

Use of behavioural stochastic resonance by paddle fish for feeding

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Stochastic resonance is the phenomenon whereby the addition of an optimal level of noise to a weak information-carrying input to certain nonlinear systems can enhance the information content at their outputs¹⁻⁴. Computer analysis of spike trains has been needed to reveal stochastic resonance in the responses of sensory receptors⁵⁻⁷ except for one study on human psychophysics⁸. But is an animal aware of, and can it make use of, the enhanced sensory information from stochastic resonance? Here, we show that stochastic resonance enhances the normal feeding behaviour of paddlefish (*Polyodon spathula*)^{9,10}, which use passive electroreceptors^{11,12} to detect electrical signals from planktonic prey¹³. We demonstrate significant broadening of the spatial range for the detection of plankton when a noisy electric field of optimal amplitude is applied in the water. We also show that swarms of *Daphnia* plankton are a natural source of electrical noise. Our demonstration of stochastic resonance at the level of a vital animal behaviour, feeding, which has probably evolved for functional success, provides evidence that stochastic resonance in sensory nervous systems is an evolutionary adaptation¹⁴.

Paddlefish feed on zooplankton, especially *Daphnia*⁹, in North American rivers where muddy turbidity limits vision. Instead, paddlefish use an electrosensory antenna to locate plankton¹³. The long flattened 'rostrum' in front of the mouth (Fig. 1a) is covered with tens of thousands of passive electroreceptors^{11,15} similar to the ampullae of Lorenzini of sharks and rays^{16,17}. The electroreceptors respond best to low frequency (0.5–20 Hz) external fields¹³, as

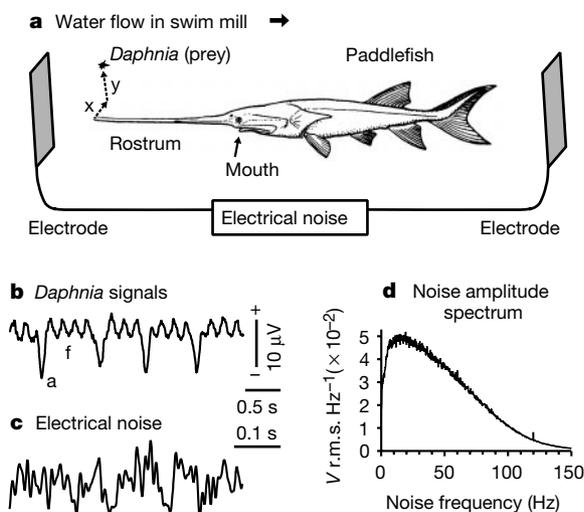


Figure 1 Experimental design. **a**, A small paddlefish (drawing reproduced with permission²⁶) fed on *Daphnia* plankton being swept towards it in a recirculating stream of water (swim mill), while noisy electrical current was passed through the water between large Ag–AgCl plate electrodes in front of and behind the fish, 95 cm apart. **b**, Electrical oscillations from a tethered *Daphnia* were correlated with rhythmic beating of the feeding legs (f) or antennae (a). The focal recording electrode was 1.2 mm behind the abdomen, with an agarose-coated plastic screen in between to block water currents. Bandwidth = 0–40 Hz. The reference electrode was distant, in 760 μS cm⁻¹ water. **c**, **d**, Sample and amplitude spectrum of the electrical noise applied during feeding.

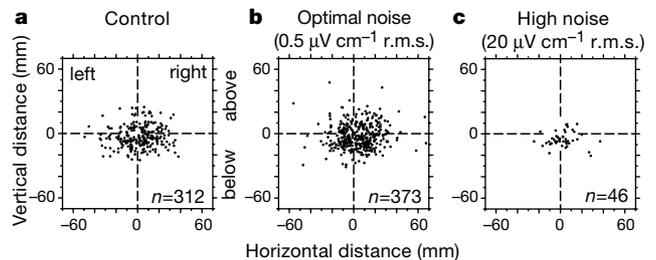


Figure 2 Spatial distributions of feeding strike locations at different noise levels. **a**, Control without noise; **b**, during an optimal amplitude of noise; **c**, during a high level of noise. Each point shows the location of an approaching *Daphnia* (plankton prey) which elicited a strike. The view is from the perspective of a fish 'looking' forward. The origin (0,0) corresponds to the midline-centre long axis of the rostrum. Distances from rostrum axis to *Daphnia* are relative. Data were pooled (see *n* values) from comparable-sized samples from each of four fish.

