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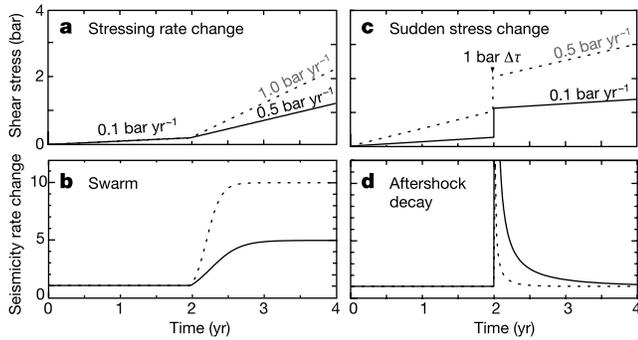


Figure 5 The rate/state effect of stress on seismicity. Details of the calculations are given in Methods. A change in the stressing rate (a) causes a swarm (b). A sudden stress change, $\Delta\tau$ (c), causes an aftershock sequence that decays inversely with time (d). The Izu swarm has several aftershock sequences embedded in it. Comparison of dashed and solid curves shows that the higher the stressing rate, the more quickly the seismicity rate reaches equilibrium. As the stressing rate change is highest close to the source, swarm seismicity appears to migrate away from an intrusion or creep site.

forecasts of damaging earthquakes during future swarms. Equally important, the Izu test suggests that a central unresolved problem of earthquake interaction—the response of seismicity to a large shock followed by viscoelastic rebound—is essentially a sudden stress change succeeded by a transient stressing rate change, which can be simulated by combining the two processes shown in Fig. 5. □

Methods

To calculate the updated daily seismicity rate, R , due to a stressing rate change (Fig. 5b), we seek the updated state variable γ from equation (11) of ref. 16, $R = r/(\gamma\dot{\tau})$, where $\dot{\tau}$ is the background stressing rate, and the background seismicity rate r is set to 1. At $t = 0$, γ is steady state, where $\gamma_{ss} = 1/(\dot{\tau})$. To evolve γ , we use equation (B17) of ref. 16, $\gamma = [\gamma_0 - \frac{1}{\dot{\tau}}] \exp\left[\frac{-\dot{\tau}t}{A\sigma_n}\right] + \frac{1}{\dot{\tau}}$, where γ_0 is the state variable before each time step, and $\dot{\tau}$ is the stressing rate. For the response to a sudden stress change $\Delta\tau$ (Fig. 5d), $\gamma = \gamma_0 \exp\left(\frac{-\Delta\tau}{A\sigma_n}\right)$, modified from equation (B11) in ref. 16. For the Izu swarm, we infer $A\sigma_n$ using the relation $A\sigma_n = t_a \dot{\tau}$. From Fig. 2a, t_a for the $M \approx 6$ shocks close to the dyke is ~ 0.3 d where the calculated stressing rate $\dot{\tau} \approx 150 \text{ bar yr}^{-1}$. The observed t_a for the background $M \approx 6$ shock in Fig. 2a is ~ 1 yr, and the background $\dot{\tau} \approx 0.1 \text{ bar yr}^{-1}$. Both estimates yield $A\sigma_n \approx 0.1$ bar, which we use here. The mean stressing rate in Fig. 4a is 32 bar yr^{-1} , and the mean t_a for the $M \approx 6$ shocks is ~ 3 d, for $A\sigma_n \approx 0.3$ bar, similar to a previous estimate²¹.

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Competing interests statement

The authors declare that they have no competing financial interests.

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Pretender punishment induced by chemical signalling in a queenless ant

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Animal societies are stages for both conflict and cooperation. Reproduction is often monopolized by one or a few individuals who behave aggressively to prevent subordinates from reproducing (for example, naked mole-rats¹, wasps² and ants³). Here we report an unusual mechanism by which the dominant individual maintains reproductive control. In the queenless ant *Dinoponera quadriceps*, only the alpha female reproduces. If the alpha is challenged by another female she chemically marks the pretender who is then punished⁴ by low-ranking females. This cooperation between alpha and low-rankers allows the alpha to inflict punishment indirectly, thereby maintaining her reproductive primacy without having to fight.

