SSCP genotyping

SSCP genotyping was performed using polymerase chain reaction (PCR) with exon 2 internal primers, amplifying a 20-bp-base portion of the PBR, and subsequent capillary electrophoresis on an ABI310 automated sequencer with modifications. These were the inclusion of a second different reverse primer (GTG GTG CAG ACA GTA CCT TCT) to increase the number of alleles detectable with SSCP. Both primer pairs together amplified 1724 (thus 74%) of the PBR that was identified by sequencing in the experimental fish. Isolated sequences cloned into plasmids were subjected to SSCP in order to standardize the fluorescent signals with sequence information. In three cases, complete PBR residues that differed only in 2–4 amino acids cannot be resolved by SSCP, such that our method underestimated the true MHC class-II diversity. In all cases, SSCP signals obtained from PCR of plasmids were also present in genotypes based on fish DNA.

Microsatellite genotyping, heterozygosity and relatedness

All fish of the first experimental block were genotyped for seven polymorphic microsatellites (GenBank accession numbers: A0103522, -54, -55, -57, -58; ref. 26) representing a total of 72 alleles. Individual heterozygosity was calculated as the number of different alleles per microsatellite locus, and as mean $\bar{h}$ taking the length difference of alleles into account27. We calculated a correlation between allele number of MHC class-IIB loci and individual heterozygosity. Relatedness coefficients $R$ (ref. 28) were calculated for all pairs of fish using a program called Relatedness 5.0 (K. F. Goodnight, available from http://gsoft.smu.edu/gsoft.html). $R$ coefficients were tested for correlation with female mating behaviour using the time difference of females among preferred/unpreferred male as variable for the female preference.

Permutation procedure

The risk that females will choose an MHC-identical male, or a male with fewer alleles, when mating completely at random was assessed in a permutation procedure. We combined 46 male and 46 female genotypes from Schohsee 100 times at random, each time counting genotypic similarity and difference in allele number.

Sexual selection experiments

Adult three-spined sticklebacks were seine-netted from an interconnected natural system of large lakes near Plön, Germany, in spring. A bit of a dorsal spine of each fish was cut for MHC class-II and microsatellite genotyping (see above). Thereafter fish were housed in individual tanks (10 litres, continuous exchange of 1 litre per hour, 18 °C, 18 h of light). Males (every second tank) were offered artificial nesting material. Only bright red males with completed nests were used for experiments. Gravid females were tested in a flow channel22. In the test compartment (25 × 20 cm long, 10 cm water level) each female was offered the choice between the two sides of the current (1.4 cm s$^{-1}$), which was video-recorded from above. The inlet compartment, which was separated upstream by a net from the test compartment, was divided laterally into halves. A peristaltic precision pump (ISMATEC MV, 100 W, 50 Hz) supplied water to the test compartment. A peristaltic precision pump (ISMATEC MV, 100 W, 50 Hz) supplied water to the test compartment. A peristaltic precision pump (ISMATEC MV, 100 W, 50 Hz) supplied water to the test compartment. A peristaltic precision pump (ISMATEC MV, 100 W, 50 Hz) supplied water to the test compartment.

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When voluntary saccadic eye movements are made to a silently ticking clock, observers sometimes think that the second hand takes longer than normal to move to its next position. For a short period, the clock appears to have stopped (chronostasis). Here we show that the illusion occurs because the brain extends the percept of the saccadic target backwards in time to just before the onset of the saccade. This occurs every time we move the eyes but it is only perceived when an external time reference alerts us to the phenomenon. The illusion does not seem to depend on the shift of spatial attention that accompanies the saccade. However, if the target is moved unpredictably during the saccade, breaking perception of the target's spatial continuity, then the illusion disappears. We suggest that temporal extension of the target's percept is one of the mechanisms that ‘fill in’ the perceptual ‘gap’ during saccadic suppression. The effect is critically linked to perceptual mechanisms that identify a target’s spatial stability. Although most observers have experienced the ‘stopped clock’ illusion, previous psychophysical experiments that have tested when subjects perceive the time of transient external events relative to saccadic eye movements have yielded contradictory results5,6. A
The tight coupling of the duration of chronostasis to the duration of the saccade suggests that the effect may be linked to the perceptual gap caused by saccadic suppression and retinal blur that occurs when we move the eyes. However, it is possible that the illusion of chronostasis is not tightly coupled to movement of the eyes per se, but occurs because subjects also shift the locus of their visual attention at around the time the eyes move. This attention shift may act as the reference point to which the target is predicated. To test this, subjects were asked either to make the usual saccade to the target or first to shift their attention to the target and then move their eyes. Figure 2a shows that the illusion of chronostasis persisted with a similar magnitude when subjects shifted their attention before moving their eyes. Control trials intermixed with the eye movement trials verified that subjects were successful in shifting the locus of their visual attention. They fixated on a central cross and had to saccade to a target appearing on the right or left of the screen. If they had been told to shift their attention to the correct side before the target appeared, their reaction time was faster than if they had been incorrectly cued (Fig. 2b).