produced by *Daphnia* prey (Fig. 1b). We studied the role of electroreceptors in feeding behaviour by video observation of small paddlefish feeding on *Daphnia* in a recirculating stream of water (swim mill)^{10,13}. The spatial distribution of strike locations (Fig. 2a) surrounds the fish, but extends further horizontally than vertically. About 95% of *Daphnia* are taken at radial distances less than 40 mm. Plankton further from the rostrum are less likely to be detected, owing to the dipole-like electric field from *Daphnia*, which decreases approximately as the inverse cube of distance. Other sensory modalities, including vision and lateral line mechanosense, are not necessary for prey capture¹³.

Our hypothesis is that when a random electric field of optimal amplitude is applied to the environment in which a paddlefish is swimming and feeding, more distant plankton can be located and captured, compared with zero-field controls, owing to the dynamical process of stochastic resonance. Our hypothesis predicts that the spatial distribution of strike locations should become more spread out (show increased scatter or variance) when an optimal level of noise is presented. The only variable in our experiments was the selected amplitude (0.05–50 μV cm⁻¹ r.m.s.) of a randomly varying electrical stimulus passed through the water in the swim mill, between plate electrodes in front of the fish and behind it (Fig. 1a). Noise of different amplitudes strongly affected the spatial distribution of strike locations. The scatter of the feeding strike locations was increased during presentation of 0.2–1 μV cm⁻¹ noise, and was maximally increased during 0.5 μV cm⁻¹ noise (Fig. 2b), compared with zero-field controls (Fig. 2a). This amplitude, 0.5 μV cm⁻¹ r.m.s., was therefore defined to be the optimal noise amplitude. Higher levels of noise caused the distribution of strike locations to become compressed (show reduced scatter); that is, only nearby *Daphnia* elicited strikes (Fig. 2c).

Quantitative comparison of the spatial strike distributions during optimal noise or controls revealed that they differed chiefly in the vertical components of strike distances ($P_S(y)$, above or below the rostrum). A histogram of vertical strike distances (Fig. 3a) showed that with optimal noise (left side), the distribution became asymmetrical, the peak was shifted to ~10 mm below the rostrum, and there were additional outliers, compared to controls (right side). The mean and median shifted downwards only slightly (-0.92 and -1.37 mm, respectively). These distributions were normalized to the total number of strikes, so increased variance necessarily causes reduced amplitude near the centre. The increase in vertical spatial variance during optimal noise, compared to zero-field controls, was a robust finding in experiments with different fish over two years.

This increase is statistically significant ($P < 0.0001$ for equal dispersion, pooled data) as illustrated in Fig. 3b. We used the nonparametric Mood test for scale¹⁸, as the distributions (Fig. 3a)

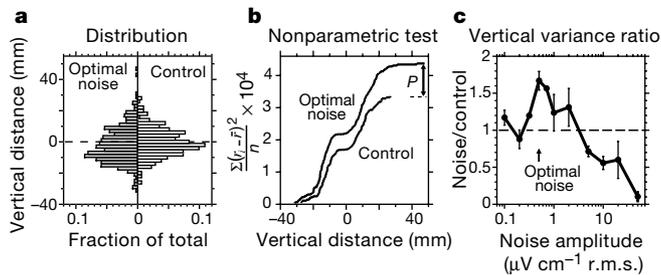


Figure 3 Spatial variance of strike locations. **a**, Normalized histograms of only the vertical components of fish-to-plankton strike distances (above or below the rostrum). **b**, Mood test¹⁸ showing increased scatter of vertical fish-to-plankton strike distances during optimal noise, seen as faster accumulation of rank variance than in control data. r_i , ranks of vertical distance values, after combining controls with optimal-noise data. \bar{r} , mean rank of combined data. n , sample size (312 for controls, 373 for optimal-noise data). P , probability was calculated for the difference between the final sums. **c**, Noise/control variance ratios (the conventional F statistic) for the vertical strike distances at different noise levels; mean \pm s.e.m. for $n = 4$ fish, two runs per fish. Points without bars are from one fish.