Queenless ponerine ants have evolutionarily lost the morphological queen caste⁵. All females are workers who can potentially mate and reproduce sexually (mated workers are called gamergates)⁵. Colonies of *D. quadriceps* have, on average, 80 adult workers and a single gamergate⁶, who has the alpha rank in a near-linear dominance hierarchy of about 3–5 high-ranking workers⁷. High-rankers are hopeful reproductives. They do little work, and one of them, usually the beta, replaces the gamergate if she dies⁷. Workers with lower ranks work, and are little involved in dominance

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interactions. Males, as in other social Hymenoptera⁸, play no active role in colony life. Mating experiments show that the gamergate is mated to a single unrelated male⁶, so that workers are either daughters of the gamergate, or—if gamergate replacement has recently occurred—daughters and sisters of the new gamergate.

A high-ranker can enhance her inclusive fitness by overthrowing the gamergate, rather than by waiting for her to die naturally, because the high-ranker is more related to her own offspring than to those of another high-ranker (Methods, Benefits of attempted gamergate replacement to pretender). When a high-ranker challenges the gamergate, the two ants may engage in short fights and chase each other inside the nest, jostling others and trampling brood⁷. Bouts of chasing are interspersed with periods of relative calm, during which the gamergate may rub her sting against the pretender⁷ ('sting smearing', Fig. 1a). Following sting smearing, the pretender is often immobilized by low-ranking workers (ref. 7, and C. Peeters, personal communication) (Fig. 1b). Immobilization can last several days, and typically results in the pretender losing her high rank. It is not clear why punishment causes loss of rank, but it is probably a combination of the stress caused by immobilization and being prevented from performing dominance behaviours. Occasionally the immobilized individual is killed outright. Here we experimentally test the hypothesis that the gamergate can trigger immobilization of a pretender by a distinct chemical signal.

When performing sting smearing, the gamergate marks the pretender with chemicals from the Dufour's gland, which empties via the sting⁹. We smeared beta workers with the Dufour's gland

content of either a gamergate, or another beta or a low-ranker, and then recorded the duration of immobilization induced (Methods, Immobilization trial). Immobilization cannot be confused with other aggressive behaviours (Fig. 1b). We applied extracts to betas because beta is the rank most often naturally smeared by the gamergate⁷.

Gamergate glands induced immobilization significantly more often and for longer than beta or low-ranker glands (Fig. 2), and with more damaging consequences. Betas lost high rank significantly more often following smearing with gamergate gland than with gland of betas and low-rankers (4 of 9 trials versus 2 of 27 trials; $P = 0.0245$, Fisher's exact test, one-tailed). Because gland extracts were all from non-nestmates (Methods, Immobilization trial), our results also show that the signal is neither colony specific nor unique to each gamergate.

Chemical analyses (by gas chromatography/mass spectrometry, GC/MS) show that Dufour's glands contain mostly hydrocarbons (alkanes, alkenes and methyl-branched alkanes with 15–30 carbon atoms), and that gamergate glands differ from beta and low-ranker glands. Gamergate glands ($n = 12$) have significantly more hydrocarbons than low-rankers' ($n = 9$, summed area of the 30 peaks of highest abundance is 3.2 times higher in gamergates, Mann-Whitney U -test: $z = -2.487$, $P = 0.013$) but not more than beta glands ($n = 7$, summed area is 1.9 times higher in gamergates but $z = -1.268$, $P = 0.205$). Furthermore, gamergate glands contain a distinctive chemical mixture with a higher proportion of high-molecular-mass hydrocarbons than low-ranker glands, with beta glands intermediate (Figs 3 and 4). This is in agreement with the results of the bioassay, and suggests that Dufour's gland chemicals are a pheromonal signal that induces immobilization, and that only the gamergate produces sufficient quantities or the correct composition or both.

