FIG. 3 Expression analysis of genes involved in spermatogenesis. Northern blot analysis with testis-specific probes reveals that several of these mRNAs are absent from CREM-mutant testis. Rehybridization of the filters with cytochrome c oxidase (COX) indicates equal loading.

METHODS. Total RNA was prepared from testis from three 10-week-old CREM−/− mice and their wild-type littermates, as in Fig. 1. Northern blot analysis of 10 μg RNA, each with probes for mouse proacrosin27, MCS26, prostaticins (obtained from American Type Culture Collection)29, TP-1 (obtained from American Type Culture Collection)29, RT-7 (provided by F. A. van der Hoorn); and COX26.

present in the CREM-mutant testis, although to a reduced extent, and so the dramatic reduction of MCS mRNA in homozygous mutant mice seems to be a direct consequence of the CREM mutation.

RT7 is a male germ cell-specific gene that is expressed at a very high level in early spermatids. Putative regulatory elements in its promoter include potential binding sites for members of the CREB/ATF transcription factor family20,21. In testis of homozygous mutant mice, RT7 mRNA is completely absent, indicating that CREM is essential for the activation of the RT7 gene.

Through targeted inactivation of the CREM gene we have demonstrated its importance in male fertility. The identification of a transcription factor that controls several genes involved in spermatogenesis and is directly or indirectly responsible for their activation is an important step in understanding the complex process of spermatogenesis. It seems likely that transcriptional control of these genes would be coordinated with meiosis because during this time transcription factors such as CREM could respond to the changes in chromatin structure that occur during the meiotic and meiotic relictions.

About one-third of infertile men fall into the category of idiopathic infertility, that is, they suffer from deficient spermatogenesis even though gonadotropic and androgenic hormone secretion are not subnormal25. The causes underlying this condition have remained unknown, and research in this area has been hampered by the lack of availability of appropriate animal models. Thus the specificity of the CREM mutation means that CREM homologous mutant mouse could become instrumental in unravelling those mammalian factors pivotal for the postmeiotic differentiation and maturation of haploid germ cells into spermatooza. It would also provide a potential model for developing a strategy to regulate male fertility.


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Broadband neural encoding in the cricket cercal sensory system enhanced by stochastic resonance

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SENSORY systems are often required to detect a small amplitude signal embedded in broadband background noise. Traditionally, ambient noise is regarded as detrimental to encoding accuracy. Recently, however, a phenomenon known as stochastic resonance has been described in which, for systems with a nonlinear threshold, increasing the input noise level can actually improve the output signal-to-noise ratio over a limited range of signal and noise strengths. Previous theoretical and experimental studies of stochastic resonance in physical2 and biological11 systems have dealt exclusively with single-frequency sine stimuli embedded in a broadband noise background. In the past year it has been shown in both theoretical and model biological data that stochastic resonance can be observed with broadband signals11,12. Here we demonstrate that broadband stochastic resonance is manifest in the peripheral layers of neural processing in a simple sensory system, and that it plays a role over a wide range of biologically relevant stimulus parameters. Further, we quantify the functional significance of the phenomenon within the context of signal processing, using information theory.

The effect of ambient noise on signal encoding was investigated in the cercal system of the cricket Acheta domestica, a well characterized mechanosensory system capable of detecting small-amplitude low-frequency air disturbances13. Movement of air particles, such as those caused by an approaching predator or conspecific, excite mechanosensory afferents that synapse on

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interneurons in the cricket’s terminal abdominal ganglion. These interneurons encode information about the velocity and direction of air-current stimuli in their patterns of spikes (action potentials). Intracellular recordings were made from the two types of these identified interneurons having the lowest observed thresholds for air displacement\(^5\). Their responses to a series of air-current stimuli presented at their directions of maximal response were recorded.

To demonstrate stochastic resonance in the conventional manner\(^2,6-9\), we presented very low amplitude (just suprathreshold) sinusoidal air-current signals of 23 Hz to the cricket in the presence of varying levels of 5–400 Hz white-noise background (Fig. 1). As has been seen in other systems, the output signal-to-noise ratio (SNR) increased with added input noise to a maximum (the stochastic resonance peak), and then fell as the input noise amplitude was increased beyond that level.