were not gaussian. Three of the four fish reached significance when tested individually ($P < 0.05$, Mood; $P = 0.09$ for the fourth fish), from samples of comparable size (93 ± 27 s.d. values per fish during optimal noise, and 78 ± 28 in controls). Another nonparametric test¹⁹ set 95% confidence limits: the vertical spatial dispersion was 1.13–1.49 times larger during optimal noise than in controls (for pooled data, and asymmetrical distributions¹⁹). The effects of different noise amplitudes are portrayed in Fig. 3c using the conventional F ratio (variance of vertical strike distances during noise of a given amplitude, divided by the control variance, averaged over the four fish; a ratio of 1 would signify ‘no effect’). By this measure, noise levels of $0.1 \mu\text{V cm}^{-1}$ or less had little effect. A maximum increase in the vertical spatial range, by ~ 1.6 -fold on average, or as much as 3-fold in some individual runs, was seen at $0.5\text{--}0.7 \mu\text{V cm}^{-1}$ noise. The F ratio declined to zero at higher noise levels ($5\text{--}20 \mu\text{V cm}^{-1}$) as only nearby plankton were captured; this was attributable to masking of the electrical signals from *Daphnia*. The reduced dispersion at $5\text{--}20 \mu\text{V cm}^{-1}$ noise was significant by the Mood test ($P < 0.05$, pooled data). Noise of $50 \mu\text{V cm}^{-1}$ almost abolished feeding.

The regions in space around the fish where stochastic resonance was most effective were ascertained by replotting Figs 2a and b as normalized probability distributions (Fig. 4a and b), such that the volume under each surface sums to a total of one. Subtracting these surfaces (strike distribution with optimal noise minus the control distribution, Fig. 4c) revealed two bands of noise-enhanced strike probability, one above the rostrum and another larger band below the rostrum, around the mouth. This banding pattern reflects the enhancement of vertical strike distances.

The horizontal components of distance ($P_S(x)$, to the left or right of the rostrum) also showed increased variance during optimal noise, even though Mood statistical tests did not reach significance. For example, optimal noise caused the sharp central peak in controls (Fig. 4a) to be split into left and right shoulders (arrows, Fig. 4b), reflecting the increased horizontal variance of these normalized distributions. A plot of F ratios for the horizontal components at different noise amplitudes (not shown) was similar in form to Fig. 3c for vertical components, but reached a smaller maximum of 1.3 at the same optimal noise level.

Noise amplitudes of $0.1\text{--}2 \mu\text{V cm}^{-1}$, including optimal noise, did not significantly alter the number of *Daphnia* captured per minute, compared to controls, over the group of four fish studied ($P > 0.05$, analysis of variance (ANOVA); not illustrated). For two of the fish, $0.5 \mu\text{V cm}^{-1}$ noise did cause elevation of the capture rate above

controls by $\sim 50\%$ on average, or as much as twofold in some individual runs. However for the other two fish the capture rates at noise levels of $0.1\text{--}1 \mu\text{V cm}^{-1}$ were similar to control rates. For all the fish, the capture rate fell progressively to zero at higher noise amplitudes ($5\text{--}50 \mu\text{V cm}^{-1}$).