The use of a chemical signal to trigger immobilization, and hence to control reproduction, raises the question of why other females do not produce and apply this signal. One possibility is that the signal can only be produced by fully fertile individuals^{10,11}, whereas betas have only partially active ovaries at most¹². Alternatively, counter-

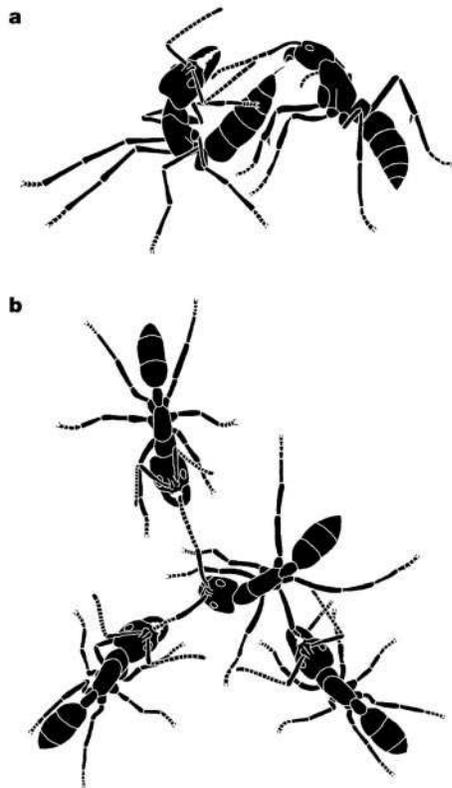


Figure 1 Dominance interactions. **a**, Sting smearing. The gamergate (left) approaches the pretender, usually from behind or from the side, briefly rubs her sting against the pretender without inserting the sting, and then runs away. Sting smearing differs from actual stinging. A stinging worker, typically a forager, bites and holds the prey and then inserts the sting into the prey to inject venom. **b**, Immobilization. One to six low-ranking workers bite and hold the appendages of the pretender for up to 3–4 days with workers taking turns (modified from ref. 7).

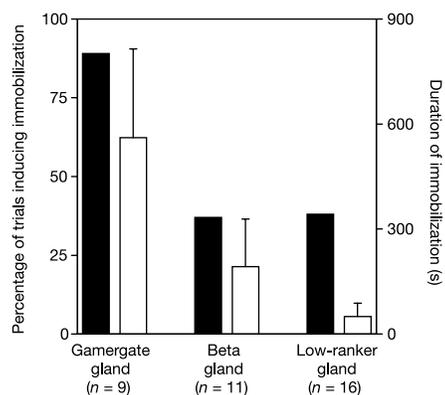


Figure 2 Bioassay results. Percentage of trials where betas were immobilized (black columns, left-hand y axis) following topical application of Dufour's gland content from either a gamergate, beta, or low-ranking worker, and average duration of immobilization (mean \pm s.e.m., white columns, right-hand y axis). Gamergate gland triggers immobilization more often (χ^2 for the three groups, 7.263; degrees of freedom d.f. = 2; $P < 0.05$. Fisher exact test, one-tailed: gamergate versus beta, $P = 0.025$; gamergate versus low-ranker, $P = 0.017$) and induces longer immobilization (Kruskal-Wallis ANOVA for the three groups: $H = 9.151$, d.f. = 2, $P = 0.0103$; Mann-Whitney U -test: gamergate versus beta: $z = -2.198$, $P = 0.028$; gamergate versus low-ranker: $z = -2.899$, $P = 0.0037$). Beta and low-ranker glands do not differ in either effect (Fisher exact test, $P > 0.05$; Mann-Whitney U -test: $z = -0.285$, $P > 0.05$).

feiting the signal could be evolutionarily unstable, because it would result in repeated gamergate replacement. This would be costly to low-rankers, because it would reduce their genetic relatedness to the gamergate's offspring. Low-rankers would, therefore, benefit by ignoring the signal. Furthermore, frequent gamergate replacement would presumably decrease colony productivity.

Our results are, to our knowledge, the first to implicate the Dufour's gland in the resolution of intracolony reproductive conflict in ants. There are, however, several precedents for the importance of the Dufour's gland in ant aggression^{9,13}. Slave-makers and workerless social parasites trigger panic and aggression among host workers with Dufour's gland chemicals^{14,15}. Queen *Leptothorax gredleri* usurp conspecific nests by smearing resident queens with Dufour's gland secretions that induce resident workers to attack their own queens¹⁶. These propaganda substances manipulate worker behaviour in a non-adaptive way, as responding workers lose inclusive fitness¹⁴⁻¹⁶. This contrasts sharply with *D. quadriceps*, where workers who prevent gamergate replacement in response to the gamergate signal increase their inclusive fitness.

