Methods

The governance scores were based on the Corruption Perception Index (CPI) produced by Transparency International[2]. This system uses independent surveys of business people and assessments by country analysts to compare national corruption levels. However, it was only initiated in 1995, so another data set, the International Country Risk Guide (ICRG), was used to provide information for earlier years. The ICRG system uses several coarse-scale factors to measure national governance levels, and a data set was available that contained information on 126 countries between 1984 and 1999. Therefore, we used stepwise linear regression analysis to produce a model that allowed $\log_{10}$ CPI scores to be calculated on the basis of these ICRG data. This model was developed using data for 1999 and included three ICRG factors, which were 'corruption' ($B = 0.074$, $P < 0.001$), 'bureaucratic quality' ($B = 0.0057$, $P < 0.001$) and 'law and order' ($B = 0.0028$, $P = 0.008$). In this system, 'corruption' measures corruption within the political system, 'bureaucratic quality' measures the ability of the bureaucracy to govern without drastic changes in policy or implementation, and 'law and order' measures both popular observance of the law and the strength and impartiality of the legal system. The model explained most of the observed variation ($F_{3,91} = 122.68$, adjusted $r^2 = 0.809$) and comparisons of predicted and actual CPI scores for 1995 and 1996 showed that the model had high levels of explanatory power, which were increased in the latter period when data on a more representative number of developing countries were available (1995: $n = 40$, $r^2 = 0.728$; 1996: $n = 53$, $r^2 = 0.860$). Therefore, we used this model to calculate CPI scores for those analyses that used biodiversity data collected before 1995.

Data obtained by the Food and Agriculture Organization on changes in forest cover between 1990 and 1995, together with information on African elephant populations[19] and black rhinoceros populations[20] were collated as the best available data on changes in widespread biodiversity elements. We also collated data on annual per capita Gross Domestic Product (GDP), Human Development Index (HDI) scores produced by the United Nations Development Programme and human population density data. Data on national conservation budgets were available from one of a range of years from 1991 to 1996 for a number of African countries[29], so these were adjusted to 1993 US$ (the median date) using deflation indexes produced by the International Monetary Fund. Spearman's rank correlation tests and stepwise multiple regression modelling were then used to identify factors that were related to national percentage change in these biodiversity components. These factors were transformed, whenever necessary, to meet the assumptions of the tests. In each case, data from countries with a restricted amount of each component were excluded, as small changes or measurement errors were more likely to produce apparently extreme results. The exclusion levels were: forest area $<30{,}000\,km^2$, elephant populations $<1{,}000$ and black rhinoceros populations <10.

We used CPI scores for 2002 to investigate the relationships between national biodiversity levels and governance. Species richness values for each country were calculated as the number of recorded mammal and bird species, using data available from the World Resource Institute. We corrected for the nonlinear relationship between species number and country area by dividing species number by A^z, where A is country area, and z is a typical value for the slope of a nested, within-continent plot of log(species number) on log(area), set here as 0.25 (ref. 30).

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Sophisticated sperm allocation in male fowl

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When a female is sexually promiscuous, the ejaculates of different males compete for the fertilization of her eggs[1]; the more sperm a male inseminates into a female, the more likely he is to fertilize her eggs[2]. Because sperm production is limited and costly, theory predicts that males will strategically allocate sperm (1) according to female promiscuity[1,3–5], (2) saving some for copulations with new females[3,6,7], and (3) to females producing more and/or better offspring[3,8]. Whether males allocate sperm in all of these ways is not known, particularly in birds where the collection of natural ejaculates only recently became possible. Here we demonstrate male sperm allocation of unprecedented sophistication in the fowl *Gallus gallus*. Males show status-dependent sperm investment in females according to the level of female promiscuity; they progressively reduce sperm investment in a particular female but, on encountering a new female, instantaneously increase their sperm investment; and they preferentially allocate sperm to females with large sexual ornaments signalling superior maternal investment. Our results indicate that female promiscuity leads to the evolution of sophisticated male sexual behaviour.