Although chronostasis is linked to voluntary saccades, the coupling is not obligatory—there is at least one condition under which the illusion is not experienced. We designed a third experiment in which the positional stability of the target counter was systematically broken. Subjects made a saccade to the target, but in some trials the computer displaced the target by more than 9 degrees during the time the eyes were moving. Under such conditions, subjects sometimes fail to notice the shift and make an unusually large corrective saccade to fixate the target. Trials were divided into three types: (1) those in which the target appeared stationary throughout; (2) those in which it was moved but the movement was not perceived by the subject; and (3) trials in which target movement was perceived by the subjects. Figure 3 shows the results of this experiment. When there was no target motion, subjects experienced the usual illusion of chronostasis when they made a saccade compared with a control condition with no movement of the eyes. However, if the target was moved and subjects noticed the movement, then no effect was found relative to control. If the shift
was not perceived, the estimates of the subjects fell somewhere between the control value and the full illusory effect. The effect of moving the target was not due to nonspecific distortion caused by the shift. The full illusion was again obtained in our final experiment, in which distracting stimuli appeared randomly 1° or 3° to the side of the target during the time the eyes were moving, and remained on the screen thereafter (Fig. 3).

Thus, backwards extension of the perception of the target only occurs when subjects perceive that the saccadic target was stationary during the period of extension. We suggest that this link between space and time occurs because of the following. When the saccade shifts the eyes from one stationary viewpoint to another, vision is degraded and it is not possible to say with certainty whether there are any changes in the position of objects during movement. However, if the saccadic target is fixated accurately at the end of the saccade, subjects can assume that it occupied approximately the same place throughout the eye movement (object constancy). Such an assumption may fulfill various functions, having already been proposed in recent theories relating to the problem of space and time occurs because of the following. When the saccade predates the target's postsaccadic state to a specific pre-motor input. The processes underlying both saccadic suppression and space constancy are active over a time period extending beyond the saccade itself. Our obtained constant values are similar to the value of 80 ms obtained for presaccadic shifts in neuron receptive fields within the lateral intraparietal area of monkeys. They also fit well with human psychophysical data on presaccadic compression of space (the systematic mislocalization of targets flashed around the time of a saccade) and saccadic suppression, which both precede saccadic onset by 50 ms or more. It therefore seems probable that presaccadic mechanisms will provide an explanation for the time course of chronostasis.

These data support ideas of conscious experience as an ongoing, often post hoc reconstruction emerging from multiple cognitive systems. Our suggestions relating to assumed continuity of target appearance fit well with ideas about object files in the visual attention literature. Here, features of a visual object (colour, form, location, and so on) are bound into a single perceptual unit (the object file) that links representational codes established across diverse cortical regions. We suggest that cross-saccadic perceptual continuity, as described here, may represent a specific case of a more general class of phenomena relating to the continuity of perception across shifting states of sensory input.

**Methods**

**Experimental design**

Subjects sat before a 14-inch colour monitor (60 Hz refresh), with their chin supported. Eye movements were recorded using electro-oculography or with an infrared eye tracker (Microguide 1000 spectacles), and sampled at 200 Hz. Stimuli were black on a white background or vice versa, subtending approximately 0.5°. The experiments were controlled by a personal computer interfaced with a 12-bit A/D card (National Instruments DAQ, 1208). Counter change was triggered when the eyes had travelled one fifth of the distance to target. We calculated the subjective second using a velocity criterion. Repeated measures designs were used throughout, with condition order counterbalanced. We calculated n for each experiment following a power analysis of initial data. Later experiments replicate experiment 1 unless stated otherwise.