Immobilization also occurs in the queenless ponerine ants *Gnamptogenys menadensis*¹⁷ and *Harpegnathos saltator*¹⁸ (reviewed in ref. 19), but is not triggered by the gamergate. Both species have several gamergates per colony, and recruitment of additional gamergates does not result in the overthrow of current gamergates. Low-rankers probably regulate gamergate number to maintain an efficient balance between egg-layers and workers, given that gamergates work less. Immobilization is directed against workers activat-

ing their ovaries^{17,18}, who are probably detected by unavoidable chemical cues of ovarian activity²⁰⁻²², rather than by an externally applied signal as in *D. quadriceps*.

Our results show sophisticated cooperation between the gamergate and low-rankers in preventing gamergate overthrow by a pretender. Cooperation is favoured because both the gamergate and low-rankers are more related to the current gamergate's offspring than to the pretender's potential offspring (Methods, Benefits of cooperative punishment to gamergate and low rankers). The cooperation integrates the ability of the gamergate to signal the identity of the pretender with the collective physical power of low-rankers to immobilize her. Immobilization in *D. quadriceps* precisely fits the definition of punishment: "individuals (or groups) commonly responding to actions likely to lower their fitness with behaviour that reduces the fitness of the instigator and discourages or prevents him or her from repeating the initial action"⁴. Loss of high rank is costly, as the punished pretender loses any hope of future reproduction (only high-rankers can replace the gamergate⁷). Immobilization in *D. quadriceps* is perhaps the clearest yet demonstration of punishment in animal societies. Similar forms of punishment, where low-rankers prevent replacement of the reproductive, could potentially occur in many insect and vertebrate societies with totipotent individuals (for example, Polistinae and Stenogastrinae wasps, Halictidae bees, cooperatively breeding birds and mammals^{23,24}). In addition, because immobilization is a mechanism by which workers prevent other workers, the pretenders, from reproducing, it is also a novel form of worker policing^{7,19,25}, analogous to

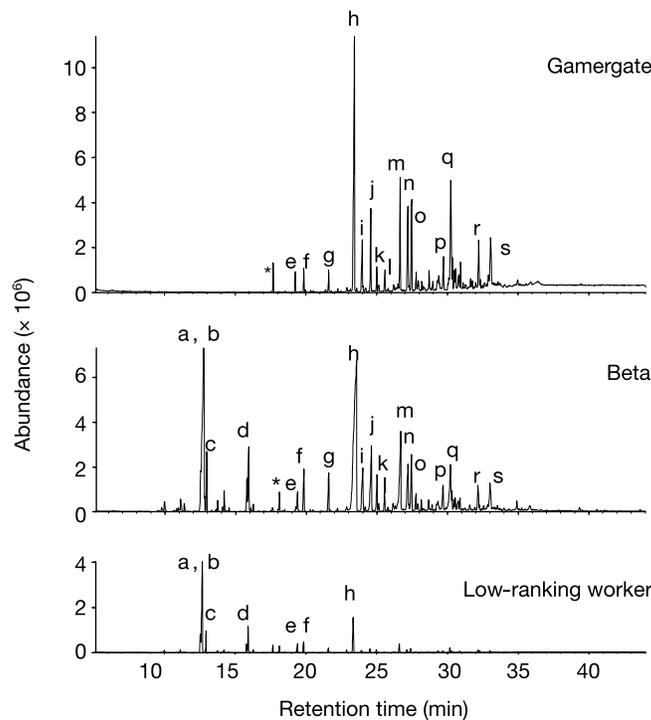


Figure 3 Chromatograms showing differences in Dufour's gland contents of workers of different ranks. Many of the hydrocarbons abundant in gamergates are rare in low-rankers and vice versa. Betas have intermediate profiles ($n = 12$ gamergates, 7 betas and 9 low-rankers). The major compounds are: heptadecadiene (a); (Z)8-heptadecene (b); *n*-heptadecane (c); (Z)9-nonadecene (d); (Z)9-heneicosene (e); *n*-heneicosane (f); *n*-docosane (g); *n*-tricosane (h); 7-,9-,11-methyltricosane (i); 3-methyltricosane (j); *n*-tetracosane (k); 11-methyltetracosane (l); *n*-pentacosane (m); 9-,11-methylpentacosane (n); 5-methylpentacosane (o); *n*-heptacosane (p); 9-,11-,13-methylheptacosane (q); nonacosane (r); and 4-,5-,6-octacosanone (s). Chemicals a–d are characteristic of betas