In the fowl, socially dominant males have privileged copulatory

access to females and females copulate with several males in each breeding cycle[9,10]. The resulting sperm competition leads males to allocate sperm differentially to females according to female promiscuity, female novelty and female reproductive quality.

We found that males differentially allocated sperm according to female promiscuity, which was simulated by experimentally exposing a male to a female in the presence of zero, one or three male competitors, and according to the male's own social status. In the absence of competitors, males minimized their sperm investment. As the number of competitors increased from one to three, dominant males increased their sperm investment, but subdominants maximized their investment in the presence of just one competitor (Fig. 1).

A male's propensity to copulate with a particular female declined with the time that a male was exposed to a female, but was renewed by replacing the female with a new female (Fig. 2). After an initial bout of frequent copulations immediately after exposure to a female, males left to forage and returned regularly to inspect the female's head closely, suggesting that males may use visual cues to recognize individual females when making copulation decisions. Once a male lost interest in a female, the female was exchanged for another and the male resumed copulating. When we removed and re-presented the same female after a male had ceased copulating with her, however, the male inspected the female but in no case copulated with her ($n = 5$ trials).

Males progressively reduced their sperm investment in a particular female according to the number of sperm that they had already inseminated into her, but increased their investment when allowed to copulate with a new female (Fig. 3a). Successive females obtained fewer sperm, confirming that male sperm reserves were depleted over successive copulations. But although males allocated progressively fewer sperm to successive ejaculations with an individual female, they increased their sperm investment when presented with a new female, indicating preferential sperm investment in new females.

In several bird species including the fowl, some copulations do not result in the transfer of semen[11–14]. Consistent with the idea that male fowl retain some of their sperm reserves for other females, the probability of a male failing to transfer sperm increased significantly over successive copulations with a particular female, regardless of the number of females to which a male had been exposed (Fig. 3b). We also replicated this experiment with a population of red jungle fowl, *G. gallus*, the wild ancestor of the domestic fowl[15], and obtained similar results (Figs 2 and 3, legend).

Males preferentially invested sperm in females with relatively large sexual ornaments. When experimentally exposed to two females simultaneously, males were more likely to copulate with (Fig. 4a) and allocate more sperm to (Fig. 4b) the female with the larger comb. Sperm allocation was also status-specific: socially dominant males biased sperm investment in favour of the large-combed female more than did subdominant males (Fig. 4b).

We investigated the adaptive significance of male preference for large-combed females. First, focal watches of free-ranging groups of four females and two males (n = 7) showed that females with larger combs (ranked according to comb size in a group) received significantly more successful copulations from both males (generalized linear model with restricted maximum likelihood estimation (REML–GLM)[16] of number of copulations, with comb rank and male status as fixed effects nested in groups, and male identity as random effect: comb rank, $X^3_{48} = 37.17$, $P = 0.023$; male status, $X^2_{48} = 8.07$, $P = 0.018$; male status × comb rank, $X^6_{48} = 24.19$, $P = 0.062$). Second, female comb size was an important predictor of mean egg mass (Fig. 4c) and mean yolk mass of eggs, after controlling for mean egg mass (yolk mass $= 1.07 \pm 0.892 + 0.43 \pm 0.077$ (egg mass) $+ 0.002 \pm 0.001$(comb mass) $- 0.002 \pm 0.001$ (body mass), $R^2 = 0.72 \pm 0.201$, $F_{3,25} = 18.5$, $P < 0.0001$; comb mass, $t = 2.13$, $n = 26$, $P = 0.045$). These results indicate that large-combed females are subject to higher sperm competition, produce larger eggs and allocate more resources to embryos.

