# MITOCHONDRIAL UNCOUPLING PROTEINS IN THE CNS: IN SUPPORT OF FUNCTION AND SURVIVAL

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Abstract | Mitochondrial uncoupling mediated by uncoupling protein 1 (UCP1) is classically associated with non-shivering thermogenesis by brown fat. Recent evidence indicates that UCP family proteins are also present in selected neurons. Unlike UCP1, these proteins (UCP2, UCP4 and BMCP1/UCP5) are not constitutive uncouplers and are not crucial for non-shivering thermogenesis. However, they can be activated by free radicals and free fatty acids, and their activity has a profound influence on neuronal function. By regulating mitochondrial biogenesis, calcium flux, free radical production and local temperature, neuronal UCPs can directly influence neurotransmission, synaptic plasticity and neurodegenerative processes. Insights into the regulation and function of these proteins offer unsuspected avenues for a better understanding of synaptic transmission and neurodegeneration.

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Mitochondria are located in the cytoplasm of all eukaryotic cells and are crucial for cell survival and function. They are most important for the conversion of latent energy, found in nutrient molecules, to stored energy in the form of ATP. Organic nutrient molecules are derived from food sources such as fats, carbohydrates and proteins. These initial macronutrient substrates must be broken down into free fatty acids, simple sugars and amino acids (metabolic intermediates) for metabolism by enzymes to a form that can be transformed into energy by mitochondria. These metabolic intermediates are translocated to mitochondria and undergo oxidative phosphorylation — a mitochondrial process that uses controlled oxidation of energy substrates (releasing a molecule's free energy) to generate a proton gradient across the inner mitochondrial membrane. The potential energy produced by the proton gradient is used to drive phosphorylation of ADP to ATP by ATP synthase.

Oxidative phosphorylation involves the coupling of electron transport, through the ELECTRON TRANSFER CHAIN (ETC), to the active pumping of protons across the inner mitochondrial membrane. This generates an electrochemical gradient that is known as the proton motive force. The proton motive force drives protons through ATP synthase, which results in ATP formation (FIG. 1a) by using an electrical gradient (membrane potential) and a small chemical gradient (pH difference). However, oxidative phosphorylation is never completely coupled in mitochondria *in vivo* or *in vitro*, and reducing this link between oxidation and phosphorylation allows electron transport to proceed without coupled ATP synthesis. Initially, this endogenous uncoupling was presumed to be an artefact of mitochondrial isolation, but it is now understood to have an important biological role in many cell types.

In the absence of artificial uncoupling agents, uncoupling results from protons leaking back into the matrix[1]. Proton leak dissipates the proton motive force and reduces the number of protons flowing through the ATP synthase, even at a normal proton extrusion rate (FIG. 1b). Because these protons return to the matrix without entering the ATP synthase, energy derived from the oxidation of substrates is 'wasted' and released as heat. This leads to the first discovered biological function of uncoupled respiration

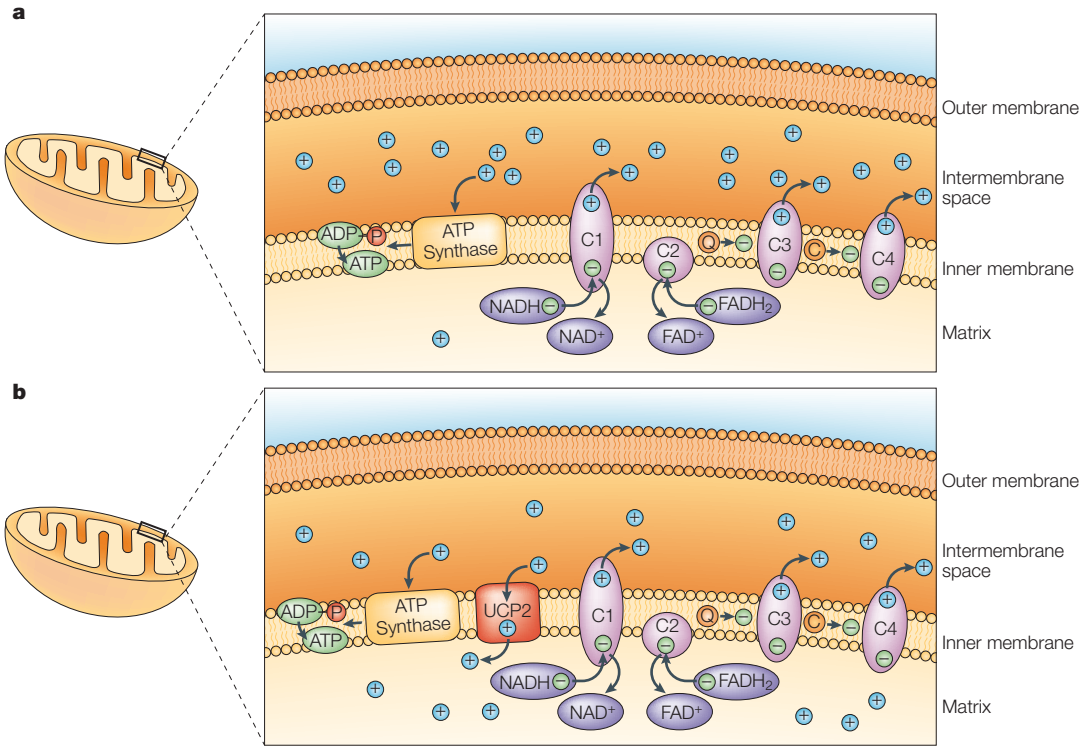


Figure 1 | **The mechanism of mitochondrial uncoupling. a** | As energy substrates enter the mitochondrial matrix, the free energy generated by oxidation is conserved in reduced molecules of NADH and $FADH_2$. NADH and $FADH_2$ then donate electrons (–) to the electron transfer chain in the inner mitochondrial membrane. As electrons flow through the enzymatic protein complexes 1, 3 and 4 (C1, 3 and 4), protons (+) are pumped from the matrix into the intermembrane space, thereby generating an electrochemical membrane potential. The membrane potential is the driving force for ATP phosphorylation. Coenzyme Q (Q) is the mobile electron carrier that accepts electrons from complexes 1 and 2 and donates them to complex 3. Cytochrome *c* (C) is the mobile electron carrier that transfers electrons from complex 3 to complex 4. **b** | Mitochondrial uncoupling mediated by uncoupling proteins (UCPs) allows controlled proton leak back into the mitochondrial matrix, thereby reducing the membrane potential.

(proton leak) in brown adipose tissue (BAT). In BAT, energy generated from the oxidation of substrates is expended by controlled proton leak through uncoupling protein 1 (UCP1) to produce heat[2]. BAT is recognized to regulate non-shivering thermogenesis in newborns, cold acclimatized and hibernating mammals, and overfed rodents[3].

