived moving direction depends on the relative strength (salience) of the two colours, as there are no other cues (for example, luminance or contrast) known to contribute to motion perception. The salience of the two colours is jointly decided by their saturation and the attentional manipulation. If the colours are equally salient, ambiguous motion is perceived.

Procedures

A baseline psychometric function using the third-order motion paradigm was constructed before the search task. The percentage of trials in which observers reported motion in the red direction was plotted as a function of red–green saturation ratios (Fig. 2a, b, black line). After the search task was finished, another measurement was made with the same third-order motion paradigm and a new psychometric function was derived (coloured line in Fig. 2a, b). The lateral shift in the psychometric function indicated an increase in the salience of target colour. To measure the lifetime of the sensitization effect, we had four observers repeat the motion paradigm at regular intervals. A new psychometric function was constructed at each visit and compared with the baseline. We take the sum of differences from all points between the new and baseline psychometric functions as an index of the size of the sensitization effect. Positive values indicate increased sensitization to red, whereas negative values indicate sensitization to green. This index is shown as a function of time (Fig. 2c, d), and the data show that there is long-term sensitization for all our observers. This sensitization persists for weeks.

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IκB kinase-α acts in the epidermis to control skeletal and craniofacial morphogenesis

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IκB kinase-α (IKK-α)[1] exhibits protein-kinase-dependent and -independent functions. Its kinase activity is required for lymphoid organogenesis[2] and mammary gland development[3], whereas a kinase-independent activity is required for epidermal keratinocyte differentiation[4]. In addition to failed epidermal differentiation, IKK-α-deficient mice exhibit abnormal skeletal and craniofacial morphogenesis[4–6]. As similar defects are not exhibited by mice that experience systemic inhibition of NF-κB[7], we postulated that the morphogenetic defects in IKK-α-deficient mice are not caused by reduced NF-κB activity but instead are due to failed epidermal differentiation that disrupts proper epidermal–mesodermal interactions. We tested this hypothesis by introducing an epidermal-specific *Ikka* (also known as *Chuk*) transgene into IKK-α-deficient mice. Mice lacking IKK-α in all cell types including bone and cartilage, but not in basal epidermal keratinocytes, exhibit normal epidermal differentiation and skeletal morphology. Thus, epidermal differentiation is required for proper morphogenesis of mesodermally derived skeletal elements. One way by which IKK-α controls skeletal and craniofacial morphogenesis is by repressing expression of fibroblast growth factor (FGF) family members, such as FGF8, whose expression is specifically elevated in the limb bud ectoderm of IKK-α-deficient mice.

IKK-α-deficient (*Ikka*$^{-/-}$) mice, which die shortly after birth, exhibit marked morphological abnormalities that include taut and shiny skin, rudimentary limbs, absent or severely truncated tail, and a short and rounded head due to shorter jaw and nasal bones[5,6,8]. Previous work revealed that IKK-α acts in a cell-autonomous manner to control terminal differentiation of epidermal keratinocytes[4]. This action of IKK-α does not depend on its protein kinase activity[4], and *Ikka*^{AA} mice expressing an inactivatable form of IKK-α exhibit normal morphogenesis[3]. As epidermal–mesodermal interactions have a major role in vertebrate morphogenesis[9], and as mice in which NF-κB activation is inhibited do not exhibit skeletal abnormalities[7], we examined whether failed keratinocyte differentiation underlies the morphogenetic defects exhibited by *Ikka*$^{-/-}$ mice. This hypothesis was tested by introducing a human *Ikka* transgene driven by the basal keratinocyte-specific promoter of the cytokeratin 14 (*CK14*) gene[10] (Fig. 1a) into *Ikka*$^{-/-}$ mice. Several transgenic lines exhibiting germline transmission and efficient expression of the *CK14–Ikka* transgene were established and two of them were crossed with *Ikka*$^{+/-}$ mice to generate *Ikka*$^{-/-}$ *CK14–Ikka* progeny. Immunoblot analysis revealed efficient expression of IKK-α in the skin of these mice, but not in other tissues, including bone (Fig. 1b). Within the skin, expression of human IKK-α encoded by the *CK14–Ikka* transgene was restricted to the basal layer of the epidermis and hair follicles (Fig. 1c), consistent with the known specificity of the *CK14* promoter[10]. Immunoblot analysis of cultured keratinocytes revealed that the transgenic human IKK-α was expressed at levels similar to those of endogenous mouse IKK-α in wild-type keratinocytes (Supplementary Fig. 1).

Notably, the *CK14–Ikka* transgene completely rescued most of the morphological abnormalities exhibited by non-complemented *Ikka*$^{-/-}$ mice (Fig. 1d). Furthermore, *Ikka*$^{-/-}$ *CK14–Ikka* mice were covered by normal, wrinkled and loose skin, exhibited well-developed limbs and tail of normal length, and their head had a fully normal appearance, entirely different from that of non-complemented *Ikka*$^{-/-}$ mice. Whereas *Ikka*$^{-/-}$ mice died within the first hour after birth, *Ikka*$^{-/-}$ *CK14–Ikka* mice survived for 1 day and during the first 12 h were as active as age-matched controls. Nonetheless, all *Ikka*$^{-/-}$ *CK14–Ikka* mice died by the second day after birth, and visual examination revealed no milk in their stomachs (Fig. 1d). The cause for the suckling failure seems to be the result of a fused oesophagus, which is identical in appearance to that of non-complemented *Ikka*$^{-/-}$ mice (Supplementary Fig. 2a). As with the epidermis, the upper part of the oesophagus contains a stratified epithelium[11], which fails to differentiate in *Ikka*$^{-/-}$ mice[8]. Indeed, the *CK14–Ikka* transgene was not expressed in the oesophagus (Supplementary Fig. 2b). As CK14 is normally expressed in the oesophagus[10,12], the failure of transgene expression in this tissue may be due to the absence of essential regulatory elements. Histological, microscopic and immunohistochemical analyses revealed that the epidermis of *Ikka*$^{-/-}$ *CK14–Ikka* mice was fully stratified, differentiated and indistinguishable from that of wild-type mice (Fig. 2a). In addition, both the epidermis and cultured epidermal keratinocytes