Our results indicate that sexual promiscuity combined with status-specific constraints causes high behavioural flexibility in male sperm allocation. Dominant and subdominant male fowl face different levels of sperm competition, and tailor their ejaculates accordingly within the constraints imposed by their status. Theory

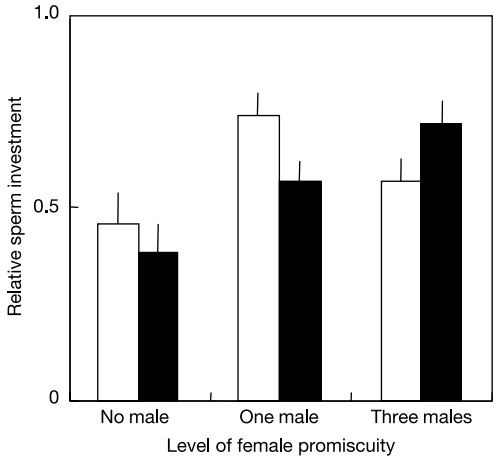

Figure 1 Differential sperm allocation and female promiscuity. Relative sperm investment of dominant (filled) and subdominant (open) male feral fowl according to female promiscuity: dominants steadily increased relative sperm investment as the number of competitors (audience) increased, whereas subdominants decreased their investment when the number of competitors increased beyond one. We measured relative sperm investment as the cumulative number of sperm transferred by a male during a trial standardized against his largest cumulative number of sperm produced in a trial across all audience treatments (generalized linear mixed model with restricted maximum likelihood estimation[16] (REML–GLMM) of relative sperm investment, with male status and audience as fixed effects, and male identity as a random effect: status, $F_{1,34} = 0.11$, $P = 0.75$; audience, $F_{1,34} = 3.90$, $P = 0.030$; status × audience, $F_{2,34} = 4.20$, $P = 0.024$). Bars indicate s.e.m.

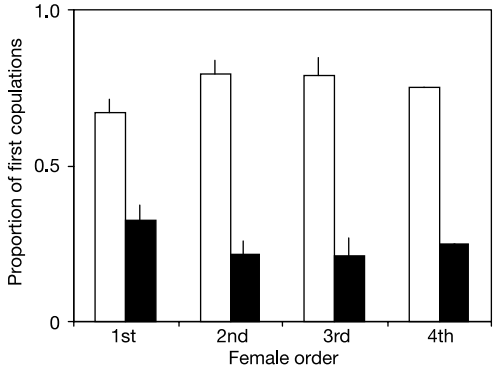

Figure 2 Preferential sperm investment in new females. In feral fowl, male propensity to copulate declined with the time that a male was exposed to a female, and increased with exposure to a new female: males copulated more frequently during the first (open) than the second (filled) half of the time that they were exposed to a female, regardless of with how many females they had previously copulated (REML–GLMM of number of copulations, with female order and first and second halves of the exposure time to a female, male identity and year as fixed effects, and duration of exposure time (min) as a covariate: female order, $F_{3,144} = 0.30$, $P = 0.83$; half, $F_{1,147} = 56.09$, $P < 0.0001$; duration, $F_{1,147} = 123.79$, $P < 0.0001$; order × half, $F_{1,143} = 1.81$, $P = 0.15$). Bars represent s.e.m. A similar pattern was seen in jungle fowl (REML–GLM; female order, $X^2_{2,39} = 3.65$, $P = 0.16$; half, $X^2_{1,41} = 3.95$, $P = 0.04$; duration, $X^2_{1,41} = 57.75$, $P < 0.0001$; order × half, $X^2_{2,37} = 0.55$, $P = 0.76$).