If ‘behavioural stochastic resonance’ occurs in natural environments, there would need to be a source of electrical noise. Figure 5a–d shows that fluctuating electrical signals resembling noise are present near populations of *Daphnia*, which form dense swarms²⁰. The noise was detectable at least 5 cm from an enclosed swarm (Fig. 5d). Its amplitude declined approximately exponentially at distances beyond 2–3 cm (Fig. 5f), with a length constant of 2–3.2 cm. The noise amplitude was approximately gaussian, and increased with the population density as well as the body size of the individual *Daphnia*. Most components of the *Daphnia* noise (Fig. 5g) were within the bandwidth of paddlefish electroreceptors¹⁵. There are some differences between the *Daphnia* noise and the noise used in the experiment. For example, the same random signal was applied to all electroreceptors in the experiment, whereas for the *Daphnia* swarm the net signal is the sum of those produced by many spatially separate generators. A paddlefish swimming amongst a swarm of *Daphnia* will encounter such electrical noise. *Daphnia* may reveal their locations to paddlefish in two ways: individual *Daphnia* produce electrical signals (as in Fig. 1b), and populations produce background noise (as in Fig. 5) that could plausibly boost the sensitivity of electroreceptors, owing to stochastic resonance, during feeding in the wild. This may explain why paddlefish respond to external electrical noise: it is tied to their food source.

Three types of observation indicated that noise did not simply induce arousal^{21,22}. First, fish showed no visible response, such as special fin movements or startle flexures, when any of the noise amplitudes, even the highest, was switched on. Second, optimal noise did not affect the capture rate in two of the fish, even though these same fish did show increased spatial variance of strike locations. Third, the frequency of a different behaviour, in which fish would turn 180° (make a U-turn) and swim downstream, was also not significantly affected by the noise, compared to controls ($P > 0.05$, ANOVA; not illustrated). Another explanation for our data is that noise might cause the *Daphnia* to produce larger electrical signals. However, $50 \mu\text{V cm}^{-1}$ noise had no significant effect on the frequency or amplitude of the electrical signature arising from rhythmic beating motions of the feeding legs and antennae (recorded as in Fig. 1b), compared to controls without noise ($P > 0.05$, Kolmogorov–Smirnov test comparing power spectra).

We interpret these results as stochastic resonance increasing the sensitivity of peripheral^{3–7} electroreceptors, or acting within the brain^{12,23,24}, or both. Increased sensitivity allows the fish to detect

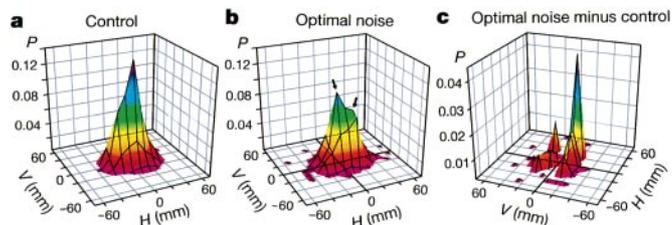


Figure 4 Probability distributions for strike locations. $P_S(x, y)$ values in controls (**a**) or during optimal noise (**b**) were obtained by binning the scatterplots in Fig. 2a and b, respectively, and normalizing to the total numbers of strikes for each. H, V , horizontal and vertical distance. The intersection of the axes corresponds to the midline centre axis of the fish’s rostrum, as in Fig. 2. **c**, Subtracting these distributions (**b** minus **a**) revealed the region of space around the rostrum where optimal noise caused an increase in the strike probability. Only positive differences are shown: $P_S(x, y)_{\text{NOISE}} > P_S(x, y)_{\text{CONTROL}}$.