Initially, little attention was paid to UCPs in non-adipose tissue. Subsequent studies clearly showed uncoupling in other tissues, although this was ascribed to the presence of mitochondrial anion carriers such as the ATP/ADP antiporter (ANT) and aspartate/glutamate antiporter (AGA), which also show uncoupling activity in a fatty acid-dependent manner, similar to UCP1 (REF. 4; BOX 1). In 1997, two additional proteins that resemble UCP1 in amino acid sequence were discovered and named UCP2 and UCP3 (REFS 5,6). UCP2 is expressed in various tissues, including the CNS, whereas UCP3 is expressed only in skeletal and cardiac muscle. Since then, UCP4 and BMCP1 (also known as UCP5) have also been identified and shown to be highly expressed in the CNS[7,8]. The resulting surge of research into the physiological function of these proteins has uncovered a unique role for neuronal UCPs in neuroprotection and neuromodulation, through controlled proton leak, in several disease and physiological processes. This review concentrates on the rapidly developing, and controversial, field of neuronal UCPs. First, we introduce the uncoupling proteins by outlining their basic structural and functional properties. We then discuss neuronal UCPs by highlighting their regional distribution in the CNS, and explain the role of UCPs in normal neuronal function. Finally, we examine the role of UCPs in neurodegeneration and offer insights into how therapeutic regulation of UCPs could be used to treat CNS disorders.

ELECTRON TRANSFER CHAIN
This comprises a series of five enzyme and protein complexes associated with the inner mitochondrial membrane. It converts energy in the form of the electron transfer potential of NADH and $FADH_2$ into the energy found in the terminal phosphate of ATP, consuming oxygen and producing water in the process.

## The UCP proteins

The UCPs are a family of mitochondrial anion-carrier proteins that are located on the inner mitochondrial membrane. UCP1 is considered to be the archetypal uncoupling protein, and much of our understanding of UCPs comes from studies involving UCP1. UCP2 and UCP3 are closely related to UCP1, and are considered to be homologues of UCP1, whereas UCP4 and BMCP1 are more divergent (REF. 1).

The UCP superfamily comprises integral membrane proteins that share structural and functional similarities. A common feature is a tripartite structure, with three repeats of ~100 amino acids, each containing two hydrophobic stretches that correspond to transmembrane alpha helices. Therefore,

Box 1 | **Uncoupling proteins are regulated by fatty acids**

**The archetypal uncoupling protein — UCP1 — is under strict control as it is activated by fatty acids that are released after adrenergic stimulation in response to cold[2]. Much debate has centred on whether the more recently discovered UCPs (UCP2–5) are also sensitive to regulation by fatty acids. The initial concern was based on the fact that UCP2 and UCP3 make up a tiny proportion of membrane-bound proteins compared with UCP1 (REF. 97). It is now becoming clear that UCP2 and 3 (UCP4 and 5 still need to be addressed) are activated by fatty acids[98,99]; indeed, it seems that UCP2 and 3 will only catalyse proton conductance in the presence of fatty acids[95,98–100]. Fatty acid activation of UCP2 occurs at a much greater concentration than for UCP1 (REFS 26,95,96), presumeably owing to the lower abundance of membrane-bound protein. The activation of uncoupling by fatty acids is sensitive to purine nucleotide inhibition and it has recently been suggested that these proteins might exist in an inhibited state that requires fatty acid activation to overcome this inhibition[101]. Furthermore, the ability of fatty acids to stimulate mitochondrial respiration has been shown in neuronal tissue and is mediated by UCP2 (REFS 26,71). The models through which fatty acids catalyse proton conductance are still under investigation (for an up-to-date review, see REFS 1,102).**

**Studies that investigate only the mRNA expression of UCPs have recently come under fire as differences in mRNA expression are not always indicative of protein levels[103]. However, caution must be extended one step further, as protein levels *in situ* do not necessarily reflect uncoupling activity because UCP activity is regulated by fatty acids and possibly superoxides (see above). Therefore, any changes in neuronal UCP mRNA or protein should be accompanied by appropriate changes in fatty acid-induced proton conductance or mitochondrial respiration.**

UCPs have six alpha-helical regions that span the lipid bilayer. The two transmembranal helices in each repeat are linked by a long hydrophilic loop, which is orientated towards the matrix side of the membrane, and the amino and carboxyl termini extend into the intermembrane space[9]. Interestingly, anion carriers and channel proteins most frequently rely on the formation of alpha-helical stretches with 12 membrane-spanning regions[10], and the functional unit of UCPs is a homodimer formed by two identical subunits that contain 12 transmembrane helices. Therefore, although UCP4 and BMCP1 are divergent in amino acid sequence compared with UCP1, they share a common protein structure with UCP1–3 that underlies functional anion-carrier proteins.

## Regional distribution of UCPs in the CNS

***UCP2.*** Studies using *in situ* hybridization have shown that UCP2 mRNA is abundantly expressed in the mouse hypothalamus, limbic system, cerebellum, choroid plexus and brainstem[11]. In the hypothalamus, the strongest hybridization signal is in the arcuate, dorsomedial, paraventricular, suprachiasmatic and ventromedial nuclei. In rats and non-human primates UCP2 mRNA is found in the same hypothalamic nuclei[12,13].

So far, there is little information about the distribution of UCP2 protein in the mouse hypothalamus, although it has been well documented in the rat and non-human primate hypothalamus. The overall distribution of UCP2-immunoreactive cell bodies in the hypothalamus is consistent with *in situ* hybridization results, with the strongest perikaryal labelling in the arcuate, paraventricular, suprachiasmatic and supraoptic nuclei in rats[14]. The presence of abundant UCP2 mRNA and protein in the hypothalamus indicates that UCP2 has an important role in metabolic, autonomic and endocrine regulation.

In the mouse, rat and non-human primate hindbrain and brainstem, there is abundant hybridization in the area postrema, medulla and dorsal motor nucleus of the vagus nerve. In rats and non-human primates, protein expression is also abundant in these regions as well as in the parabrachial nucleus, the nucleus of the solitary tract, the spinotrigeminal tract, the raphe nucleus and the locus coeruleus[13,14]. There is significant mRNA and protein expression in the midbrain, the substantia nigra and ventral tegmental area. Moreover, UCP2 in the substantia nigra is co-expressed with tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis[15].

There are also species differences in the expression of UCP2: UCP2 mRNA is abundant in the cerebellum of mice but not rats, whereas mRNA expression in the hippocampus is strong in rats but not mice. Future studies need to address whether protein expression in these regions also differs, as regulation might occur at the level of transcription or translation.

