Perceptual bias for rising tones

An approaching sound source creates a pattern of rising intensity that can specify the arrival time of the source[1–3]. Here we found that listeners reliably overestimated the change in level of rising level tones relative to equivalent falling level tones. In a natural environment this overestimation could provide a selective advantage, because rising intensity can signal movement of the source towards an organism. The bias was stronger at higher levels, suggesting that rising loudness is even more critical when a sound source is either close or loud. These results suggest a privileged status of dynamic rising loudness for harmonic tones and an asymmetry in the neural coding of harmonic dynamic intensity change.

We played synthetic vowel sounds that rose or fell equally in level to 12 subjects (Table 1). Listeners indicated change in loudness by positioning a cursor along an unmarked visual analogue scale, without considering either the direction of change or overall loudness. The left end of the scale was labelled "no change" and the right end "large change".

Rising level tones seemed to change more than falling level tones despite having the same actual change in level ($F_{(1,11)}$ = 32.74, P<0.001; Fig. 1a and Table 2), indicating that direction of change is an important (and previously unaddressed) factor in the perception of dynamic loudness change between two stimulus intensities. Furthermore, loud sounds seemed to change more than soft sounds despite having the same change in level ($F_{(3,33)}$ = 40.34, P<0.001). A significant interaction showed that the difference between rising and falling level sounds was greater for loud sounds than for soft sounds ($F_{(3,33)}$ = 12.81, P<0.001). None of these findings is predicted by traditional psychophysical laws derived using static stimuli, indicating that there are dramatic differences between static and dynamic loudness perception.

We then twice replicated the procedure with 12 new listeners in each experiment, using 1-kHz sinusoids in one experiment and white noise in the other. As was found with vowel sounds, rising level sinusoids were perceived to change in loudness more than falling ones ($F_{(1,11)}$ = 25.81, P<0.001; Fig. 1b), and loud sinusoids more than soft ones ($F_{(3,33)}$ = 25.92, P<0.001). The difference between rising and falling level sinusoids was greater for loud sounds than for soft ones ($F_{(3,33)}$ = 6.40, P<0.005). Perceived change in loudness for rising and falling level white noise was not significantly different ($F_{(1,11)}$= 0.08; Fig. 1c). However, noise at high levels changed in loudness more than noise at low levels despite having the same actual change in level ($F_{(3,33)}$ = 27.21, P<0.001). There was also a small but significant interaction of change direction and intensity range ($F_{(3,33)}$ = 3.54, P<0.05).

These results suggest that there is an asymmetry in the neural coding of harmonic rising and falling intensity sweeps, either in the response patterns of the peripheral auditory system, or in the summation of these patterns higher in the auditory pathway. The asymmetry occurred with harmonic sounds but not with broadband noise. This may be consistent with dynamic localization priorities in a natural environment. Harmonic sounds are produced by a wide variety of biological sources[4], and harmonic structure can facilitate source segregation and identification[5,6]. Approaching such a source, or anticipating its approach, can be a critically important environmental event. However, naturally occurring continuous broadband noise is less common, and can be the result of multiple sources such as crowd noise, or dispersed phenomena such as wind or rain. Dynamic localization of these types of sources may not be as important.

Behavioural research has shown that listeners can use dynamic intensity change to guide locomotion and to anticipate the arrival of a sound source[7,8]. Physiological research has shown that more intense stimuli have a lower neural response latency and higher response amplitude[9,10]. Taken together these findings suggest that dynamically changing intensity is an environmentally important cue, and that loud (or close) sounds have greater perceptual importance than soft sounds. Preferential responses to rising intensity harmonic sounds may provide an organism with an advantage in preparing for contact with a sound source, or an increased margin of safety on its approach. The increased perceptual disparity between rising and falling level tones found at higher intensities suggests that the louder the source, or the closer a source is to the organism, the more important rising intensity becomes. Although many stimulus parameters have yet to be investigated, these results suggest that rising intensity harmonic sounds have perceptual priority.

Table 1 **Conditions of intensity change.**

Rising		Falling	
Onset	Offset	Onset	Offset
60	75	75	60
65	80	80	65
70	85	85	70
75	90	90	75

Intensity changed continuously (linearly in dB s^{-1}) between onset and offset over a stimulus duration of 1.8 s.