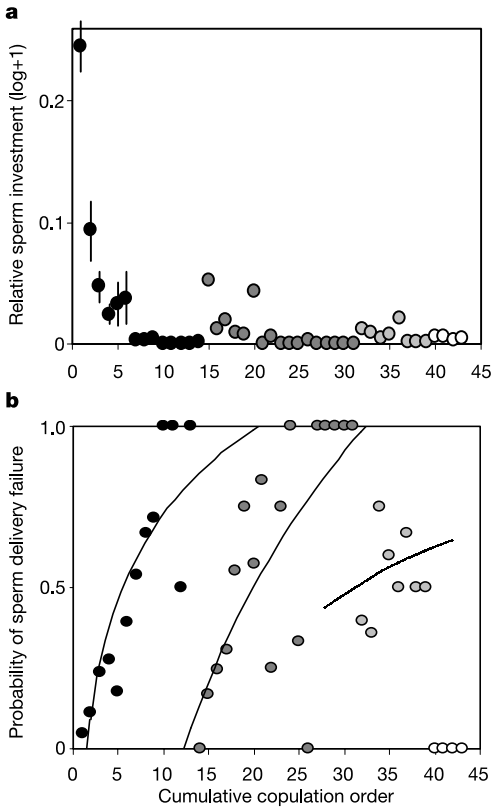

Figure 3 Preferential sperm investment in new females (continued). **a**, Relative sperm investment of male feral fowl declined over successive inseminations with the same female and was renewed by the presence of a new female. Relative sperm investment during a trial was measured as the number of sperm in an ejaculate standardized against the largest ejaculate that a male produced across all females in the same trial (REM–GLMM of relative sperm investment, with female order as a fixed effect, sexual familiarity measured as the order of copulation of a male within each female as a covariate, and year and male identity as random effects: female order, $F_{3,367} = 10.57$, $P < 0.0001$; sexual familiarity, $F_{1,367} = 38.40$, $P < 0.0001$; order × familiarity, $F_{3,364} = 3.31$, $P = 0.76$). Bars represent s.e.m. A similar pattern was seen in male jungle fowl (REML–GLM with female order as a fixed effect, and sexual familiarity as a covariate: female order, $X^2_{2,163} = 23.80$, $P < 0.0001$; sexual familiarity, $X^2_{1,163} = 12.61$, $P = 0.0004$; order × familiarity, $X^2_{2,161} = 0.03$, $P = 0.986$). Consistent with differential sperm allocation, sperm investment in the first ejaculate with a female was not lower than that in the last ejaculate with the previous female. Males tended to invest more sperm in the first ejaculate with a new female than in the last ejaculate with the familiar female (Wilcoxon paired tests: number of sperm in first ejaculate with second female versus last ejaculate with first female: feral fowl, $Z = -2.29$, $P = 0.02$, sign test $P = 0.057$, $n = 17$; jungle fowl, $Z = -0.16$, $P = 0.116$, sign test $P = 0.69$, $n = 7$. First ejaculate with third female versus last ejaculate with second female: feral fowl, $Z = -0.52$, $P = 0.60$, sign test $P = 0.69$, $n = 6$; jungle fowl, $Z = -1.83$, $P = 0.07$, sign test $P = 0.125$, $n = 6$). **b**, Mean probability of semen delivery failure over successive copulations in male feral fowl. Males were more likely to copulate without ejaculating semen the more they copulated with a female (REML–GLM of mean probability of failure of semen transfer, with female order, male identity and year as fixed effects, and sexual familiarity as a covariate: female order, $X^2_{2,292} = 15.91$, $P = 0.0012$; sexual familiarity, $X^2_{1,292} = 59.39$, $P < 0.0001$. Similar results were found in male jungle fowl (female order, $X^2_{2,163} = 16.91$, $P < 0.0002$; sexual familiarity, $X^2_{1,163} = 14.72$, $P < 0.0001$). In **a**, **b**, black symbols indicate first female; dark grey, second; light grey, third; open, fourth.

