crowd control. For example, in violent street incidents associated with demonstrations or sporting events, it is essential to understand the conditions under which small groups can gain control of the crowd, and how rapidly and in what form this perturbation or transition in behaviour could spread.

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Metallurgy

High nickel release from 1- and 2-euro coins

The amount of nickel is regulated in European products that come into direct and prolonged contact with human skin[1] because this metal may cause contact allergy, particularly hand eczema[2–4]. Here we show that 1- and 2-euro coins induce positive skin-test reactions in sensitized individuals and release 240–320-fold more nickel than is allowed under the European Union Nickel Directive. A factor contributing to this high release of nickel is corrosion due to the bimetallic structure of these coins, which generates a galvanic potential of 30–40 mV in human sweat.

We performed skin tests with 1- and 2-euro coins in seven patients known to have nickel-contact allergy. After 48 and 72 h with these coins fixed by transparent tape onto their skin, all seven patients showed a strong reaction, with erythema, infiltration and formation of vesicles; they showed no reaction to 1% zinc chloride in Vaseline or to 1% copper sulphate in water.

In a quantitative nickel-release test (the European Standard EN 1811; ref. 5), the 50-cent coin did not release a measurable amount of nickel, as expected. However, we found that the 1- and 2-euro coins released more nickel than pure nickel itself (Fig. 1).

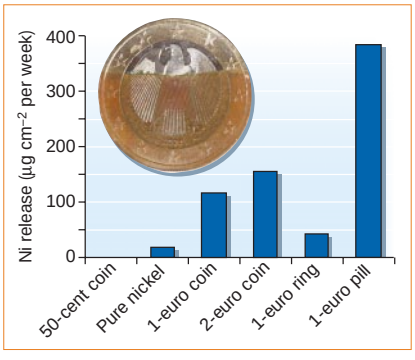

Figure 1 Release of nickel from euro coinage compared with that from pure nickel in artificial sweat, as measured by the EN 1811 standard reference test[5] (values here have not been divided by 10, as specified for the test). Release of nickel from the bimetallic 1- and 2-euro coins is higher than from pure nickel. Inset, corrosion of a 1-euro piece after partial immersion in artificial sweat for 36 h.

This was particularly high from the inner component (the 'pill') of the 1-euro coin, but not for the outer component (the 'ring'). These values are among the highest nickel-release rates ever measured on coins (see refs 6, 7 for a comparison).

In the 1-euro coin, the ring is made of a yellow alloy ('nickel brass') that consists of copper with 20% zinc and 5% nickel by weight; the white ('cupro-nickel') pill is copper with 25% nickel by weight; in the 2-euro coin the ring is cupro-nickel and the pill is nickel brass. As 1- and 2-euro coins are bimetallic, we measured the galvanic potential between the two metals with a high-impedance voltmeter after mechanically separating the pill and ring of a freshly minted coin and immersing them in either artificial sweat or saturated NaCl solution.

We found a difference in electrode potential between the two metals (the yellow metal was more negative and the white more positive) that was dependent on time and on the electrical resistance of the connector. After immersion for 24 h at ambient temperature in either solution, the potential difference between the two alloys stabilized at 40 mV (it was about 30 mV during the first 10 hours, then drifted slowly upwards) for a resistance of 100 kΩ.

It is well known that a current can enhance galvanic corrosion and thereby cause more nickel release. In thin irregular electrolyte layers such as sweat deposits, galvanic corrosion should occur primarily near the bimetallic junction because of the high resistance to lateral current flow in the thin layer. We measured electrochemically the relative rates of corrosion of each alloy, and found that the yellow metal dissolves at least five times faster than the white in the active-corrosion range (results not shown). Therefore, although the yellow component contains only one-fifth of the nickel of the white, its rate of nickel release is as high as that from the white component, or possibly higher, because the contact areas of the two alloys with the skin are about the same.

Corrosion of the 1-euro coin is visible after immersion for 36 hours in artificial human sweat: the colours changed to brown and the surface structure was damaged (Fig. 1, inset). No corrosion is evident, however, in a Swiss 1-franc coin, which consists of 25% nickel and 75% copper, under these conditions (results not shown).