Table 2 **Means and standard deviations for each condition.**

	Intensity change (dB)							
	60–75	75–60	65–80	80–65	70–85	85–70	75–90	90–75
Complex tone								
Mean	30.99	18.80	47.04	21.58	62.34	23.80	72.41	36.79
s.d.	16.80	13.72	12.78	11.26	14.51	10.81	13.34	17.67
Sinusoid								
Mean	39.46	28.43	55.68	39.78	70.09	39.48	79.90	51.16
s.d.	15.83	21.62	14.17	12.92	10.53	15.03	12.75	19.99
White noise								
Mean	23.93	31.36	38.38	36.94	52.91	49.10	61.28	51.53
s.d.	12.64	17.63	14.34	16.84	19.88	10.22	24.28	16.80

Visual analogue scale units.

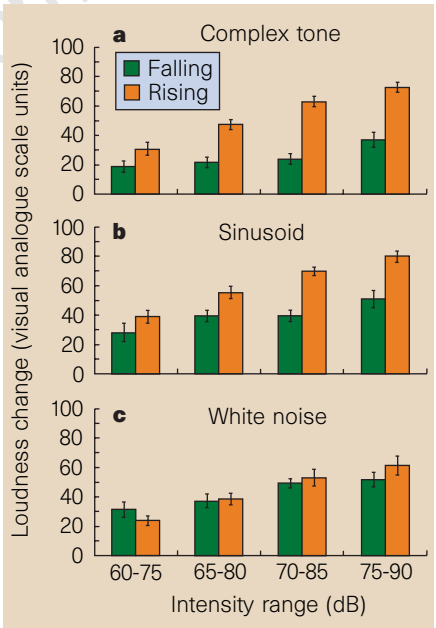

Figure 1 Perceived loudness change for each condition in each experiment. Stimuli were generated by a 16-bit sound card, fed into Sony MDR-v600 headphones, and had 10-ms onset and offset ramps. Sampling rate was 44.1 kHz for white noise and sinusoids, and 8 kHz for synthetic vowel tones. The vowel fundamental was 100 Hz with formants at 450, 1,450 and 2,450 Hz. Listeners received eight randomly ordered practice trials (one of each stimulus type) followed by each stimulus 10 times in random order. Means for each listener in each condition were used in the analysis.

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p14^{ARF} links the tumour suppressors RB and p53

Most human cancers show perturbation of growth regulation mediated by the tumour-suppressor proteins retinoblastoma (RB) and p53 (ref. 1), indicating that loss of both pathways is necessary for tumour development. Loss of RB function leads to abnormal proliferation related to the deregulation of the E2F transcription factors, but also results in the activation of p53, which suppresses cell growth. Here we show that E2F-1 directly activates expression of the human tumour-suppressor protein p14^{ARF} (the mouse homologue is called p19^{ARF}), which binds to the MDM2–p53 complex and prevents p53 degradation[2–5]. These results complete a pathway linking abnormal proliferative signals, such as loss of RB, with the activation of a p53 response, through E2F-1 and p14^{ARF}. They suggest that E2F-1, a protein inherently activated by cell-cycle progression, is part of a fail-safe mechanism to protect against aberrant cell growth.

We established inducible expression of E2F-1 in Saos-2 cells, a p53-null human osteosarcoma cell line that expresses low but detectable levels of endogenous p14^{ARF}. Activation of E2F-1 expression in these cells resulted in accelerated entry into DNA synthesis and apoptotic cell death (data not shown), as previously described following the transient expression of E2F-1 (ref. 6). Western blot analysis showed that the rapid increase in E2F-1 level following induction was closely followed by a dramatic increase in the level of p14^{ARF} (Fig. 1a). Such an increase in p14^{ARF} was not seen following the induction of two transcriptionally inactive E2F-1 mutants — E2F-1(1–374), a deletion mutant that lacks the transactivation domain, and E2F-1(132E), a point mutant that is unable to bind DNA[7] (Fig. 1a). Both of these mutant E2F-1 proteins have lost the ability to enhance cell-cycle progression, although E2F-1(1-374) retains p53-independent apoptotic activity[6]. Levels of cyclin A, which is also the product of an E2F-regulated gene, remained unchanged in these cycling cells following the induction of wild-type E2F-1. This finding is consistent with the observation that the greatest changes in expression of cyclin A would be observed in cells re-entering the cell cycle from quiescence. This indicates that the activation of p14^{ARF} expression by E2F-1 is not simply a consequence of cell-cycle progression. Expression of the two E2F-1 mutants, which inhibited cell-cycle progression, also led to the expected decrease in cyclin A expression.

