Uncoupling the agony from ecstasy

Deactivating a single protein could prevent one of the drug's most dangerous effects.

The recreational use of amphetamine-type stimulants can produce a marked and sometimes lethal increase in body temperature. Here we show that mice deficient in a mitochondrial protein known as UCP-3 (for uncoupling protein-3) have a diminished thermogenic response to the drug MDMA (3,4-methylenedioxymethamphetamine, nicknamed 'ecstasy'; Fig. 1) and so are protected against this dangerously toxic effect. Our findings indicate that UCP-3 is important in MDMA-induced hyperthermia and point to a new therapeutic direction towards solving an increasing public-health problem.

A United Nations report estimates that there has been a 70% increase in the worldwide recreational use of MDMA from 1995 to 2000, with a consequent increase in the number of hospital cases and deaths[1,2]. Most deaths result from a syndrome of persistent hyperthermia that leads to the breakdown of skeletal muscle (rhabdomyolysis), with subsequent kidney and other organ-system failure. The molecular basis for MDMA-induced hyperthermia is unknown.

Skeletal muscle may have a role in adaptive thermogenesis[3], with heat being generated through ryanodine-receptor-mediated calcium cycling and, possibly, as a result of the action of mitochondrial uncoupling proteins. These proteins divert the energy produced by cellular respiration from driving ATP synthesis to producing heat instead. Brown fat is rich in mitochondria containing uncoupling protein-1 (UCP-1), which is an important thermoregulatory mediator.

Although the related proteins UCP-2 and UCP-3 share more than 50% sequence identity with UCP-1, it is unclear whether they also participate in thermoregulation[3]. For example, mice bred to lack UCP-3 (UCP-3$^{-/-}$ or UCP-3 'knockout' mice) have normal basal body temperatures and adapt appropriately to cold exposure[4,5], suggesting that the function of UCP-3 is not to regulate body temperature under normal physiological conditions. We therefore investigated whether this uncoupling protein could mediate the pathological thermogenic response that occurs as a result of MDMA toxicity.

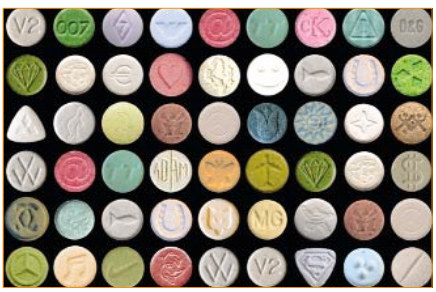

Figure 1 Hot stuff: ecstasy, which is popular among clubbers, can trigger a potentially lethal increase in users' body temperature.

Because UCP-3 is predominantly expressed in skeletal muscle and MDMA markedly increases the temperature of skeletal muscle[6], we compared the thermogenic response to this agent in wild-type and UCP-3$^{-/-}$ mice. Wild-type mice showed a dose-dependent increase in skeletal muscle temperature after administration of MDMA (Fig. 2a): the temperature peaked within the first hour after administration but remained raised for at least two hours; their rectal temperatures showed a similar response (Fig. 2b).

In contrast, although the baseline temperature of UCP-3$^{-/-}$ mice was indistinguishable from that of wild-type animals (wild type 38.6±0.08 °C; UCP-3$^{-/-}$ 38.7±0.08 °C; *P*, not significant), these mice showed a significantly blunted rise in both skeletal-muscle and rectal temperature following MDMA administration (Fig. 2a, b).

As the skeletal muscle of UCP-3$^{-/-}$ mice still expresses small amounts of UCP-2 (refs 4,5), UCP-2 may be mediating the small temperature increase seen in the UCP-3$^{-/-}$ mice. Alternatively, other thermogenic mechanisms unrelated to uncoupling (such as vasoconstriction mediated by the α_1-adrenergic receptor, for example) may be responsible[6].