***UCP4 and BMCP1/UCP5.*** Although both UCP4 and BMCP1 are more widespread in the brain than UCP2, there is little information about their detailed localization in the CNS. UCP4 transcripts detected by northern blot analysis were found in most brain tissues, with low levels in the spinal cord, medulla and substantia nigra[7]. Interestingly, these areas are relatively abundant in UCP2 mRNA. *In situ* hybridization of BMCP1 in mice revealed a substantially different distribution compared with that of UCP2 mRNA. There was particularly high BMCP1 mRNA expression in the cortex, hippocampus, thalamus, amygdala and hypothalamus[8,16]. Further studies[16] revealed abundant BMCP1 protein expression in the cortex, thalamus and hippocampus, as well as in the cerebellum, basal ganglia and spinal cord. Moreover, the immunoreactive cells were almost all neurons. Future research will be necessary to clarify the anatomical distribution of UCP4 and BMCP1.

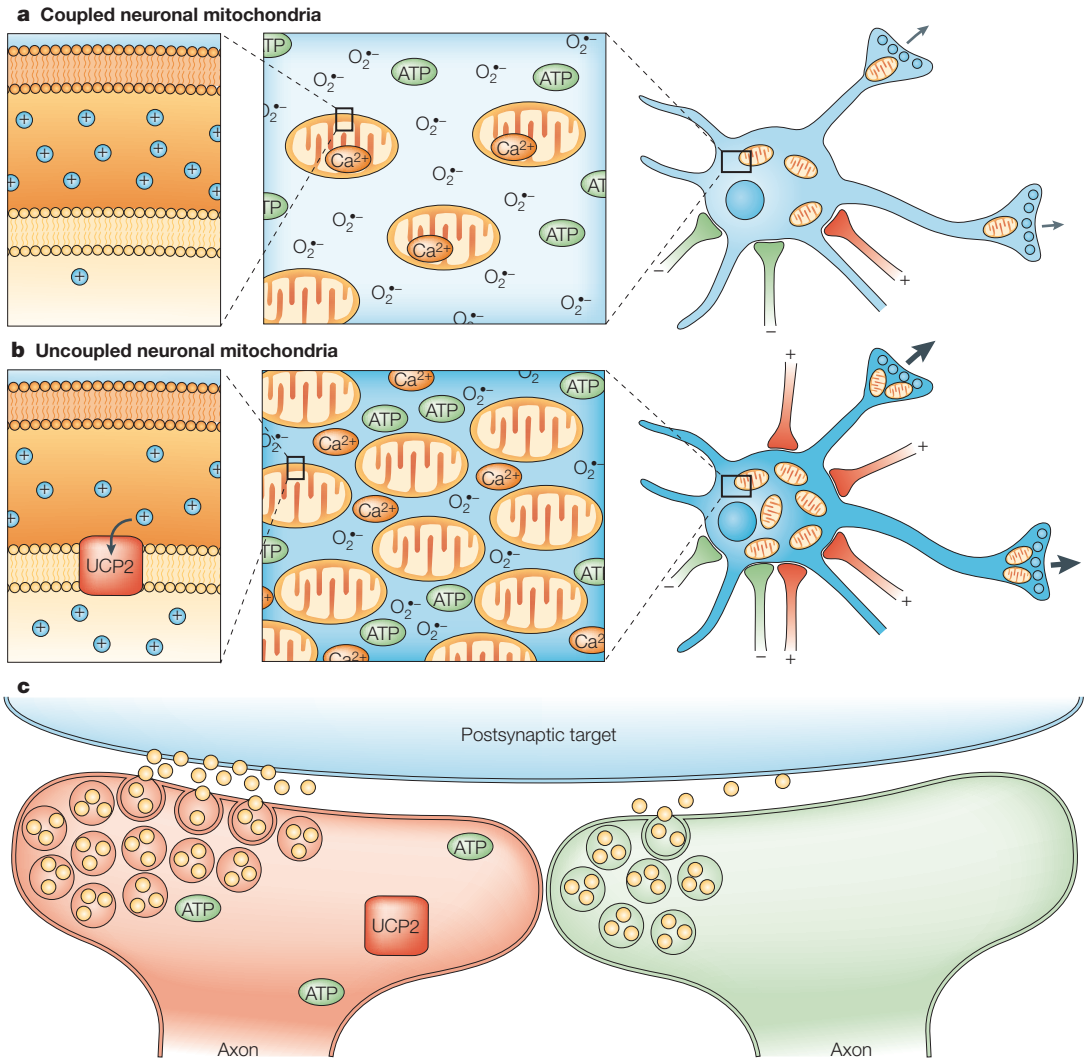


Figure 2 | **Proposed mechanism through which neuronal uncoupling proteins can regulate neuronal function.** **a** | Coupled mitochondria have a large mitochondrial membrane potential across the inner mitochondrial membrane. A large membrane potential promotes strong proton drive through ATP synthase, and, as a consequence, enhances reactive oxygen species (ROS) production and mitochondrial calcium influx, both of which are known to promote neuronal dysfunction. Eventually, this coupled mitochondrial state limits synaptic plasticity and neurotransmission. **b** | Increased uncoupling protein (UCP) activity allows controlled proton re-entry (leak) into the mitochondrial matrix without affecting ATP synthase activity. Consequently, ROS production is diminished and mitochondrial calcium efflux is increased. Although acute uncoupling reduces mitochondrial ATP production due to decreased proton drive through ATP synthase, chronic uncoupling leads to mitochondrial proliferation and greater ATP production per cell. Therefore, uncoupled neurons are readily amenable to dynamic fluctuations in neuronal activity and adapt rapidly and efficiently through enhanced synaptic plasticity and neuronal transmission. **c** | Shows UCP-induced mechanisms that lead to enhanced neurotransmission (left). By dissipating the mitochondrial membrane potential, uncoupling leads to local heat generation. It is therefore proposed that local temperature gradients increase neurochemical diffusion through extracellular compartments to postsynaptic neuronal targets. In addition, elevated cellular ATP promotes active processes — such as vesicle formation, transportation and exocytosis — at the presynaptic nerve terminal, thereby promoting neurotransmission.

## UCPs in normal neuronal function

UCPs function by allowing controlled dissipation of the proton motive force, which is established by actively pumping protons against their concentration gradient as electrons flow through the ETC. The activation of UCPs allows protons to re-enter the mitochondrial matrix and, in the process, reduces the driving force for ATP synthesis by mitigating proton flow through ATP synthase. The mitochondrial membrane potential exists as the electrical difference, established by protons, across the inner mitochondrial membrane. Therefore, the nature of uncoupling activation allows us to predict a decrease in mitochondrial membrane potential. Transfecting cultured cells with UCP2 (REF. 17), UCP4 (REF. 7) or BMCP1 (REF. 16) results in a decrease in mitochondrial membrane potential, which indicates that uncoupling activity is consistent in neuronal UCPs. The overall impact of uncoupling by neuronal UCPs on neuronal function might result from any or all of several mitochondrial effects (discussed below; FIG. 2).