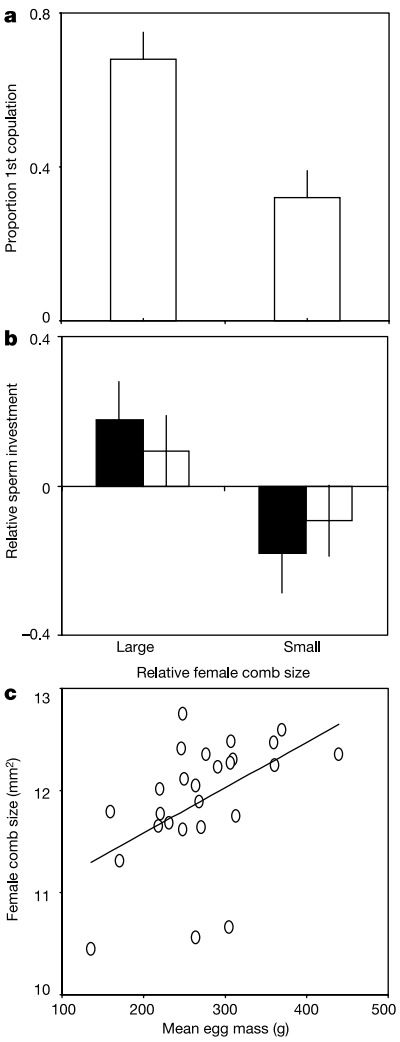

Figure 4 Mate choice and strategic sperm allocation by male fowl. **a**, When presented with two females simultaneously, male feral fowl were significantly more likely to mount the female with a relatively large comb. In each trial, females were randomly designated as A or B and their relative comb size was ranked as either large or small. The probability that female A was mounted first by a male was analysed in relation to whether she had a large or small comb (REML–GLM of binary measure of whether female A was mounted first, with female comb rank and male status as fixed effects, male identity and male groups as random effects, and female relative body mass and body size (A − B) as covariates: female comb rank, $F_{2,53} = 8.96$, $P = 0.0042$). This preference was independent of male social status (male status × female comb rank nested within male pairs: $F_{2,48} = 0.35$, $P = 0.71$; all other effects not significant). **b**, Male feral fowl allocated significantly more sperm to females with relatively large combs (REML–GLMM of relative sperm investment measured as the number of sperm invested in female A minus that invested in female B in each trial, with copulation order to control for the effect of sperm depletion, female comb rank and male status as fixed effects, male identity and male group as random effects, and relative female body mass and body size as covariates: copulation order, $F_{1,119} = 57.23$, $P < 0.0001$; female comb rank, $F_{1,119} = 11.58$, $P = 0.0009$), but dominant males (filled) invested more sperm than did subdominant males (open) in large-combed females (status × comb rank nested within male pairs: $F_{2,119} = 3.91$, $P = 0.02$; all other effects not significant). **c**, Comb size, x, was a predictor of the average egg mass produced by a female feral fowl, y ($y = 10.7 \pm 0.45 + 0.004 \pm 0.002x$; $R^2 = 0.24 \pm 0.54$, $F_{1,25} = 7.75$, $P < 0.01$; comb size: $t = 2.78$, $n = 26$, $P < 0.01$). Removing the three lowest data points strengthens the regression ($R^2 = 0.357 \pm 0.31$, $P < 0.003$).

predicts that males increase their sperm investment in a female with increasing risk of sperm competition (that is, the competition for fertilization between the ejaculates of different males generated by female promiscuity[1]). When females obtain sperm from several males however, sperm competition is certain and males are predicted to maximize sperm investment in those females that copulate with just one other male, and to decrease sperm investment progressively as the number of males that inseminate a female increases (increasing sperm competition intensity[1,3]).

The response of dominant males to different levels of sperm competition is consistent with the strategy predicted under risk of sperm competition[1,3], and the sperm allocation by subdominant males with that predicted under sperm competition intensity[1,3]. Dominant males have privileged control over copulations, and the presence of other males may represent an increased risk of another male inseminating the same female (but not necessarily an increased number of males inseminating this female). As subdominant males cannot prevent females from copulating with other males[10], the presence of other males is likely to result in more intense sperm competition. Although copulation success co-varies with male status in the fowl, most subdominant males still experience multiple copulations[9], and therefore may be selected to save sperm for less-competitive copulation opportunities. In addition, status may be condition-dependent in male fowl[17], and thus sperm investment may be more costly for subdominant males.