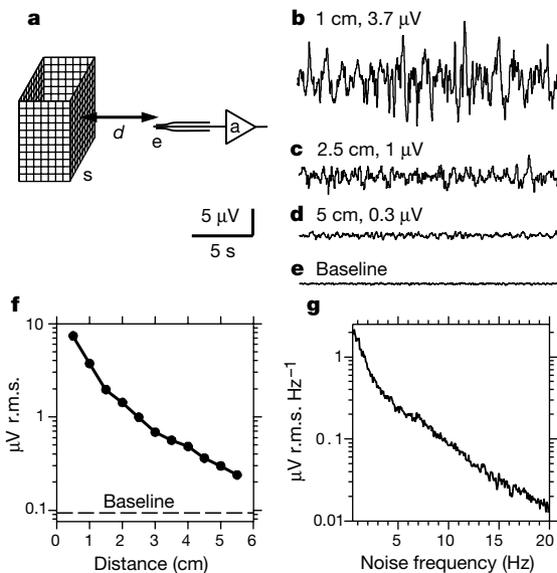


Figure 5 Electrical noise from *Daphnia* populations. **a**, An enclosure with sides of plastic screen (s), $5 \times 5 \times 3.3$ cm, contained 200 *Daphnia*, of ~ 3 mm body length. The side nearest a movable recording electrode (e) was coated with agarose to block water currents. d, horizontal distance. a, amplifier. The reference electrode was distant, in $760 \mu\text{S cm}^{-1}$ water. **b–d**, Sample recordings of *Daphnia* noise, at the distances listed, with r.m.s. amplitudes for the 0.5–20 Hz bandwidth that paddlefish electroreceptors are most sensitive to¹³. **e**, Control recording when plankton were absent, showing the electrode + instrumentation baseline (0.094 μV r.m.s.). **f**, Exponential falloff of r.m.s. amplitude in the 0.5–20 Hz band at distances beyond 3 cm. **g**, Amplitude spectrum of the swarm electrical noise at a distance of 10 mm, averaged over 54 windows, after baseline subtraction.

plankton at greater distances, increasing prey availability. The possibility that neural pathways from the brain might modulate the sensitivity of peripheral electroreceptors is unlikely, as such pathways have not been found²⁵. The optimal noise amplitude, 0.5–0.7 $\mu\text{V cm}^{-1}$, equivalent to a current density of 0.38–0.53 nA cm^{-2} , is close to the reported behavioural limit of sensitivity of ampullary electroreceptors in other freshwater fish¹⁶ ($\sim 1 \mu\text{V cm}^{-1}$). That these two numbers are comparable is expected for stochastic resonance: noise of comparable amplitude to a weak signal will enhance threshold crossings at a detector^{1–4}. Behavioural stochastic resonance may be an advantageous approach for measuring the sensitivity limits of sensory systems because, in contrast to the 50% behavioural response criterion used conventionally, stochastic resonance generates a well defined peak near the ‘threshold’.

Methods

Fish, swim mill and plankton

Data were from four juvenile paddlefish, 3–5 months old, 18–20 cm total length, deprived of food for 1–2 days before use. Experiments were run at night, when young paddlefish feed more briskly. The fish fed on individual *Daphnia* one-by-one in the viewing chamber of a ‘swim mill’^{10,13}, an apparatus for recirculating a stream of water, in a closed environmental room with only invisible near-infrared illumination. Paddlefish swim forward continuously, are ram ventilators¹⁰ and instinctively orient to swim into the oncoming stream. The water velocity ($\sim 10 \text{ cm s}^{-1}$) was adjusted to match normal swimming velocity, so a fish remained stationary relative to two co-aligned infrared video cameras which viewed the fish from the side and below (using a mirror), whose images were combined and videotaped¹³. An upstream collimator gave laminar flow, sweeping *Daphnia* in straight lines across the viewing chamber, parallel to the long axis of the fish’s rostrum. Controlled parameters included the water conductivity ($760 \mu\text{S cm}^{-1}$ at 21–22 °C), the low density of plankton in the water (kept at ~ 2 per litre by adding aliquots of 10 as a fish consumed them, through tubing from an adjacent room, and monitoring the density during experiments using a special particle counter), and the *Daphnia* size (2.64 ± 0.18 mm). Control studies showed that the distribution of plankton across a transverse section of the viewing chamber was uniform except near the walls.

Applied noise

Noise from a General Radio 1390B generator was low-pass filtered by an 8-pole Bessel filter set to 50 Hz, attenuated, and then commanded a constant-current linear isolation unit driving the stimulus electrodes (Fig. 1a). A third plate electrode grounded the water. The noise amplitude was monitored during experiments by recording differentially between the stimulus electrodes. The optimal noise amplitude was verified offline using two separate movable focal horizontal recording electrodes, one 30 cm downstream from the other, while applying noise between the stimulus plates. The same approach verified less than 3% variation in r.m.s. stimulus amplitude across a transverse section of the fish viewing chamber.