Though previous studies of stochastic resonance have been formulated within the context of the SNR, it is the total information encoded about the signal that is the biologically relevant quantity to consider. We have quantified the encoding accuracy in terms of Shannon’s transinformation (or mutual information) rate\(^10\) across the entire range of air-current frequencies and amplitudes to which these particular neurons are known to respond\(^11\). The test signals were broadband (5–400 Hz) white-noise air-current waveforms, presented at multiple r.m.s. amplitudes. The waveforms presented as additive noise were also 5–400 Hz white-noise air currents, constructed to be uncorrelated with the test-signal waveforms. As the organism has no way of discriminating signal from noise in this, or any other, experiment, white-noise waveforms were chosen for both, to allow the study of information flow in a sensory system. Our approach was to assess the extent to which the additive noise affected the transinforma-

**FIG. 1** Results of the standard\(^2,6-9\) stochastic resonance experimental procedure. Very low amplitude (near threshold) sine-wave stimuli in the region of best sensitivity (23 Hz) were presented with varying levels of broadband (5–400 Hz) white-noise background air disturbances. *a*, Output power spectrum of the spike-train response patterns for the broadband white noise presented alone. Average is over 12 presentations of a 3.2-s segment of white noise. Inset is a portion of the noise waveform. *b*, Output power spectrum for the same white noise as presented above along with a 23-Hz sine wave of r.m.s. amplitude 1/25 that of the noise. Insets are portions of both waveforms, presented simultaneously to the system at the direction of the interneuron’s peak response. *c*, Output SNR for varying amplitudes of input noise, calculated by the standard formula\(^2,6-9\). SNR = 10 log[(S/N(f))\(^2\)], where N(f) is the amplitude of the noise power density at the stimulus frequency when presented alone, and S is the area under the signal above the noise in the joint presentation case. For the 23 Hz sine wave at this amplitude, a stochastic resonance peak was observed near an added input noise level of 25 times the r.m.s. amplitude of the stimulus.

**FIG. 2** Results of presentations of broadband stimuli, covering the entire frequency and amplitude sensitivity ranges of the neurons, alone and with varying levels of broadband noise. *a*, Transinformation rates as a function of added input noise amplitude for three low-amplitude signals (~0.06, 0.09 and 0.12 mm s\(^{-1}\) r.m.s. velocity, bottom to top curves, respectively). Note that information rates always increased with increasing signal strength, as would be expected. Stochastic resonance peaks are seen for all three. Error bars represent the s.e.m. in the transinformation, calculated by jackknife bootstrapping statistics.\(^13,14\) *b*, Transinformation rates as a function of noise for six higher amplitude signals (~0.33–1.5 mm s\(^{-1}\) r.m.s. velocity, bottom to top curves respectively). In these cases the neuron was encoding well with no noise, and additional input noise degraded the encoding rate. METHODS. Forty different segments of broadband (5–400 Hz) white noise air-current signals, each 3.2 s in duration, were presented at 17 different mean intensity levels, spanning most of the dynamic range of the interneurons. Each signal was presented either alone or simultaneously with an uncorrelated 5–400 Hz white noise background at one of 17 possible intensities. Several independent white-noise stimuli were used for each stimulus/noise amplitude pair, to eliminate possible stimulus-specific responses. Multiple presentations of each stimulus/noise combination were made at each intensity, and trials were completely randomized with regard to stimulus waveform, stimulus amplitude, noise waveform and noise amplitude. The interstimulus waiting period was 3 s. Total rate of information transmitted about the stimulus waveform by the elicited spike trains is plotted. Note that the information rate is directly related to the SNR through Shannon’s formula\(^12\): R = \(\int \frac{df}{df} \cdot \log(1 + SNR)\) for gaussian distributions of signal and noise, such as those used here.

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tion between the test signal and the cell’s response. We calculated the coherence between the signal and the elicited spike trains as a function of frequency. This was accomplished by calculating the cross-correlation of the test-signal waveform and the spike trains across all frequencies, and then normalizing the result by the product of their autocorrelations\(^{11,12,13}\). The rate, \( R \), at which information was transmitted by each spike-train response about the corresponding signal was then computed through Shannon’s formula for transinformation with independent white noise\(^{10}\):

\[
R = \int_0^\infty \delta f \log_2 \left( \frac{S + N}{N} \right)
\]

(where \( S \) and \( N \) are the powers of the signal and the noise, respectively), and by exploiting the fact that for frequency independent linear stimulus coding, \( (S + N)/N = 1/(1 - g) \), where \( g \) is the coherence function\(^{11,12}\). Coherences between each signal waveform and its corresponding spike train were computed in the presence of several different noise waveforms, each of which were uncorrelated with the signal. Additionally, several signal waveforms were presented at each amplitude, and multiple repetitions of each signal/noise waveform combination were presented. Thus, we ensured that the measure of the accuracy of the neuronal information channel represented signal encoding, and not encoding of the noise background.