Given that the fatal effects of MDMA in both rodents and humans correlate with the degree of hyperthermia[7], we tested whether UCP-3$^{-/-}$ animals were protected against a lethal challenge. Although the toxicity of MDMA in mice is less consistent than in other species[7], a 25% lethal dose (LD_{25}) is estimated as about 50 mg of drug per kg body weight[8]. Consistent with this, wild-type animals given increasing doses of MDMA under defined conditions succumbed within 4 h of drug administration, with one in five of the animals dying at a dose of 20 mg kg^{-1} and three in ten dying at 40 mg kg^{-1} (Fig. 2c). However, none of the UCP-3$^{-/-}$ animals died after administration of either dose (n = 5 and n = 10, respectively).

Our results indicate that UCP-3 is required for the rise in skeletal and core temperature that is associated with MDMA administration. Endogenously produced reactive aldehydes can also stimulate UCP-3 activity[9], and there are some structural similarities between this class of molecule and MDMA and/or its metabolites[7]. Further investigation is needed to determine whether ecstasy can directly stimulate uncoupling activity.

The temperature response in individuals

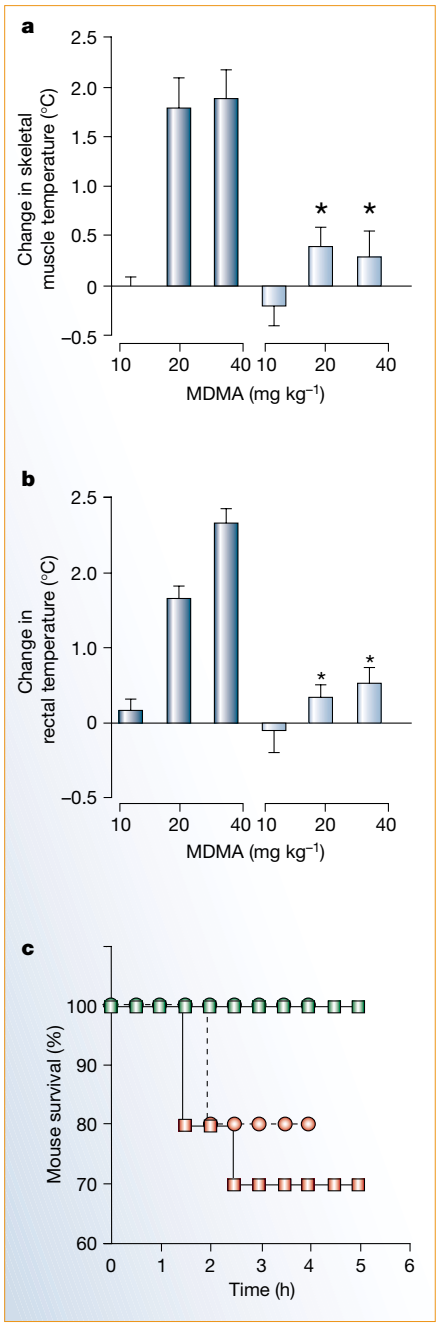

Figure 2 Mice deficient in the mitochondrial uncoupling protein UCP-3 (UCP-3$^{-/-}$ mice) are protected against the hyperthermic effects of MDMA. **a, b,** Group-housed wild-type (filled bars) and UCP-3$^{-/-}$ (white bars) mice were injected with increasing doses of MDMA and the temperature of their skeletal muscle (a) and rectum (b) recorded 1 h later. Temperatures are plotted as the change from baseline temperatures before MDMA administration. Asterisks indicate that $P < 0.01$ when compared with wild-type controls. **c,** Percentage of mice surviving MDMA administration (circles, 20 mg kg^{-1}; squares, 40 mg kg^{-1}): filled symbols, wild-type mice; open symbols, UCP-3$^{-/-}$ mice. UCP3$^{-/-}$ mice were provided by M. Reitman. Further experimental details are available from the authors.

who take excessive amounts of MDMA varies, and this variation could relate to the uncoupling activity of their skeletal muscle. For example, it is known that thyroid-hormone treatment can exacerbate the hyperthermic effects of amphetamines[10] and that UCP-3 is upregulated by thyroid hormone[11]. It is possible that UCP-2 and/or UCP-3 could also mediate the toxicity of agents such as ephedrine, methamphetamine and cocaine, recreational stimulants that can produce a similar hyperthermic response and which are related to MDMA. Our demonstration that UCP-3 is a molecular mediator of the thermogenic response to ecstasy suggests that agents designed to target uncoupling proteins could provide the basis for an important new therapeutic direction.