and low-rankers, e–h are common to all groups, and i–s are characteristic of gamergates and betas. Chemicals present in lower quantities include: *n*-pentadecane; 9-hexadecene; *n*-hexadecane; octadecene; nonadecadiene; *n*-nonadecane; octadecenal; tricosene; pentacosene; 3-methylpentacosane; hexacosene; *n*-hexacosane; 11-, 13-methylhexacosane; 5-methylhexacosane; heptacosene; 7-methylheptacosane; 5-methylheptacosane; 3-methylheptacosane; octacosene; 5-,9-,5,15-,5,17-dimethylheptacosane; *n*-octacosane; 9-,11-,13-methyloctacosane; *n*-nonacosane; 9-,11-,13-,15-methylnonacosane; *n*-triacontane; and 9-,11-methyltriacontane. Asterisk indicates the contaminant isophthalate.

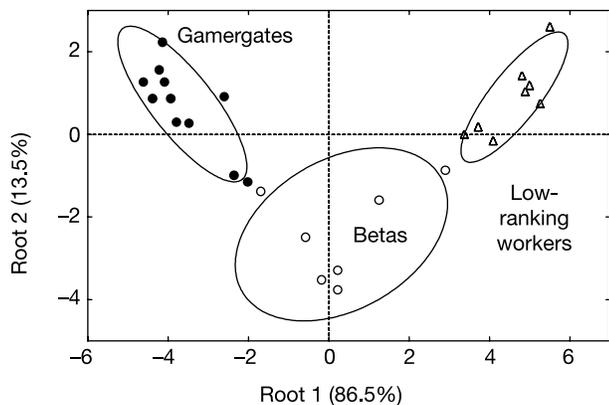


Figure 4 Discriminant analysis of Dufour's glands. The analysis separates gamergates, betas and low-rankers ($n = 12, 7$ and 9 , respectively), with no overlap of 95% confidence ellipses. The analysis was performed on the 5 factors of highest value resulting from a principal component analysis of the standardized proportions²⁰ of the 30 hydrocarbon peaks representing 0.5% or more of the total area. These 5 factors represent 92.9% of the total variance of the 30 hydrocarbon peaks.

the killing of worker-laid eggs in honeybees^{26,27} and wasps^{28,29} but acting in the context of policing breeder replacement rather than male production. Another difference is that policing by immobilization directly targets the 'selfish' worker who tries to reproduce, whereas policing by egg-killing targets only the products of selfish reproduction. □

Methods

Study animals

18 colonies of *D. quadriceps* were collected at the State University of Feira de Santana (Bahia, Brazil) in February 1998, February 2000 and October 2000. All individuals were individually marked (in Sheffield), and dominance interactions were recorded to determine social ranks. 'Blocking' and 'gaster rubbing' behaviours readily allow alpha and beta ants to be identified⁷.

Benefits of attempted gamergate replacement to pretender

High-rankers are usually daughters of the gamergate. They are not directly in conflict over reproduction with a mother gamergate, because they are as related to her offspring (0.25 brothers, 0.75 sisters), on average, as to their own offspring (0.5). However, a high-ranker can benefit by early replacement of a mother gamergate as a result of competition with other high-rankers, because she is more related to her own offspring (0.5) than to a sister's offspring (0.375)⁷. Additionally, a high-ranker would benefit from gamergate overthrow if, following recent gamergate replacement or colony fission, the gamergate is a sister (own offspring 0.5 versus sister's offspring 0.375).

Benefits of cooperative punishment to gamergate and low-rankers

The gamergate and low-rankers cooperate to prevent gamergate replacement because both parties are more related to the current gamergate's offspring than to the pretender's potential offspring (gamergate: own offspring 0.5 versus daughter's 0.25; low-rankers: mother gamergate's 0.5 versus sister's 0.375)⁷. Cooperation is still favoured if gamergate replacement has recently occurred. The new gamergate is then the low-rankers' sister, and pretenders are either sisters or nieces to low-rankers. Low-rankers favour the gamergate because they are equally or more related to the gamergate's offspring than to the pretenders'. Low-rankers additionally benefit from preventing replacement, because replacement itself would be costly. In particular, there would be a delay in brood rearing, because it takes approximately 6 weeks for a replacement alpha to mate⁶ and activate her ovaries fully³⁰. Furthermore, the colony would lose one worker, approximately 1% of the workforce, as the gamergate dies when overthrown⁷.