The decline in male sexual interest in the same female and its resuscitation with a new female is known as the Coolidge effect[18]. The Coolidge effect has been thought to mediate differential sperm allocation, enabling a male to distribute sperm more evenly and adaptively across multiple females[3,19]. Because the number of sperm inseminated by a male into different females has been seldom quantified, the adaptive significance of the Coolidge effect was previously unknown. Our study shows experimentally that a male's propensity to copulate is matched by the number of sperm transferred, thus indicating that the Coolidge effect may be adaptive to males.

Our study also shows that under sexual promiscuity male choice of partners occurs at two levels: behaviourally and cryptically through differential sperm allocation. Although the male comb is known to be important in partner choice in the fowl[20], the function of the female comb has received less attention. Male preference for more ornamented partners is explained by the superior condition and reproductive investment of these females. Prudence in sperm investment and preferential sperm allocation to more ornamented females by dominant males are consistent with dominant males having privileged access to females[10], and indicate that dominant males may be more competitive when they inseminate high-quality females, suggesting an important but neglected reason for why high-quality males may produce high-quality offspring. □

Methods

Study populations

We studied a population of feral fowl (11–16 males, 28–32 females), free-ranging at the Tovetorp Zoological Research Station, Sweden[9,10,21,22], in June–July 2000, April–September 2001 and April–June 2002. The female order experiment was also replicated in a red jungle fowl population[21] (16 males, 30 females) at the same site (June–July 2000). Males were kept isolated from females and sexually rested for at least 2 d to allow them to replenish their sperm reserves[11]. We collected natural ejaculates by presenting males with a live female fitted with a harness for collecting the ejaculate and held in a soliciting position to minimize the possibility that female behaviour would influence male copulatory response[9,23]. All males were fully habituated to human presence and no alteration in the birds' normal behaviour was observed during the trials. After copulation, ejaculates were always collected in the same manner[9] and the sperm were counted[24]. Ejaculates obtained with this technique are similar in volume to ejaculates naturally inseminated by males and subsequently ejected by females[9].

Female promiscuity experiment

We collected natural ejaculates of male feral fowl allowed to copulate *ad libitum* with a female in the presence of three, one and no other males. Males were kept in pens in groups of four, two and single males: 16 males were exposed to the four-male treatment (four groups with two top- and two bottom-ranking[25] males), and were rearranged into eight pairs of one top- and one bottom-ranking male (the top- with the third-ranking, and the second- with the fourth-ranking) in the two-male treatment. Eight of these sixteen males (four top- and four bottom-ranking males, randomly chosen) were also exposed individually to the one-male treatment. We randomized the order of treatments across males. Males were allowed singly out of the pen to copulate with a female less than 5 m from the pen in full view of the other group members.

Female novelty experiment

Each male was isolated 2 h before each trial and then allowed to copulate *ad libitum* with a female until he had lost sexual interest in her (at least 10 min had elapsed since he last ejaculated), after which the female was replaced by a new one. The new female was kept out of sight of the focal male until the familiar female was removed so that a male was never exposed to more than one female at any given time. In 2000, we used ten male feral fowl, which were each replicated (mean ± s.e.m.) 2.20 ± 0.29 (with first female), 1.78 ± 0.28 (first and second) and 2.00 ± 0.41 (first, second and third) times. In 2002, we replicated the experiment on 12 different male feral fowl, which were each exposed to one trial.

All 22 male feral fowl copulated and ejaculated sperm when exposed to a first female; 17 (77%) ejaculated sperm when presented with a second female, and 6 of these 17 (53%) ejaculated sperm with a third female. These six males also copulated with a fourth female, and one of them (17%) ejaculated sperm with this female (Figs 2 and 3). Male jungle fowl were exposed to one trial each: seven of nine (78%) ejaculated sperm when presented with a second female, six of these seven (86%) also copulated with a third female.