Edward M. Mills*, Matthew L. Banks†, Jon E. Sprague†, Toren Finkel*

**Cardiovascular Branch, NHLBI, National Institutes of Health, Bethesda, Maryland 20892-1622, USA*

†Department of Pharmaceutical and Biomedical Sciences, Ohio Northern University, Ada, Ohio 45810, USA

e-mail: j-sprague@onu.edu

1. Landry, M. J. J. *Psychoact. Drugs* **34,** 163–169 (2002).
2. Dar, K. J. & McBrien, M.E. *Intensive Care Med.* **22,** 995–996 (1996).
3. Lowell, B.B. & Spiegelman, B.M. *Nature* **404,** 652–660 (2000).
4. Gong, D.-W. *et al. J. Biol. Chem.* **275,** 16251–16257 (2000).
5. Vidal-Puig, A. *et al. J. Biol. Chem.* **275,** 16258–16266 (2000).
6. Sprague, J. E., Banks, M. L., Cook, V. J. & Mills, E. M. *J. Pharm. Exp. Therap.* **305,** 159–166 (2003).
7. Green, A. R. *et al. Pharm. Rev.* **55,** 463–508 (2003).
8. Fantegrossi, W.E. *et al. Psychopharmacology* **166,** 202–211 (2003).
9. Echtay, K.S. *et al. EMBO J.* **16,** 4103–4110 (2003).
10. Halpern, B. N., Drudi-Baracco, C. & Bessirard, D. *Nature* **204,** 387–388 (1964).
11. Gong, D.W., Karas, M. & Reitman, M. *J. Biol. Chem.* **272,** 241129–24132 (1997).

Competing financial interests: declared none.

Photonic crystals

Imaging by flat lens using negative refraction

The positive index of refraction in conventional optical lenses means that they must have curved surfaces to form an image, whereas a negative index of refraction allows a flat slab of a material to behave as a lens and focus electromagnetic waves to produce a real image[1]. Here we demonstrate this unique feature of imaging by a flat lens, using the phenomenon of negative refraction in a photonic crystalline material. The key advance that enabled this observation is the design of a photonic crystal[2,3] that has suitable dispersion characteristics to achieve negative refraction over a wide range of angles.

Although negative refraction has been demonstrated at microwave frequencies in a quasi-homogeneous metamaterial[4], imaging by a flat lens is severely constrained because of large dissipation[5,6] and anisotropy in the metamaterial. Plane-wave negative refraction at specific incident angles has been demonstrated at microwave frequencies by using a metallic photonic crystal prism[7] and a dielectric photonic crystal[8]. However, to focus a diverging beam from a point source, it is necessary for the material to exhibit all-angle negative refraction[2] as well as low absorption.