Protocol

After one fish was transferred to the swim mill for 10 min, *Daphnia* were added and the fish allowed to feed for 15 min, giving a total habituation time of 25 min before data were collected. Continuous video recording was then begun, divided into 8-min epochs throughout the 48–80 min of data collection, always alternating between an 8-min control epoch (no noise), and an 8-min presentation of a noisy electrical current, whose amplitude could be selected. Results for an 8-min noise presentation were always normalized to results in the next 8-min control epoch, to compensate for any nonstationarity. The order of noise amplitudes was randomized using random numbers. To present one entire sequence of noise amplitudes to a fish required 2–4 experimental sessions, as each session was restricted to less than 95 min of feeding to limit satiation. Each of the four fish received two complete sequences of noise amplitudes. One of the fish received additional noise presentations at amplitudes around the optimum.

Measurement of videotaped strike locations

The analysis for Figs 2–4 was ‘blinded’ to avoid bias: assistants who were unaware of the experimental protocol analysed the tapes from start to end. Feeding strikes were identified by the fish opening its mouth and lunging at an approaching *Daphnia*, usually capturing it. The strike time on the videotape was noted. Then the videotape was backed up in pause mode until the *Daphnia* was in a vertical plane at the rostrum tip¹³ (see x and y arrows in Fig. 1a), a point chosen to be before the fish had reacted. If the fish’s rostrum was turned less than 15° from straight ahead¹³, ensuring valid fish-to-plankton distance measurements because *Daphnia* approached along paths nearly parallel to the rostrum’s long axis, the image was digitized. A software cursor was used to measure the location of the *Daphnia* and the rostrum tip centre in both camera views. Subtracting these gave the relative pixel separation from the fish to the *Daphnia*, both horizontally and vertically. Pixel distances were converted to millimetres using the image of a 10-cm spatial calibration bar, and corrected for camera perspective error. We measured 4,891 strikes for Fig. 3c and other parts of Figs 2–4, an unbiased 73% sample (the other strikes were not measured because fish were turned more than 15°). For Mood tests, the median vertical distance was made equal for noise and control groups¹⁸.

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The amygdala modulates prefrontal cortex activity relative to conditioned fear

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Animals learn that a tone can predict the occurrence of an electric shock through classical conditioning. Mice or rats trained in this manner display fear responses, such as freezing behaviour, when they hear the conditioned tone. Studies using amygdalotomized rats have shown that the amygdala is required for both the acquisition and expression of learned fear responses^{1–3}. Freezing to a conditioned tone is enhanced following damage to the dorsal part of the medial prefrontal cortex⁴, indicating that this area may be involved in fear reduction. Here we show that prefrontal neurons reduce their spontaneous activity in the presence of a conditioned aversive tone as a function of the degree of fear. The depression in prefrontal spontaneous activity is related to amygdala activity but not to the freezing response itself. These data indicate that, in the presence of threatening stimuli, the amygdala controls both fear expression and prefrontal neuronal activity. They suggest that abnormal amygdala-induced modulation of prefrontal neuronal activity may be involved in the pathophysiology of certain forms of anxiety disorder.

To test whether neurons in the medial prefrontal cortex are involved in mechanisms of fear modulation, mice were implanted with two recording electrodes in the dorsal part of this cortical area. The whole experiment comprised four phases, each separated by a 3-day period: habituation phase (5 days), first training phase (2 days), second training phase (5 days) and test phase (1 day). The habituation and test phases took place in a recording chamber with behavioural and electrophysiological equipment for assessing freezing and recording multi-unit activity, respectively. The training phase took place in a different chamber. We used two conditioned stimuli: conditioned fear excitation (CS: 20-s tone) and conditioned