***'Thermal' synapses.*** An exciting aspect of controlled mitochondrial uncoupling by neuronal UCPs is their ability to influence temperature in the microenvironment of presynaptic nerve terminals, thereby providing a mechanistic basis for temperature as a neuromodulator[14]. There is a crucial distinction between thermogenesis pertaining to core body temperature and energy dissipation in the form of heat at the mitochondrial level. Clearly, UCP1 is the key protein involved in thermogenesis in BAT, whereas UCP2–5 contribute little to the maintenance of core body temperature[18,19]. Indeed, CNS areas rich in UCPs (such as the hypothalamus) have a higher local temperature than areas relatively devoid of UCPs (for example, the striatum and thalamus, which have a 2.7% maximal significant reduction compared with the hypothalamus), despite steady core body temperature[14]. The increase in temperature has been shown to be associated with increased uncoupling activity, which indicates that heat generation in neuronal microenvironments is affected by UCPs[14]. Considering that UCPs are expressed in neuronal mitochondria, that they frequently accumulate in axon terminals close to synaptic vesicles and synaptic membranes, and that UCP activation leads to heat generation, it is reasonable to postulate that this presynaptic temperature change affects synaptic neurotransmission and transmitter reuptake.

Recent evidence indicates that temperature gradients created by neuronal UCPs enhance the migration of chemical signals from a cell source to high-affinity receptors on cells for the signal, especially in brain regions where there are mismatches in innervation and receptor localization[20]. For example, D1 dopamine receptors are highly expressed in the nucleus accumbens shell, but dopamine terminals are almost completely absent. However, a high density of dopamine terminals surrounds this region and patches of UCP2 immunoreactivity have been observed between the mismatched regions. This suggests that UCP2 might enhance diffusion and convection of dopamine into the nucleus accumbens shell by generating local temperature gradients[20], as chemical diffusion is temperature dependent. This highly provocative hypothesis awaits experimental proof.

***Calcium regulation.*** Mitochondria have a large capacity for calcium uptake and are an important site of calcium storage in cells. Mitochondrial calcium influx or efflux (through the potential-driven calcium uniporter) depends on the inner mitochondrial membrane potential[21]. A significant increase in mitochondrial membrane potential underlies elevations in calcium influx into mitochondria, whereas a significant fall in membrane potential releases mitochondrial calcium. Mitochondria have evolved as important cytosolic calcium buffers, because calcium cannot be metabolized like other second-messenger molecules. Therefore, intracellular calcium must be tightly regulated to maintain homeostasis. Brief exposure of cultured neurons to uncoupling agents such as FCCP (carbonyl-cyanide-4-(trifluoromethoxy)-phenylhydrazone) and dinitrophenol reduced mitochondrial membrane potential, inhibited mitochondrial calcium uptake and prevented cell death[22]. These results indicate that uncoupling lowers the mitochondrial membrane potential, limits mitochondrial calcium overloading and decreases the potential for apoptotic events. Moreover, UCP2 expression in cardiomyocytes produced a partial lowering of the mitochondrial membrane potential that was sufficient to restrict mitochondrial calcium influx[23]. Therefore, the ability of neuronal UCPs to regulate the mitochondrial membrane potential underlies their ability to regulate neuronal calcium homeostasis. As mitochondria are numerous in presynaptic terminals, and the calcium milieu of axon terminals is important in vesicle trafficking, fusion, release and recycling, regulation of presynaptic calcium concentrations by UCPs is expected to directly affect neurotransmission.

***ATP production and mitochondrial biogenesis.*** When considering the biological functions of UCPs, there is a fine line between cellular protection and cellular degeneration. Tissue-specific functions are emerging: for example, in the pancreas, UCP2 has been linked to the pathophysiology of type 2 diabetes[18]. In this instance, the presence of UCP2 in pancreatic beta cells promotes cell degeneration owing to ATP depletion, which, in turn, leads to reduced insulin secretion[1]. Intriguingly, however, the induction of mitochondrial uncoupling through UCP2 in the hippocampus[24] and UCP3 in the muscle[25] elevates cellular ATP and ADP levels. Although mitochondrial uncoupling suppresses ATP production per mitochondrion, UCPs trigger mitochondrial proliferation in neuronal[24,26] and adipose tissue[27], thereby elevating ATP and ADP for a given cell. Peroxisome proliferative activated receptor-γ (PPARγ) co-activator 1 (PGC1), a cold-inducible co-activator of nuclear receptors, stimulates mitochondrial biogenesis and respiration in muscle cells through induction of UCP2 (REF. 28). Furthermore, high aerobic capacity reduces certain parameters of cardiovascular disease, including serum insulin levels, and increases UCP2 expression in skeletal muscle[29]. Therefore, the ability of neuronal and muscle tissue to undergo mitochondrial biogenesis and enhance ATP production after increased uncoupling might underlie tissue-specific UCP function.

***Neuronal UCPs and ROS production.*** Neuronal UCPs are crucial for reducing the production of reactive oxygen species (ROS) and consequent oxidative stress. This is one of the most attractive explanations for ways in which neuronal UCPs might reduce neurodegenerative pathology. An increase in mitochondrial membrane potential promotes ROS production[30] close to the inner mitochondrial membrane, as it increases 'random' single electron transfer reactions from components of the ETC to molecular oxygen. Increased mitochondrial uncoupling through UCP2 decreases ROS production or oxidative stress, and is neuroprotective in response to pharmacological and physical insults[15,24,31].