Immobilization trial

Dufour's glands of gamergates, betas and low-rankers were dissected ($n = 9, 11$ and 16 , respectively). Gamergate glands were thick, beige-brown and filled with a yellow oily secretion whereas beta and low-ranker glands were thin and pale-coloured. Histological studies show that gamergate glands have a thicker epithelium and biosynthetically more active cells than worker glands (J. Billen, personal communication; T.M. and F.L.W.R., unpublished work). The Dufour's glands, tubes approximately 10 mm × 1 mm in gamergates, were cut in half across the length. One half was used for chemical analysis (below). The other half was applied on the cuticle of a beta worker from another colony to simulate sting smearing. We could not smear a beta with nestmate Dufour's glands because

it is impossible to treat a beta with her own gland. In addition, using the gland of the colony's gamergate would orphan the colony and increase worker aggressiveness'. Before application of the gland, the beta was chilled for 3–5 minutes to prevent her from struggling. The beta was then returned to her nest, where she rapidly warmed up and was video-recorded for 30 min. When several workers simultaneously immobilized the beta, we summed the durations of immobilization inflicted by each worker, to reflect the higher intensity of immobilization. Betas were used in 1–8 trials carried out at 1–121-day intervals. The beta was reused soon afterwards only when the previous trial had resulted in zero or little immobilization. The venom gland also empties by the sting. The destructiveness of the bioassay and the limited supply of gamergates prevented us from studying the effect of venom. However, venom is unlikely to be involved in sting smearing and gamergate behaviour. The venom sac is full in foragers, who use venom to kill large arthropod prey, but empty in gamergates (T.M., unpublished work).

Chemical analysis

Half of each Dufour's gland was extracted in 0.5 ml hexane. 100 µl of the extract was dried by evaporation under nitrogen flow, re-dissolved in 2 µl hexane and analysed with a high performance (HP) 5890 GC/MS apparatus fitted with a 30 m × 0.25 mm internal diameter column covered with a 5% diphenyl–95% polysiloxane phase (1.0 µm thickness, Rtx-5, Restek). The carrier gas was helium (1 ml min⁻¹), and injections were splitless. The temperature was initially kept at 100 °C for 3 min, then increased by 15 °C min⁻¹ to 170 °C, then by 5 °C min⁻¹ to 300 °C and finally kept at 300 °C for 10 min. Linear and methyl-branched compounds were identified by retention times, equivalent chain length, NIST MS library and common fragmentation patterns. These identifications were not confirmed by co-injection of reference compounds and are, therefore, tentative. Double bounds were determined by derivatization with dimethyl disulphide, and their stereochemistry was determined by equivalent chain length and co-injection. Chirality of methyl-branched compounds was not determined. The numbers of individuals used for the bioassay and for chemical analysis vary, because some individuals were available for GC/MS when no colony was available for the bioassay (for example, a beta had been replaced recently, or the gamergate had been dissected).

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An ultra-sparse code underlies the generation of neural sequences in a songbird

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Sequences of motor activity are encoded in many vertebrate brains by complex spatio-temporal patterns of neural activity; however, the neural circuit mechanisms underlying the generation of these pre-motor patterns are poorly understood. In songbirds, one prominent site of pre-motor activity is the fore-brain robust nucleus of the archistriatum (RA), which generates stereotyped sequences of spike bursts during song¹ and recapitulates these sequences during sleep². We show that the stereotyped sequences in RA are driven from nucleus HVC (high vocal centre), the principal pre-motor input to RA^{3,4}. Recordings of identified HVC neurons in sleeping and singing birds show that individual HVC neurons projecting onto RA neurons produce bursts sparsely, at a single, precise time during the RA sequence. These HVC neurons burst sequentially with respect to one another. We suggest that at each time in the RA sequence, the ensemble of active RA neurons is driven by a subpopulation of RA-projecting HVC neurons that is active only at that time. As a population, these HVC neurons may form an explicit representation of time in the sequence. Such a sparse representation, a temporal analogue of the ‘grandmother cell’⁵ concept for object recognition, eliminates the problem of temporal interference during sequence generation and learning attributed to more distributed representations^{6,7}.