Female reproductive quality experiment

Eight male pairs were kept in pens in visual (but not physical) contact with a group of four females. Male hierarchies were assessed[25]. The focal male was isolated 2 h before the trial and then presented with two females from the group simultaneously for 1 min, after which he was allowed to copulate once with one of them. Copulation with the other female was then encouraged by placing a wire cage over the first female. If copulation with a female did not occur within 15 min, sperm investment in that female was considered zero. After males were sexually rested, the trial was repeated but the order of copulation with each female was reversed (thus, a male could copulate with each female on two occasions, 48 h apart).

Each male was replicated using four different groups of females: with two groups, males were allowed to choose their first copulation partner; with the remaining two, the copulation order was predetermined. Thus, male mate choice was assessed by two replicates and sperm allocation by all four replicates. Female comb area was calculated with Photoshop (Adobe) from a digital image in standard light conditions and females were ranked according to their relative comb size in all trials.

Eggs were collected for 51 d (12 May to 2 July 2002), dissected into the constituent parts, baked at 40 °C for 12 h, and dry-weighed to 0.0001 g. Maternity was established through yolk staining with lipid dyes fed to females. We measured female body size using PC1 of principle component analysis of head, tarsus and wing length (79.2% of variation explained).

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Truncated TrkB-T1 mediates neurotrophin-evoked calcium signalling in glia cells

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The neurotrophin receptor TrkB is essential for normal function of the mammalian brain[1–3]. It is expressed in three splice variants. Full-length receptors ($TrkB^{FL}$) possess an intracellular tyrosine kinase domain and are considered as those TrkB receptors that mediate the crucial effects of brain-derived neurotrophic factor (BDNF) or neurotrophin 4/5 (NT-4/5). By contrast, truncated receptors (TrkB-T1 and TrkB-T2) lack tyrosine kinase activity and have not been reported to elicit rapid intracellular signalling[4]. Here we show that astrocytes predominately express TrkB-T1 and respond to brief application of BDNF by releasing calcium from intracellular stores. The calcium transients are insensitive to the tyrosine kinase blocker K-252a and persist in mutant mice lacking $TrkB^{FL}$. By contrast, neurons produce rapid BDNF-evoked signals through $TrkB^{FL}$ and the $Na_v1.9$ channel[5,6]. Expression of antisense TrkB messenger RNA strongly reduces BDNF-evoked calcium signals in glia. Thus, our results show that, unexpectedly, TrkB-T1 has a direct signalling role in mediating inositol-1,4,5-trisphosphate-dependent calcium release; in addition, they identify a previously unknown mechanism of neurotrophin action in the brain.

A short, pulse-like application of the TrkB ligand BDNF (0.73 nM, 50 ms) to cultured astrocytes evoked a Ca^{2+} wave that spread over a distance of more than 150 μm (Fig. 1a). Ca^{2+} transients recorded in single astrocytes consisted of a fast peak, which was often followed by a biphasic recovery phase (Fig. 1b, c). Applying vehicle alone or BDNF together with a BDNF scavenger (polyclonal antibodies to BDNF) was ineffective in eliciting Ca^{2+} signals (Fig. 2h). Similar astrocytic Ca^{2+} waves can be elicited by glutamate or ATP and represent a prominent form of long-range intercellular signalling in the brain[7–10]. Notably, however, the concentrations needed for their induction by other neuroactive substances are several orders of magnitudes higher. Thus, BDNF, which is effective at subnanomolar concentrations, is the most potent endogenous agonist for the production of glia Ca^{2+} signals described so far.