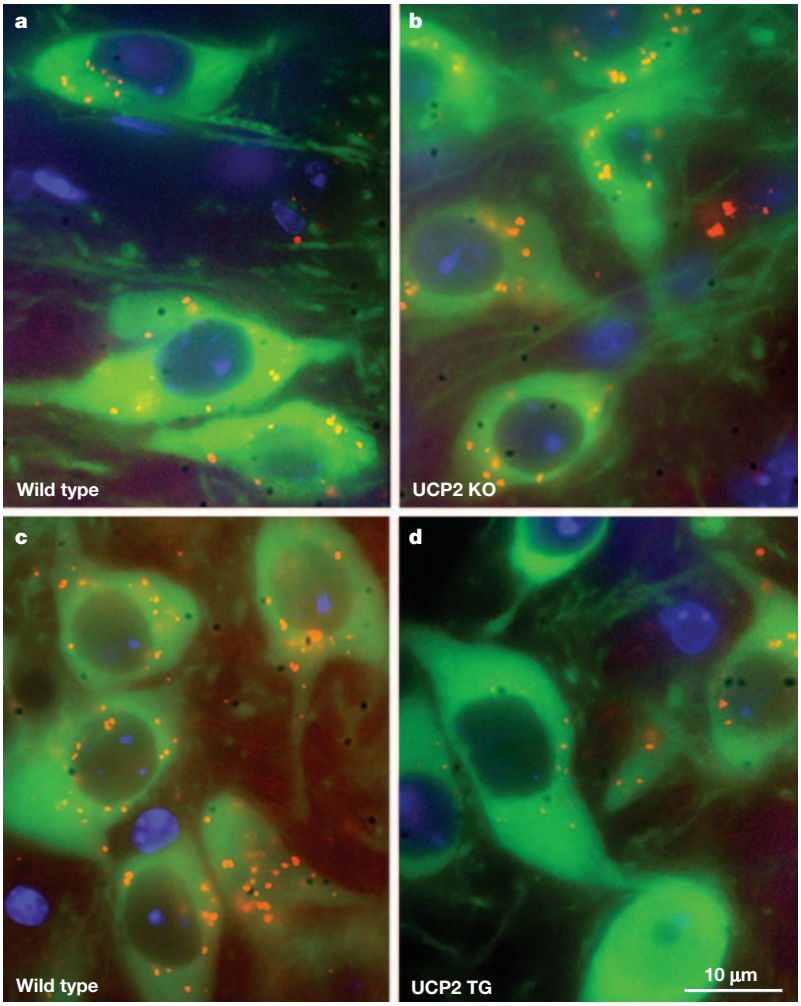


Figure 3 | **Uncoupling protein 2 reduces reactive oxygen species production *in vivo*.** **a**,**b** | Representative photomicrographs of *in situ* reactive oxygen species (ROS) production (red) assessed by the oxidation of dihydroethidium to ethidium by $O_2^{\bullet-}$ in identified tyrosine hydroxylase neurons (green) in the substantia nigra of wild-type mice (**a**) and uncoupling protein 2 (UCP2)-knockout (KO) mice (**b**). Note the elevated number of ROS-producing mitochondria in UCP2-knockout mice. **c**,**d** | Photomicrographs showing triple exposure for ethidium (red), Hoechst nuclear staining (blue) and tyrosine hydroxylase immunofluorescence (green) in wild-type mice (**c**) and transgenic (TG) mice that express human UCP2 (**d**). Tyrosine hydroxylase immunofluorescence reveals a lower number of ROS-producing mitochondria in transgenic compared with wild-type animals. Modified, with permission, from REF. 26 © (2005) Society for Neuroscience.

In addition, using mice that lack UCP2, we have shown that UCP2 can buffer *in vivo* ROS production in the absence of cell stress[26] (FIG. 3). This is further supported by the ability of UCP2 to regulate mitochondrial production of hydrogen peroxide[32]. Overexpression of UCP4 decreases basal mitochondrial ROS production in cultured neurons and limits ROS production after exposure to neurotoxins[33]. Similarly, BMCP1-expressing cells suppress mitochondrial ROS production and enhance uncoupling activity[16].

It has also been suggested that UCP2 dissipates ROS from the mitochondrial matrix rather than reducing the production of ROS in isolated brain mitochondria[34]. UCP2 is thought to act as a channel, facilitating transport of mitochondrial ROS into the cytosol, where it could undergo rapid dismutation and be scavenged by antioxidant defensive mechanisms. Regardless of the exact mechanism, the net effect of increased neuronal uncoupling through UCP2, 4 and 5 leads to a diminution of oxidative stress. The ability of mitochondrial uncoupling to trigger mitochondrial biogenesis (see above) prevents a reduction in cellular ATP-generating capacity and, at the same time, allows a reduction in ROS production to levels that can be scavenged by intracellular antioxidative mechanisms. In this manner, it is proposed that UCPs reduce ROS production without compromising ATP production.

Intriguingly, superoxides themselves can induce the expression of UCPs, including UCP2 (REFS 35,36), and activate proton transport through UCP1–3 (REFS 37,38). Moreover, products of oxidative stress, such as 4-hydroxy-2-noneal, may also regulate mitochondrial uncoupling[39]. Therefore, ROS-induced uncoupling might act as a simple feedback mechanism to limit the extent of oxidative cell damage, which could lead to protection against neurodegenerative diseases and ageing (FIG. 4). However, activation of UCPs by superoxide is a controversial issue that has not yet been independently verified[40]. First, the XANTHINE PLUS XANTHINE OXIDASE SYSTEM generates high levels of superoxide that might not represent a physiological effect, and second, this model also involves oxygen consumption in the process of generating superoxides, thereby affecting mitochondrial respiration in the reaction mixture[40].

However, using the xanthine oxidase metabolism system, it seems that the superoxide anion is responsible for the enhanced uncoupling activity, as it was reversed by the addition of superoxide dismutase but unaffected by catalase, which indicates that uncoupling was not caused by hydrogen peroxide[37]. Moreover, superoxide-induced uncoupling was not prevented by ATP-sensitive potassium channel blockers, inhibitors of the adenine translocase or inhibitors of the mitochondrial permeability transition, which suggests that it is specific to uncoupling proteins[37]. Superoxide-induced mitochondrial uncoupling requires fatty acids, is inhibited by GDP and is absent from the skeletal muscle of UCP3-knockout mice[37]. Although exogenously generated superoxide can activate UCP2 in isolated kidney and spleen mitochondria[38], a role for superoxide activation of neuronal UCPs remains to be established.

***Synaptic transmission and plasticity.*** During synaptic transmission, synapses are exposed to repeated bouts of oxidative and metabolic stress as a result of fluctuating ion gradients and ATP usage. Mitochondria are crucial for meeting the synaptic demand for ATP and for regulating calcium homeostasis[41]. Synaptic function is altered in response to conditions that affect UCP expression, including hypo- and hyperthermia[42–45] and oxidative stress[38,46]. Therefore, the abilities of UCP2, 4 and 5 to decrease mitochondrial membrane potential and free radical production, and to increase mitochondrial biogenesis (see above) in neurons implies that neuronal UCPs modify the local production of a temperature gradient, ATP, free radicals and calcium

XANTHINE PLUS XANTHINE OXIDASE SYSTEM
An exogenous system used to generate superoxide and study the molecular and cellular consequences of superoxide production.