We conclude that the actual release of nickel from the present 1- and 2-euro coins exceeds the value acceptable for prolonged contact with human skin (as defined by European Union directive 94/27; ref. 1) by a factor of between 240 and 320 (Fig. 1). Whether or not this is acceptable by European standards hinges on the meaning of "prolonged" contact. Further investigation is warranted not only into the epidemiological implications of such high-nickel-releasing coins but also into the factors that promote nickel release, such as the crevice between the pill and the ring — a potential corrosion site.

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Gene therapy

Biological pacemaker created by gene transfer

The pacemaker cells of the heart initiate the heartbeat, sustain the circulation, and dictate the rate and rhythm of cardiac contraction[1]. Circulatory collapse ensues when these specialized cells are damaged by disease, a situation that currently necessitates the implantation of an electronic pacemaker[2]. Here we report the use of viral gene transfer to convert quiescent heart-muscle cells into pacemaker cells, and the successful generation of spontaneous, rhythmic electrical activity in the ventricle *in vivo*. Our results indicate that genetically engineered pacemakers could be developed as a possible alternative to implantable electronic devices.

In the early embryonic heart, each cell possesses intrinsic pacemaker activity. The mechanism of spontaneous beating in the early embryo is remarkably simple[3] — the opening of L-type calcium channels causes depolarization, and the subsequent opening of transient outward potassium channels leads to repolarization. As development

progresses, the heart differentiates into specialized functional regions, each with its own distinctive electrical signature. The atria and ventricles become electrically quiescent, and the overall rate and rhythm is determined by a small number of pacemaker cells situated within compact 'nodes'.

We investigated the possibility that pacemaker activity is latent in adult ventricular myocytes and is normally repressed by the inward-rectifier potassium current (I_{K1}). This current, which is intensely expressed in the atrium and ventricle but not in nodal pacemaker cells, is encoded by the *Kir2* gene family[4]. I_{K1} stabilizes a strongly negative resting potential and should therefore suppress excitability. We tested this idea by producing dominant-negative inhibition of *Kir2*-encoded inward-rectifier potassium channels in the ventricle.

Replacement of three amino-acid residues in the pore structure of Kir2.1 (introduction of alanine residues at positions 144–146: GYG144–146AAA, here designated as Kir2.1AAA) creates a dominant-negative construct that suppresses current when expressed together with wild-type Kir2.1 (refs 5, 6). We packaged Kir2.1AAA and green fluorescent protein (GFP) into a bicistronic adenoviral vector (AdEGI–Kir2.1AAA) and injected this construct into the left ventricular cavity of guinea-pigs (*Cavia porcellus*) during transient cross-clamping of the great vessels; transduction was successful in about 20% of ventricular myocytes, as determined by GFP fluorescence. Myocytes isolated 3–4 days after *in vivo* transduction with Kir2.1AAA showed roughly 80% suppression of I_{K1}.

Non-transduced left ventricular myocytes isolated from AdEGI–Kir2.1AAA-injected animals, as well as GFP-positive cells from control AdEGI-injected hearts, showed no spontaneous activity, but did generate single action potentials when subjected to depolarizing external stimuli (Fig. 1a). By contrast, Kir2.1AAA-transduced myocytes exhibited one of two phenotypes: a stable resting potential from which prolonged action potentials could be elicited by external stimuli (7 of 22 cells; results not shown), or spontaneous activity (Fig. 1b).

The spontaneous activity, which was observed in all cells in which I_{K1} was suppressed below 0.4 picoamps per picofarad (at -50 mV), resembles that of genuine pacemaker cells: the maximum diastolic potential (-60.7 ± 2.1 mV, 15 out of 22 Kir2.1AAA cells, $P < 0.05$, *t*-test) is relatively depolarized, with repetitive, regular electrical activity initiated by gradual 'phase-4' depolarization and a slow upstroke[1,7]. Kir2.1AAA-transduced pacemaker cells responded to β-adrenergic stimulation (by isoprenaline) just as nodal cells do, by increasing their pacing rate[7].