With a similarly high efficiency, BDNF depolarizes neurons[5]. In contrast to the propagating Ca^{2+} wave in astrocytes, however, BDNF puffs cause neuronal Ca^{2+} signals that are spatially restricted, for example, to small portions of spiny dendrites[11]. These neuronal BDNF-induced Ca^{2+} transients are caused by Ca^{2+} influx through voltage-gated Ca^{2+} channels after the $TrkB^{FL}$-mediated depolarizing action of saxitoxin-sensitive Na^+ channels[6,11]. As expected from these previous studies, saxitoxin (STX) blocked BDNF-evoked Ca^{2+} signals in cultured hippocampal neurons ($n = 41$; Fig. 1c). As it has been suggested that BDNF-evoked Ca^{2+} transients in glia are mediated by $TrkB^{FL}$ receptors[12,13], we anticipated that STX would be also effective in astrocytes. STX, however, did not affect glia Ca^{2+} transients ($n = 4$; Fig. 1c). But whole-cell recordings obtained from astrocytes showed a BDNF-induced inward current (Fig. 1d; $n = 7$). The nature of

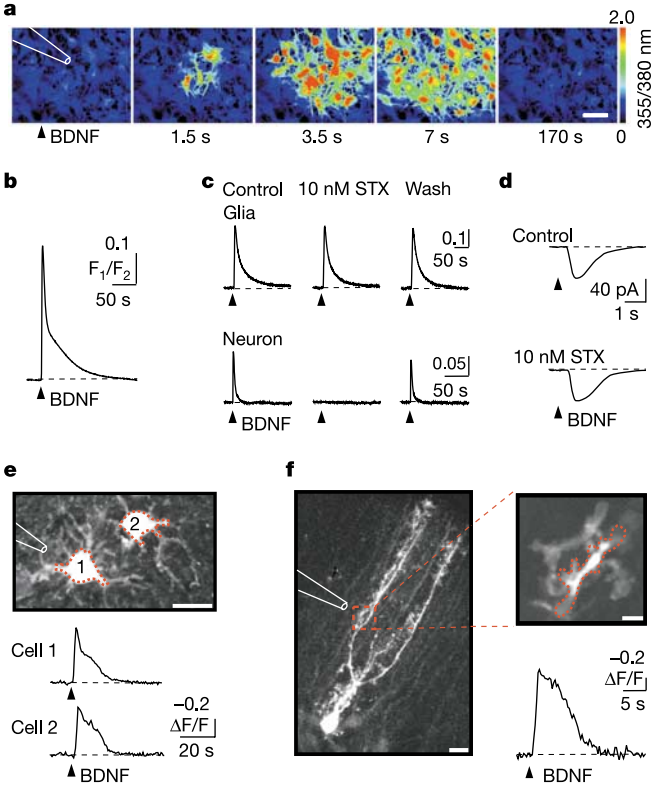

Figure 1 Ca^{2+} signalling in glia cells evoked by focal application of 0.73 nM (20 ng ml^{-1}) BDNF. **a**, BDNF-evoked Ca^{2+} wave in cultured astrocytes. **b**, Glia Ca^{2+} transient in a single astrocyte. F_1, F_2: fluorescence emission at 355 nm and 380 nm excitation, respectively. **c**, **d**, Influence of the Na^+ channel blocker STX on Ca^{2+} transients in astrocytes and CA1 pyramidal neurons (**c**) and on a BDNF-evoked inward current in astrocytes (**d**). **e**, Image of two putative glia cells in the hippocampal stratum radiatum and BDNF-evoked Ca^{2+} signals in these cells. ΔF/F: change in fluorescence emission divided by the baseline fluorescence. **f**, Images of a Bergmann glia cell and its processes, and BDNF-evoked Ca^{2+} signal in the glia process. In **a**, **e**, **f**, the position of the BDNF ejection pipette is shown schematically; scale bars, 50 μm (**a**), 10 μm (**e**), 10 μm and 2 μm (**f**). Dotted red lines in **e** and **f** indicate the area from which the Ca^{2+} measurement was taken.