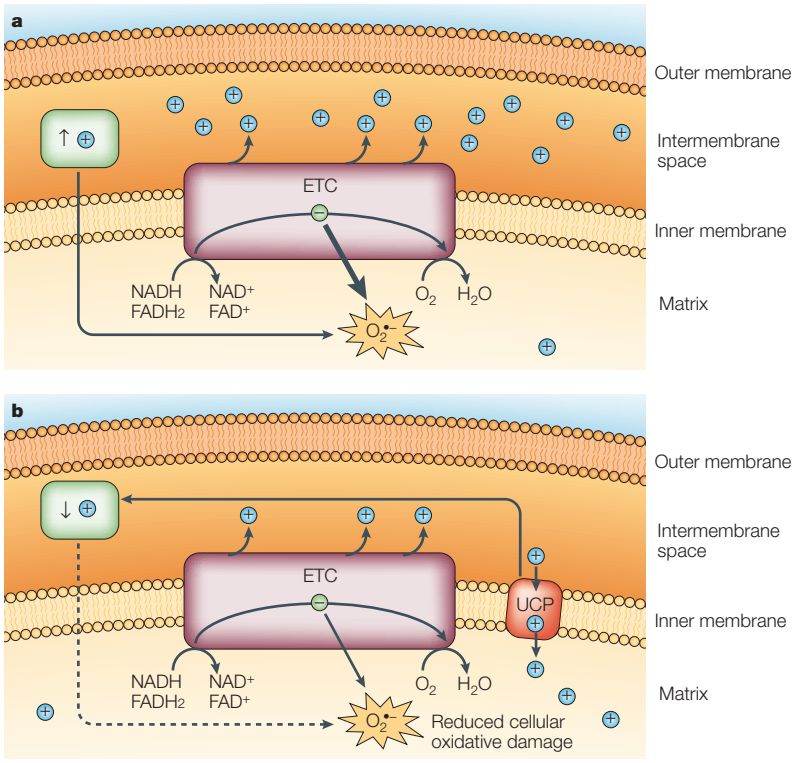


Figure 4 | **Superoxides activate uncoupling proteins via a mitochondrial feedback loop. a** | An increase in the mitochondrial membrane potential (↑+) promotes reactive oxygen species (ROS) production through 'random' single electron transfer reactions from components of the electron transfer chain (ETC) as energy-carrying electrons are passed from energy substrates (NADH, $FADH_2$) to oxygen, the final electron acceptor. If this increased ROS production is too great to be controlled by intrinsic enzymatic antioxidant mechanisms, such as superoxide dismutases 1 and 2 (SOD1 and 2) or catalase, mitochondrial and cellular oxidative stress will result. However, it seems that mitochondria have evolved additional antioxidant mechanisms to combat enhanced ROS production. One such possible mechanism is the potential ability of superoxide ($O_2^{\bullet-}$) to activate uncoupling proteins (UCPs)[37,38]. **b** | This simple feedback model predicts that increased production of $O_2^{\bullet-}$ (panel **a**) activates UCPs in the inner mitochondrial membrane. This results in greater proton influx into the matrix and a reduction in the mitochondrial membrane potential (↓+), which leads to diminished $O_2^{\bullet-}$ production. This represents an exciting aspect of the regulation of neuronal UCPs, as superoxide-induced uncoupling could help to limit ROS production during ageing and restrict the pathogenesis of neurodegenerative diseases. It should be noted that superoxide activation of UCPs remains controversial.

regulation in synapses. It should be noted that PC12 CELLS transfected with rat UCP2 show reduced dopamine secretion and intracellular ATP concentrations[17]. However, we must interpret *in vitro* studies with caution. The level of overexpression is crucial, as excessive mitochondrial uncoupling compromises cell function by reducing the mitochondrial membrane potential and ATP concentrations. Indeed, UCP2 protein levels were extremely high in transfected neurons compared with control cells[17]. Therefore, it is not surprising that ATP levels and dopamine secretion were reduced. We suggest that there is a distinct difference between the acute activation of uncoupling proteins for a limited time period and chronic neuronal UCP function. Acutely, before mitochondrial proliferation takes place, increased uncoupling should impair the ability of mitochondria to produce ATP, possibly leading to a period of ATP decrease. However, increased uncoupling is a trigger for mitochondrial proliferation (although the molecular mechanism for this is not known), at least in certain neuronal populations. This, in turn, leads to increased numbers of uncoupled mitochondria[24], which results in an overall increase in ATP concentrations. Such increases have been found in animals with elevated hippocampal uncoupling levels due to UCP2 overexpression[24].

The extension, movement and number of mitochondria in dendritic compartments correlates with the morphological plasticity of spines, which indicates that the postsynaptic distribution of mitochondria is essential and limiting for the support of synapses[47]. This indicates that mitochondria are more directly involved in synapse regulation than was previously thought. As UCPs — UCP2 in particular — seem to affect mitochondrial number[24], it is reasonable to assume that UCP2 might directly influence synaptic plasticity.

***Pain and tolerance.*** Pain sensation and ethanol tolerance/sensitivity involve both central and peripheral mechanisms. Neuronal UCPs are expressed in subcortical regions and the spinal cord, where UCP2 is specifically located in primary sensory afferents at various brain sites[13]. In the dorsal horn of the spinal cord, UCP2 is co-expressed with substance P afferents in the substantia gelatinosa and projects onto NMDA (*N*-methyl-D-aspartate) receptor-concentrating neurons[48], both of which mediate nociceptive stimuli[49]. These anatomical studies indicate that UCP2 is involved in pain and temperature regulation. Indeed, mice that overexpress UCP2 have a greater pain threshold and show decreased ethanol sensitivity compared with wild-type mice, whereas UCP2-knockout mice show increased ethanol sensitivity[50]. Furthermore, UCP2 expression is inversely correlated with the impairment of pain and temperature sensation that is induced by ethanol. Therefore, UCP2 activation after ethanol exposure might decrease recovery time after ethanol intoxication, whereas UCP2 inhibition might decrease tolerance to ethanol. So far, there are no data on the role of UCP4 or BMCP1 in pain regulation. The mechanisms through which UCP2 regulates pain and ethanol sensitivity are unknown, but might relate to the regulation of ATP concentrations, calcium homeostasis and free radical production, all of which have been tied to pain and temperature sensation[51,52].

## UCPs in neurodegeneration

The effects of neuronal UCPs on synaptic plasticity and synaptic neurotransmission have yet to be fully determined. However, the evidence described above pinpoints neuronal UCPs as crucial proteins for the maintenance of mitochondrial homeostasis. A lack of neuronal UCPs initiates mitochondrial dysfunction (decreased ATP, increased oxidative stress and calcium dysregulation) and might influence plasticity and neurotransmission, all of which influence neurodegenerative pathologies. Below, we summarize some of the disorders that have been either directly linked to or proposed to be influenced by mitochondrial UCPs.

PC12 CELLS
A cloned rat pheochromocytomal cell line that retains a number of chromaffin cell characteristics, including the synthesis and secretion of catecholamines and the expression of various neuropeptide genes. PC12 cells are often used to study the cell biology of neuronal genes after transfection.