For the lowest amplitude decade of the three-decade sensitivity range of these cells, a peak in the transinformation rate was seen at an added noise level of approximately 3.2 times the r.m.s. signal amplitude (Fig. 2a). Theory predicts that such a peak should exist\(^{12}\). However, the peak occurred at an almost constant ratio of noise-to-signal input powers across an order of magnitude of stimulus amplitudes, and not at decreasing noise amplitudes for increasing signal amplitudes. This result was unexpected and we suggest it is a consequence of adaptation. For higher input signal amplitudes, added input noise had a purely detrimental effect on signal encoding (Fig. 2b).

As the signal waveform contained all frequencies from 5 Hz to 400 Hz, the effect of added noise could be evaluated independently at each frequency (Fig. 3). For a neuron sensitive to relatively broadband stimuli, such as the interneurons studied here, stochastic resonance would only be a functionally significant phenomenon if it proved beneficial to signal encoding across a large portion of the frequency range of interest, and not just for the restricted range of frequencies suggested by classical transition-rate theory\(^{13,14}\). For the cells studied here, significant improvements in SNR were indeed observed across most, or all, of the range of frequencies to which these neurons showed sensitivity.

We also calculated the average amount of information carried by individual spikes in the presence of different background noise levels, by dividing the calculated transinformation rates by the mean spike rates in each case. Plots of the bits of transinformation per spike against background-noise amplitude for low stimulus amplitudes demonstrated the same general stochastic resonance phenomenon (Fig. 4). Thus, the accuracy of individual spike placement actually increased with certain levels of added input noise.

Considerable improvements in signal encoding were observed in the perithreshold stimulus regime though the addition of external noise. The stochastic resonance-related increases in transinformation rate for broadband signals were as high as 150\%, and up to 600\% increases in SNR were observed for sinusoidal signals. Although the magnitude of the stochastic resonance effect varied under the wide variety of the signal and noise conditions tested, every cell in our experimental set (14 cells in 12 animals) showed a significant degree of encoding enhancement through stochastic resonance.

Air displacements during the attack of the wasp Liris niger, a predator of A. domestica, have been reported to be in the same

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**Figure 3** Coherence of the elicited spike trains with the signal in the presence and absence of background noise, across the frequency range of an example cell. Integrating \( 1/(1 - \text{coherence}) \) over all frequencies gives the transinformation rate (see text) as plotted in Fig. 2. Frequency-dependent coherence is shown for a 5–400 Hz white-noise signal presented at very low amplitude alone (lower line of points) and along with uncorrelated 5–400 Hz white-noise backgrounds with 3.2 times the r.m.s. amplitude of the signal (upper line of points). The plotted points are the averages of multiple presentations of identical signals with several different uncorrelated background noise waveforms. Input is the coherence of the cell’s spiking response to the same 5–400 Hz signal presented at 50 times greater r.m.s. amplitude, where encoding quality is near maximal. Note that an enhancement of the response was evident over a broad frequency sensitivity range of the cell (\( \sim 5–60 \) Hz), and that the noise-enhanced coherence function had the same general shape as the high stimulus amplitude curve. That is a broad peak was centered at about 25 Hz, a shoulder was centered at around 50 Hz and the coherence dropped to insignificance by 70 Hz. In general, coherence improvements due to added noise were observed across most or all of the frequency operating range for the majority of the cells analysed. Improvements in coherence of the spike trains with the stimulus cannot simply be the result of additional power in that frequency, as the stimulus and noise were uncorrelated. Thus, the spikes were actually improving their encoding of the signal.

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**Figure 4** Average bits of information per spike for four low-amplitude signals as a function of background noise amplitude (\( \sim 0.04, 0.06, 0.09 \) and \( 0.12 \) mm s\(^{-1}\)) r.m.s. velocities, bottom solid curve to top dashed one, respectively). Transinformation rates in each experimental condition were divided by the mean spike rate of the neuron for that condition, giving the average number of bits of information carried per spike. Note that bits per spike always increased with increasing signal strength. Over an entire decade of signal amplitudes, a peak very similar in shape to the standard stochastic resonance peak was seen. Thus, the addition of input noise increased the cells’ spiking probability, increased the amount of transmitted information, and also increased the precision with which individual spikes were being placed to encode the stimulus.
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frequency range (5–50 Hz), and escape behaviour has been reliably observed for velocities in the range where we observed stochastic resonance effects (below 1 mm s⁻¹)². Escape responses are also reliably evoked for this velocity range from a similar system in the cockroach, where it has been noted that the difference between an escape response and no behaviour was a matter of a few interneuron spikes. Clearly, stochastic resonance must be considered as having significance within the context of neural coding and computation.