Electrocardiography revealed two phenotypes *in vivo*: normal sinus rhythm (Fig. 1c) with simple prolongation of the QT interval (results not shown), or an altered cardiac rhythm indicative of spontaneous ventricular foci. In 40% of animals after transduction with Kir2.1AAA, premature beats of ventricular origin could be distinguished by their broad amplitude, and were found to 'march through' to a beat that was independent of, and more rapid than, that of the physiological sinus pacemaker (Fig. 1d). In these proof-of-concept experiments, the punctate transduction required for pacing occurred by chance rather than by design, in that the distribution of the transgene throughout the ventricles was not controlled. Nevertheless, the foci of induced pacemakers caused the heartbeat to originate from the ventricles.

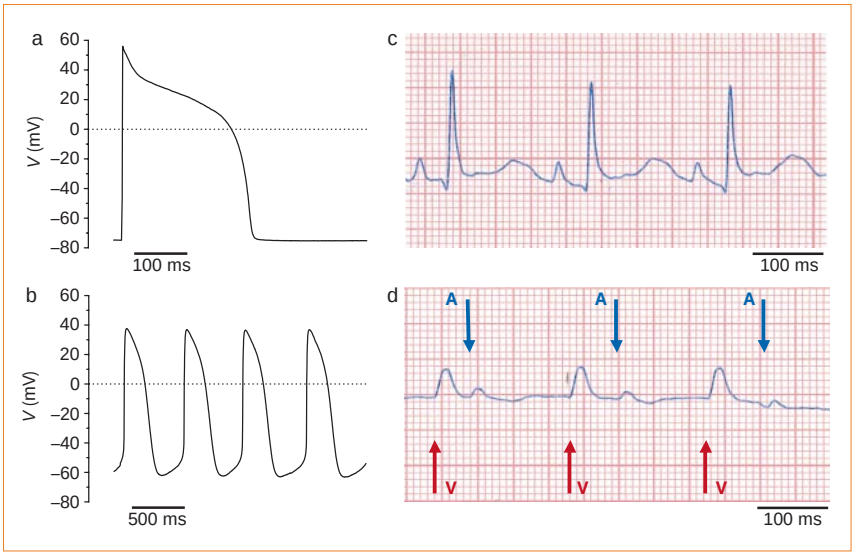

Figure 1 Suppression of Kir2.1 channels unleashes pacemaker activity. **a,** Stable action potentials evoked by depolarizing external stimuli in control ventricular myocytes. **b,** Spontaneous action potentials in Kir2.1AAA-transduced myocytes with suppressed inward-rectifier potassium current (I_{K1}; pacemaker phenotype). **c,** Baseline electrocardiograms in normal sinus rhythm (control). **d,** Ventricular rhythms for the pacemaker phenotype 72 h after transduction of Kir2.1AAA. P waves (A, arrow) and wide QRS complexes (V, arrow) 'march through' to their own rhythm.

Our findings provide new insight into the biological basis of pacemaker activity. Such activity has been thought to require the highly localized expression in nodal cells of 'pacemaker genes', such as those of the *HCN* family[8], although an important role for I_{K1} has also been recognized[7]. Exposure to barium induces automaticity in ventricular muscle and myocytes because of its time- and voltage-dependent blocking of I_{K1} (refs 9, 10). However, barium also permeates L-type calcium channels in mixed solutions of Ca^{2+} and Ba^{2+} (ref. 11) and slows inactivation[12], effects that make it difficult to interpret barium's effect strictly in terms of I_{K1}. Our dominant-negative approach is durable and regionally specific; the barium effect is not.

Our direct experimental evidence indicates that specific suppression of Kir2 channels can give rise to pacemaker activity in ventricular myocytes. The crucial factor for pacing is the absence of the strongly polarizing I_{K1}, rather than the expression of certain genes (although such genes may play an important modulatory role in genuine pacemaker cells)[13]. As genetic suppression of I_{K1} converts myocytes to pacemaker cells, localized delivery of constructs such as Kir2.1AAA to the myocardium may eventually be useful in the creation of biological pacemakers for therapeutic purposes.

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