***Epilepsy.*** Epilepsy affects more than 1% of the population worldwide and is characterized by excessive excitatory activity that is harmful to neurons as it triggers molecular pathways that lead to excitotoxic neuronal death[53]. Mitochondria have a salient role in excitotoxic cell death that specifically relates to cellular calcium homeostasis and calcium influx during periods of vigorous neuronal excitation[21,22]. Conditions that promote excitotoxicity involve increased calcium load and heightened energy demand and underlie a marked elevation in the formation of mitochondrial ROS[54–56]. As mitochondrial calcium influx depends on the mitochondrial membrane potential, and activation of all three neuronal UCPs reduces this potential, it is reasonable to suggest that mitochrondial uncoupling activity influences neuronal survival by reducing excessive calcium influx. Indeed, several studies have shown that mitochondrial uncoupling *in vitro* diminishes mitochondrial calcium loading and can inhibit excitotoxic cell death[22,57,58]. The potential neuroprotective application of mitochondrial uncoupling agents (such as dinitrophenol) has also been shown after the induction of striatal damage by quinolinic acid (an NMDA agonist)[59].

Interestingly, epileptic insults induce apoptosis through various substances, including hydrogen peroxide ($H_2O_2$) and nitric oxide (NO) with toxic free radical metabolites[60–62]. However, cell death after exposure to $H_2O_2$ and NO is reduced in PC12 cells transfected with UCP2 (REF. 24). Furthermore, mice that overexpress human UCP2 show increased neuronal survival in hippocampal CA1 cells and decreased oxidative stress after exposure to kainic acid — a drug that is commonly used to induce excitotoxic cell death. Overexpression of human UCP2 also induces hippocampal mitochondrial biogenesis and enhances ATP production[24]. Therefore, an increase in the number of partially uncoupled mitochondria can produce the same or greater amounts of ATP than a normal number of tightly coupled mitochondria in wild-type animals without a corresponding increase in free radical production. The idea that mitochondrial proliferation is important for neuronal survival during epilepsy is supported by the fact that surviving hippocampal neurons in humans with epilepsy contain more mitochondria than normal[63].

Kainic acid also induces neuronal expression of UCP2 in selected brain regions that are associated with epilepsy, including the CA1 subfield of the hippocampus, the dorsal endopiriform nucleus, and the entorhinal and piriform cortices[64]. Given the potential neuroprotective role of UCP2 in restricting ROS production and modulating calcium handling, the induction of neuronal UCP2 expression after kainic acid injection might be an attempt to protect cells from oxidative stress. Indeed, the strongest induction of UCP2 expression appeared in area CA1, which is resistant to kainic acid-induced cell death[65].

Increased UCP2 expression in immature brains protects against kainic acid-induced excitotoxic cell death and reduces ROS formation[66]. Furthermore, UCP2 expression and function have been shown to be increased in neonatal brains by the fat-rich diet of maternal milk. Substituting this for a low-fat diet reduced UCP2 expression and uncoupling activity, and permitted neuronal injury. This highlights the physiological importance of fatty acid-induced uncoupling activity (BOX 1) and also underscores the value of juvenile nutrition given the prevalence of epilepsy in infants and children[67,68]. In addition, a ketogenic diet, which is a high-fat, low-protein, low-carbohydrate diet, can be effective against medically intractable epilepsy, especially in children[69]. A ketogenic diet upregulates UCP2 and UCP4 expression, increases uncoupling activity and decreases ROS production in the hippocampus of juvenile mice[70]. These results strongly support a neuroprotective role for UCPs in epilepsy and also accentuate the importance of diet for UCP function.

***Parkinson's disease.*** Although gain-of-function studies can provide important insights into the molecular function of UCP2, complications in interpretation can occur owing to improper insertion of the transgenic protein into the mitochondrial inner membrane. Therefore, studies involving loss-of-function mutants might be more useful. In a mouse model of Parkinson's disease, UCP2-knockout mice were found to be more susceptible to the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Specifically, mice that lack UCP2 have reduced, but not ablated, mitochondrial uncoupling in the substantia nigra (FIG. 5), increased *in vivo* ROS production (FIG. 3) and reduced mitochondrial number per neuron compared with wild-type controls[26]. In addition, mice that express human UCP2 show increased mitochondrial uncoupling and decreased *in vivo* ROS production (FIG. 3) compared with wild-type controls, which confirms the validity of gain-of-function studies. These changes occurred in the absence of cell stressors, thereby indicating that UCP2 is crucial for normal nigral dopamine function. The lack of UCP2 resulted in greater nigral dopaminergic cell loss and reduced striatal dopamine content compared with wild-type mice after exposure to MPTP (FIG. 6). Conversely, human UCP2 expression in transgenic mice decreased MPTP-induced dopamine cell loss compared with that seen in wild-type controls (FIG. 6). This has also been confirmed in mice that overexpress UCP2 selectively in catecholaminergic neurons[71]. Importantly, these studies illustrate that maintaining UCP2 function helps to protect against nigral dopamine neuronal degeneration and reduces susceptibility to harmful endogenous or exogenous toxins. Moreover, dietary restriction and 2-deoxyglucose administration, which increases UCP2, 4 and 5 levels (REFS 33,70), improves behavioural outcomes and reduces dopaminergic neurodegeneration in models of Parkinson's disease[72].

COENZYME Q (CoQ) is constitutively expressed in the ETC of mitochondria, where it acts as the first mobile electron acceptor, shuttling electrons from complexes 1 and 2 and donating them to complex 3. In the CNS, CoQ induces nigral mitochondrial uncoupling and

MPTP
(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). A toxic by-product of the chemical synthesis of a meperidine analogue that induces a parkinsonian syndrome that is almost indistinguishable from Parkinson's disease. MPTP is commonly used to study cellular and molecular aspects of Parkinson's disease in mice and monkeys, as it specifically induces dopaminergic neurodegeneration in the substantia nigra.

COENZYME Q
(2,3-dimethyloxy-5-methyl-6-multiprenyl-1,4-benzoquinone; also known as ubiquinone). A mobile electron carrier from complexes 1 and 2 to complex 3 of the electron transfer chain that is located in the hydrophobic domain of the inner mitochondrial membrane. It also acts, with vitamin E, to provide anitoxidative protection.