The activity of the E2F-1 mutants suggests that activation of p14^{ARF} expression is dependent on the transcriptional activity of E2F-1. Analysis of the sequence of the region upstream of human exon 1β, which contains the initiation codon for p14^{ARF}, revealed the presence of a potential E2F-1 binding site, GCGGGAAA (see Supplementary information). We therefore isolated sequences encompassing the human exon 1β promoter and created a reporter plasmid in which the expression of the luciferase gene was regulated by p14^{ARF} promoter sequences. Co-transfection experiments showed that, although wild-type E2F-1 efficiently activated transcription from this promoter, a transactivation-defective E2F-1 mutant had no activity (Fig. 1b). To confirm the transcriptional activation of the endogenous p14^{ARF} gene by E2F-1, we performed northern blot analyses of the E2F-1-inducible cells (Fig. 1c). As expected, E2F-1 transcription increased within 4 hours of induction, followed by a significant induction of the p14^{ARF} message. These results show that E2F-1 expression results directly in the transcriptional activation of p14^{ARF}, although we have also observed increased p14^{ARF} stability following E2F-1 activation (unpublished data).

We propose a model in which E2F-1, by activating p14^{ARF}, protects cells from oncogenic changes that result in abnormal proliferation (Fig. 1d). Our results provide a role for p14^{ARF}, which is not required for DNA damage-induced stabilization of

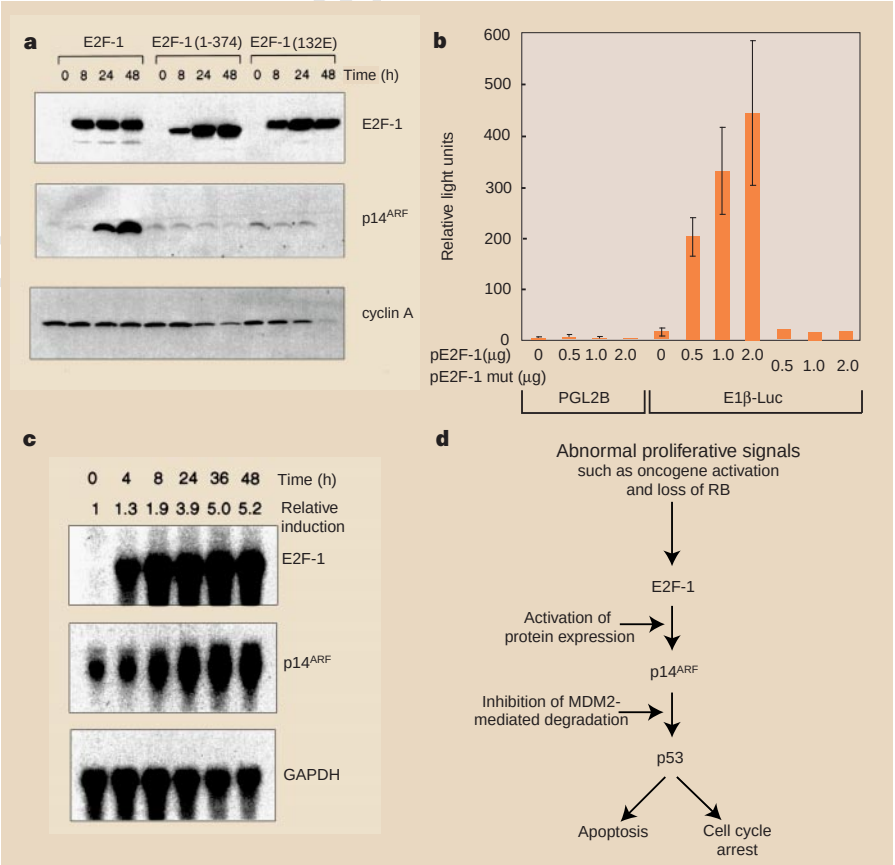

Figure 1 E2F-1 induced p14^{ARF} protein expression. **a,** Western blot analysis of Saos-2 cells inducibly expressing wild-type E2F-1, E2F-1(1-374) and E2F-1(132E). The expression of E2F-1, p14^{ARF} and cyclin A was monitored at the indicated time points following induction of E2F-1 by doxycycline. **b,** E2F-1 directly activates expression from the p14^{ARF} promoter. Activation of a reporter luciferase gene (E1b–Luc) under the control of the exon 1b promoter region following the expression of increasing amounts of wild-type, but not transcriptionally inactive, mutant E2F-1. E2F-1 does not activate transcription from a control reporter construct lacking the exon 1b sequences (PGL2B). **c,** E2F-1 induced p14^{ARF} mRNA expression. RNA was collected from the E2F-1-inducible cells at the indicated time points after the induction of E2F-1 by doxycycline, and northern blot analysis was carried out using probes to E2F-1 and p14^{ARF}. Equivalent loading was confirmed using a GAPDH probe. Quantification of the increase in p14^{ARF} expression following normalization for GAPDH is also shown. **d,** Model depicting the pathway linking RB and p53.