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β-Amyloid-mediated vasoactivity and vascular endothelial damage

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Deposits of β-amyloid are apparent in ageing and Alzheimer's disease¹, but the role of this peptide in neurodegeneration is unclear. The free-radical theory of ageing may also account for Alzheimer-type degeneration and consequently links between free-radical generation and β-amyloid have been sought. We demonstrate here that β-amyloid interacts with endothelial cells on blood vessels to produce an excess of superoxide radicals, with attendant alterations in endothelial structure and function. The superoxide radical can scavenge endothelium-derived relaxing factor and produce potent oxidizing agents, which can cause lipid peroxidation and other degenerative changes. The alterations in vascular tone and endothelial damage are prevented by the oxygen radical scavenger superoxide dismutase. These observations suggest a normal vasoactive role for β-amyloid as well as a mechanism by which β-amyloid may play a role in vascular abnormalities and neurodegeneration mediated by free radicals.

We examined the relationship between β-amyloid peptides (Aβ 1–39, 1–40, 1–42) and oxygen radical formation in a system known

FIG. 1. a, β-Amyloid-induced contraction of blood vessel with intact endothelium. Freshly excised rat aorta was cut into ring segments 3 mm in length. The tissue was then mounted on stainless steel hooks attached to a force transducer and maintained in Krebs buffer (95% CO₂ in O₂) at 37°C. b, The buffer was changed every 15 min. After incubation for 60 min the viability of the blood vessel with intact endothelium was established by contracting the tissue using PE. Subsequent addition of acetylcholine demonstrated the characteristic relaxation of blood vessel. The vessel preparations were washed 15 min before various manipulations. The endothelium was removed from some tissues by rubbing the inside of the lumen with a spatula for 6–10 s followed by subsequent rinsing with buffer. Removal of the endothelial membrane was confirmed by testing for absence of relaxation to acetylcholine as suggested. The arrow indicates the addition of PE. The constrictor response to β-amyloid was significantly greater than the addition of 10 μM acetylcholine. In the presence of SOD, the contraction was reduced to 0.04 ± 0.02% (n = 5). Values represent the mean ± s.e.m. of five separate experiments. b, Showing the effect on acetylcholine relaxation response of rat aorta after a single dose of PE with and without pretreatment with β-amyloid.

Trace shows effect of pretreatment with a single dose (10⁻⁴ M) of β-amyloid on relaxation response. Aorta was precontracted with 10⁻⁴ M PE and relaxed with increasing doses of acetylcholine as arrowed (1, 10⁻⁷ M; 2, 10⁻⁶ M; 3, 5, 10⁻⁵ M; 4, 10⁻⁴ M; 5, 10⁻³ M; 6, 10⁻² M; 7, 10⁻¹ M; 8, 5 × 10⁻¹ M; c). The acetylcholine relaxation response is shifted to the right but maintains its sigmoidal form, indicating attenuation of acetylcholine-induced relaxation. The relaxation by acetylcholine is shown under control conditions and following 15 min incubation with 10⁻⁵ M β-amyloid protein. The aorta was precontracted submaximally with 10⁻⁵ M PE. The concentration of acetylcholine used was 10⁻⁴ M to 10⁻³ M. The values of β-amyloid represent the mean ± s.e.m. of five or more experiments. The cumulative percentage relaxation is statistically significantly lowered by β-amyloid protein at all points after 10⁻⁴ M acetylcholine (P < 0.05 or less). β-amyloid pretreatment opposes the effects of lower doses of acetylcholine. At higher doses of acetylcholine greater percentage changes in relaxation occur, preserving the sigmoidal response. Pretreatment of the blood vessel with SOD (150 units ml⁻¹) 30 s before the addition of β-amyloid blocked the attenuation of acetylcholine-induced relaxation. The relaxation induced after pretreatment with SOD + β-amyloid was significantly greater than that produced in vessels pretreated with β-amyloid alone at acetylcholine doses of 10⁻⁶ M or greater (P < 0.05 or less).

FIG. 2. a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z

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