fear inhibition (CI: 20-s light). An electric shock was the unconditioned stimulus (US). In the first phase of training, five CS–US pairings were presented with a variable intertrial interval (60–180 s) on each of two consecutive days. Following this fear-conditioning phase, animals were divided into two groups (CI–CS paired and CI–CS unpaired groups). All animals underwent a second training phase in which five CS–US pairings were randomly mixed with five additional trials, where either the CS was preceded immediately by the CI and the US was withheld (CI–CS paired group) or the CI was presented alone (CI–CS unpaired group). The intertrial interval ranged from 60–180 s. Animals in the CI–CS paired group were then trained according to a Pavlovian conditioned inhibition procedure⁵ that reduces behavioural changes normally attributed to fear excitation. For example, it has been reported that, following conditioning (light–shock and tone–light compounds), rats displayed less fear-potentiated startle to the light CS when it was preceded by the tone CI⁶. We hypothesized that animals in the CI–CS paired group would display less freezing than animals in the CI–CS unpaired group, which is considered to be a good control group for conditioned inhibition⁷.

To test the effects of both CI and CS on spontaneous neuronal activity in the medial prefrontal cortex, multi-unit recordings were performed in the recording chamber on the last day of the experiment

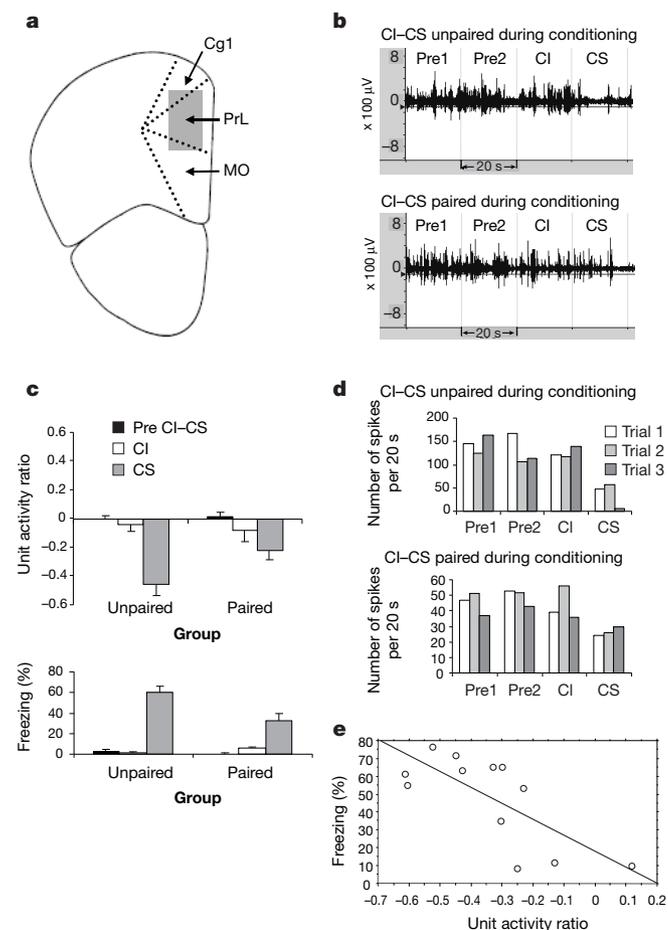


Figure 1 Conditioned inhibition: electrophysiology and behaviour. **a**, Electrode placements in the prefrontal cortex (grey area) Cg1: cingulate area 1; PrL and MO: prefrontal and medial orbital cortices, respectively³⁰. **b, d**, Representative changes in activity before (Pre 1 and 2) and during CI–CS presentation. **c**, Top, mean unit activity ratios. CS presentation caused a greater decrease in prefrontal activity in unpaired animals than in paired animals ($F_{1, 10} = 5.9$; $P < 0.05$). Bottom, mean percentage freezing. Unpaired mice displayed more freezing than paired mice ($F_{1, 10} = 7.77$; $P < 0.02$). **e**, Relationship between behavioural and electrophysiological data from all 12 animals ($r = 0.73$; $P = 0.0063$).