Songbirds produce highly stereotyped, learned vocalizations^{8,9}. Zebra finch (*Taeniopygia guttata*) song consists of a complex pattern of sounds with spectral and temporal modulation over a wide range of timescales¹⁰. A basic acoustic element is the song syllable, which may itself be composed of a complex sequence of sounds varying on a 10-ms timescale, or even less¹¹. Several distinct song syllables are organized into a single, repeated pattern of about 1 s in duration,

called a song motif. Two pre-motor nuclei have been identified for their importance in song generation: nucleus RA and nucleus HVC¹². Premotor HVC neurons project onto RA neurons, which in turn project with a myotopic mapping onto motor neurons of the vocal organ¹³, and to respiratory brain areas¹⁴. During singing, RA neurons generate a highly stereotyped, complex sequence of action potential bursts, each precisely correlated to the song vocalization on a submillisecond timescale^{1,15}. The average burst duration is roughly 10 ms, and each RA neuron generates a unique pattern of roughly ten bursts per song motif, such that on average 12% of RA neurons are active at any time (A. Leonardo, and M.S.F., unpublished data) (Fig. 1a).

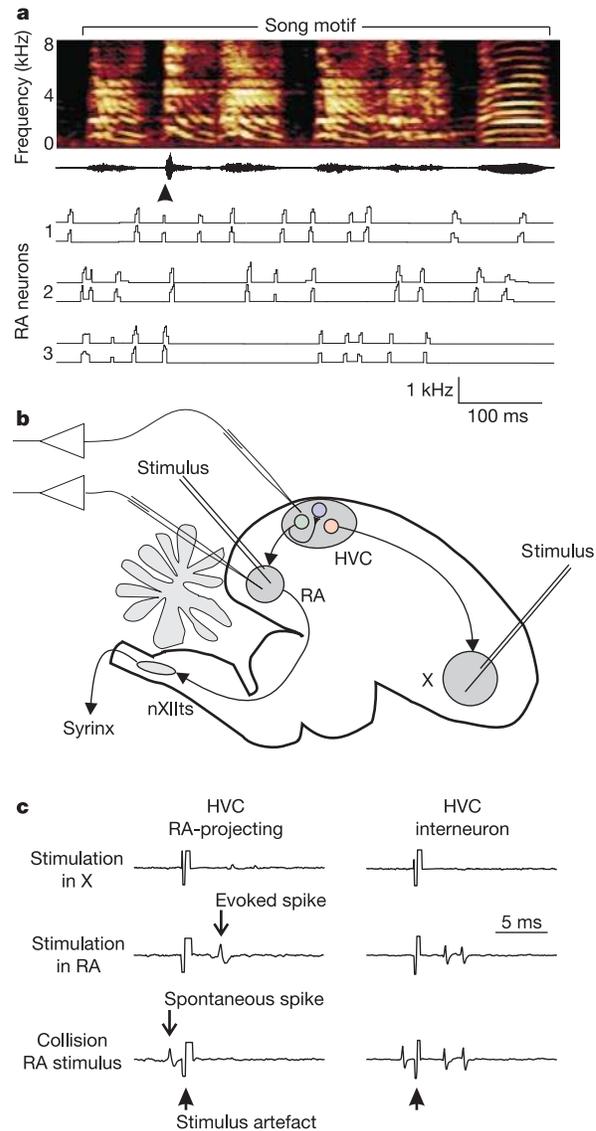


Figure 1 RA sequences and identification of HVC neurons. **a**, Neurons in nucleus RA generate complex sequences of brief action potential bursts during song vocalizations. Spectrogram (top) and acoustic signal of the song motif, and plots of instantaneous firing rate (bottom) of song-related spike activity in three different RA neurons recorded in one zebra finch. Neural activity is aligned using the onset of the second syllable of each motif (arrowhead). Two renditions are displayed for each neuron. **b**, Single-unit recordings were made in pre-motor nuclei HVC and RA. HVC neurons were antidromically identified by electrical stimulation in RA and area X. RA projects to vocal motor neurons in the nucleus of the twelfth nerve (nXIIts). **c**, RA-projecting neurons and putative interneurons could be activated from RA but not from area X. Stimulation in RA, triggered by spontaneous spikes, resulted in spike collision for RA-projecting neurons but not for interneurons.