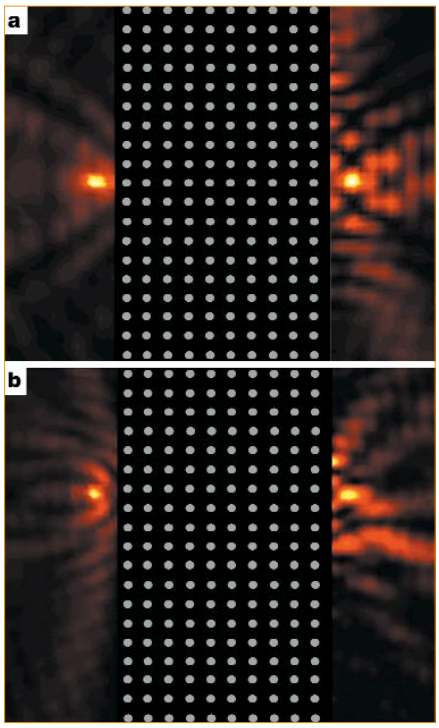

Figure 1 Imaging by a flat lens. **a**, Microwave electric-field-intensity map on a cross-sectional view of the two-dimensional source (left)–image (right) system for the flat lens. **b**, Displacement of the image by 4 cm when the source is moved up by the same distance. Both panels are to scale, corresponding to dimensions 37.5 cm × 30.0 cm. The intensity scale on the source side varies from −20 dB (yellow) to −48 dB (black), and on the image side from −30 dB to −75 dB.

Figure 1a shows the image of a microwave point source of frequency 9.3 gigaherz (GHz) (wavelength 3.22 cm) placed at a distance of 2.25 cm from a two-dimensional flat lens made of a photonic crystal fabricated from an array of cylindrical alumina rods (see supplementary information). On the far side, a high-quality image is seen at a distance of 2.75 cm. Note that for the sub-wavelength source there is an image of similar size. This image is observed only in a narrow frequency range, between 9.0 and 9.4 GHz, with the best focus at 9.3 GHz. Outside this narrow band, at all other frequencies between 2 and 12 GHz, a single focus point is not seen.

These observations can be understood by examining the band structure of the photonic crystal and corresponding equi-frequency surfaces (EFS), from which an effective refractive index, n_{eff}, can be defined and calculated[9]. In the present geometry, the central axis of the diverging beam is along the (1,0) direction of the square lattice crystal. Just below the top of the second band, between 9.0 and 9.4 GHz, the EFS move inward with increasing frequency, consistent with a negative effective index of refraction. The condition for negative refraction $n_{\mathrm{eff}}(\theta) < 0$ holds for a diverging beam of sufficiently large angle, enabling formation of the image. Figure 1 therefore also represents a demonstration of wide-angle negative refraction by a photonic crystal.

The effective refractive index is necessarily angle-dependent. Inside the crystalline lens, the electromagnetic field is highly modulated and simple ray diagrams applicable in homogeneous media cannot be employed. However, upon emerging, the waves create a focus of the divergent beam emanating from the point object.

The unique properties of the flat lens described here lead to entirely new perspectives on imaging and potentially to new applications. A particular advantage of the photonic crystalline material is its scalability to submicron dimensions, ensuring several possible applications from microwave to optical frequencies.

Patanjali V. Parimi, Wentao T. Lu, Plarenta Vodo, Srinivas Sridhar*

Department of Physics and Electronic Materials Research Institute, Northeastern University, Boston, Massachusetts 02115, USA

**e-mail: s.sridhar@neu.edu*

1. Pendry, J. B. *Phys. Rev. Lett.* **85,** 3966–3969 (2000).
2. Luo, C. *et al. Phys. Rev. B* **65,** 201104(R)(4) (2002).
3. Notomi, M. *Phys. Rev. B* **62,** 10696–10705 (2002).
4. Shelby, R. A., Smith, D. R. & Schultz, S. *Science* **292,** 77–79 (2001).
5. Houck, A. *et al. Phys. Rev. Lett.* **90,** 137401(4) (2003).
6. Iyer, A. K. *et al. Opt. Express* **11,** 696–708 (2003).
7. Parimi, P. V. *et al. arXiv:* cond-mat/0306109.
8. Cubukcu, E. *et al. Nature* **423,** 604–605 (2003).
9. Foteinopoulou, S. & Soukoulis, C. M. *Phys. Rev. B* **67,** 235107(5) (2003).

Supplementary information accompanies this communication on *Nature*'s website.

Competing financial interests: declared none.