**Experiment 1**

Thirty subjects (18 male, mean age 28.2, standard deviation, s.d. 7.4) completed four conditions: saccade of 3° and 2° and two matched control conditions. In the two saccade conditions, subjects fixated on a cross on one side of the screen, initiated the trial with the depression of a key, and then made a voluntary saccade to a target ‘0’ on the other side of the screen. Eye movement triggered a change of digit to a ‘1’, which remained on the screen for 0.30–1.600 ms; subsequent digits (2, 3) remained on the screen for 1 s each, culminating in the appearance of a ‘4’. Subjects indicated whether the time that they saw the ‘1’ was longer or shorter than that for the subsequent digit. Trials where the first saccade recorded did not exceed 90% of the total distance to target were excluded and immediately repeated. In control trials, subjects fixated on a ‘0’ at equivalent eccentricity that changed to become the judged digit (1) 500 ms after the subject’s key press. The computer controlled the duration of the first digit by a modified binary search (MOBS) procedure that homed in on a single, matched estimate (low boundary 400 ms, high boundary 1,600 ms, initial presentation random 800–1,200 ms, five reversals to terminate). Four estimates
were obtained per condition, then corrected post hoc to match the time that the ‘1’ was on screen after target fixation. Ten subjects (9 male, mean age 30.5, s.d. 7.8) completed a control experiment. They estimated the duration of the first digit when a counter moved 24° to the point of fixation in 100 ms (six screen refreshes), compared with the usual stationary control. A further control experiment (n = 10, 9 male, mean age 31.4, s.d. 7.6) varied the time from saccade onset to the initial counter change by triggering this change either one fifth or four fifths of the way into a 55° saccade (randomly within the same block; separate self-terminating MOBS).

**Experiment 2**

The data of 12 subjects was included in experiment 2 (10 male, mean age 32.8, s.d. 9.3). In addition to a control, subjects completed two conditions requiring 12° saccades to a counter (as experiment 1) with or without deliberate, prior covert shifting of attention. Every other trial was a reaction time task in which a target flashed the central target and then made a speeded 12° saccade to the appearance of a target ‘0’ to the left or right. An uninformative cue (an arrow pointing to the left or right near fixation) directed attention before the appearance of the ‘0’ in attention-shift blocks.

**Experiment 3**

Twenty-two subjects performed experiment 3 (15 male, mean age 32.8, s.d. 9.1). We tested three conditions: a 20° saccade to a stationary counter; a 20° saccade in which the counter shifted +0–9° synchronously with triggering of counter onset; and a control. All eye movement data were obtained within a single block type in which subjects made the standard timing judgment and also indicated whether the counter had moved during the saccade. Presentation was controlled by three randomly interleaved (equally probable) self-terminating MOBS. The first of these controlled target time intervals for the stationary counter (as in experiment 1), the latter two controlled the size of the target shift in a hypo- or hypermetric direction (0–9°) according to whether the movement was perceived. This ensured that most of the shift trials were close to the subject’s point of shift perception, whether perceived or not. For shift trials, the target time interval was randomly generated in the range 400–1,600 ms. Trials were divided between perceived and unperceived shift conditions post hoc. For all conditions, matched time estimates were generated using logistic regression. Subjects initially completed four experimental blocks and four short control blocks, with a single additional block completed where fitted logistic regression lines exceeded P = 0.05.

**Experiment 4**

Ten subjects participated in experiment 4 (7 male, mean age 29.4, s.d. 7.5). We compared four conditions: a 20° saccade to a stationary counter; an identical saccade with a random, lower-case alphabetical character appearing 3° from the counter (hyper- or hypometrically) at trigger time; a saccade with the character appearing 3° from the counter; and a control. Data for the first three conditions was obtained within a single block type, using three randomly interleaved and self-terminating MOBS.

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**Haemoglobin C protects against clinical Plasmodium falciparum malaria**


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Haemoglobin C (HbC; β6Glu→Lys) is common in malaria-prone areas of West Africa, especially in Burkina Faso. Conclusive evidence exists on the protective role against severe malaria of haemoglobin S (Hbs; β6Glu→Val) heterozygosity, whereas conflicting results for the HbC trait have been reported and no epidemiological data exist on the possible role of the HbCC genotype. In vitro studies suggested that HbCC erythrocytes fail to support the growth of P. falciparum, but HbC homozygotes with high P. falciparum parasitaemia have been observed. Here we show, in a large case–control study performed in Burkina Faso on 4,348 Mosis subjects, that HbC is associated with a 29% reduction in risk of clinical malaria in HbAC heterozygotes (P = 0.0008) and of 93% in HbCC homozygotes (P = 0.0011). These findings, together with the limited pathology of HbAC and HbCC compared to the severely disadvantaged HbBS and HbSC genotypes and the low β^3 gene frequency in the geographic epicentre of β^3*, support the hypothesis that, in the long term and in the absence of malaria control, HbC would replace HbB in central West Africa.

Since hominziation the human genome has been under selective pressures for resistance to infectious diseases. For example, West African populations are able to escape the infection altogether, with complete protection from Plasmodium vivax achieved through the fixation of a Duffy silent allele (fy)15. In other cases, polymorphic