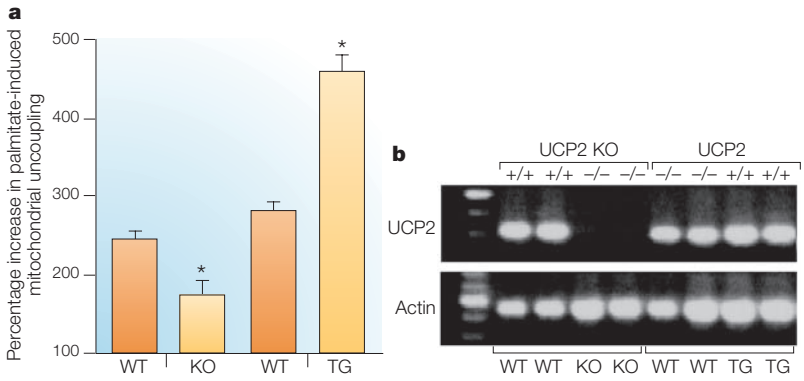


Figure 5 | **Fatty acid-induced uncoupling activity in UCP2-knockout mice and mice that overexpress human UCP2.** Uncoupling protein 2 (UCP2) is a highly active proton transporter in the presence of fatty acids[37]. **a** | The fatty acid palmitate increases uncoupling activity in isolated mitochondria from the substantia nigra/ventral tegmental area of transgenic (TG) mice that overexpress human UCP2 compared with wild-type mice (WT). By contrast, UCP2-knockout mice (KO) show reduced mitochondrial uncoupling compared with wild-type controls. The data are expressed as the percentage increase above OLIGOMYCIN-INDUCED STATE 4 RESPIRATION. These studies show that the maximum uncoupling ability of isolated mitochondria of the substantia nigra was lower in UCP2-knockout mice and higher in transgenic mice that express human UCP2 (REF. 26). Therefore, in the brain, as in peripheral tissues, UCP2 functions as a natural and physiological conditional uncoupler. **b** | Polymerase chain reaction (PCR) analysis revealed a complete absence of UCP2 mRNA in knockout mice, whereas mice expressing human UCP2 showed an approximately twofold increase in UCP2 mRNA. Asterisks indicate significant differences. Modified, with permission, from REF. 26 © (2005) Society for Neuroscience.

prevents dopamine cell loss after MPTP administration in non-human primates[15], and UCP2 is expressed in nigral neurons that express tyrosine hydroxylase, the rate-limiting step in dopamine synthesis. CoQ also attenuates MPTP-induced loss of striatal dopamine and dopaminergic axons in mice[73] and has been used in clinical trials to slow the functional decline that is associated with early Parkinson's disease[74]. 1-methyl-4-phenylpyridinium ions ($MPP^+$) and paraquat (nigrostriatal dopaminergic toxins that have been implicated in Parkinson's disease[75]) induce ROS production and subsequent oxidative stress that is inhibited by CoQ10 treatment[76,77]. Therefore, neuronal CoQ is important for uncoupling activity, attenuating ROS production and restricting the pathogenesis of Parkinson's disease.

***Ischaemia and traumatic brain injury.*** Several pathophysiological events contribute to neuronal damage and death after traumatic brain injury and stroke/ischaemia, including excitotoxicity, calcium handling, ROS formation and subsequent oxidative stress. All neuronal UCPs have been implicated in these pathogenic mitochondrial processes.

Mattiasson *et al.*[34] first showed that UCP2 has an important neuroprotective role in ischaemia in an elegant set of experiments that took advantage of the neuroprotection provided by ISCHAEMIC PRECONDITIONING. cDNA array analysis of hippocampal tissue from preconditioned rats identified *Ucp2* as a potential neuroprotective gene. In follow-up experiments, they found that cultured cortical neurons that overexpressed UCP2 showed inhibited activation of the apoptotic signal caspase 3 and 50% less neuronal death after oxygen–glucose deprivation than untransfected or control cultures[34]. Furthermore, they also reported that experimental stroke and traumatic brain injury are associated with diminished brain damage and enhanced neurological recovery in mice that overexpress human UCP2 (REF. 34). Mild uncoupling also reduces neuronal cell death[24,26,34], and fatty acid-induced uncoupling is significantly greater in mice that overexpress UCP2 (REFS 26,34). Finally, Mattiasson *et al.* also found that UCP2 shifts the release of ROS from the mitochondrial matrix to the extramitochondrial space, where they can be more readily degraded by cytosolic antioxidants such as catalase[34].

Acute traumatic brain injury also causes upregulation of UCP2, similar to that seen after kainic acid administration[34] into neurons and microglia in areas that normally do not express UCP2 (REF. 78). UCP2 activity was induced in an acute neurodegeneration model (in which the entorhinal cortex is lesioned), and its early induction after the lesion was inversely correlated with caspase 3 activation. Moreover, traumatic brain injury increased ROS production and lipid peroxidation[79], and reducing this ROS overload after the injury conferred neuroprotection[80]. Indeed, inducing mitochondrial uncoupling using 2,4-dinitrophenol after traumatic brain injury diminishes tissue loss, oxidative damage and calcium loading, and improves behavioural outcomes[66]. Furthermore, 2,4,6-trinitrophenol (a 2,4-dinitrophenol analogue) cannot uncouple mitochondria and consequently offers no neuroprotection, which illustrates that uncoupling activity is crucial for neuroprotection. Fasting increases the expression of neuronal mitochondrial UCPs, and fasting after injury yields similar results to those of 2,4-dinitrophenol[66,81]. These findings strongly implicate UCPs in the promotion of neuronal survival after acute brain trauma and stroke/ischaemia. However, a recent report indicates that UCP2 deficiency promotes resistance to cerebral ischaemia by increasing neuronal antioxidant levels[81], although this is thought to reflect a chronic adaptation to the lack of UCP2. Future research will be required to resolve the seemingly inconsistent intricacies of neuronal UCP function.

***Alzheimer's disease.*** Alzheimer's disease affects almost 2% of the population in industrialized countries and the risk is significantly increased in individuals of more than 70 years of age. It is characterized by neurofibrillary tangle formation in neuronal perikarya and extracellular deposits of amyloid-β protein[41]. Tangles and plaques are found predominantly in brain areas that regulate learning, memory and emotional behaviours, including the hippocampus, cortex, basal forebrain and brain stem[82]. Interestingly, these brain regions also express neuronal UCPs (see above). Although there are no studies directly linking neuronal uncoupling activity to Alzheimer's disease, neuronal UCPs might regulate many of the pathogenic mechanisms that lead to neuronal degeneration, such as increased oxidative damage, perturbed cellular calcium homeostasis,

OLIGOMYCIN-INDUCED STATE 4 RESPIRATION
A state of mitochondrial respiration that requires oligomycin to prevent ADP phosphorylation (state 3 respiration) by blocking protons from interacting with ATP synthase. State 4 respiration is a direct measure of mitochondrial uncoupling activity.

ISCHAEMIC PRECONDITIONING
A process that occurs after sublethal ischaemic insults. Neurons activate defensive mechanisms, such as cellular calcium buffering and antioxidants systems, that counteract ischaemic damage.

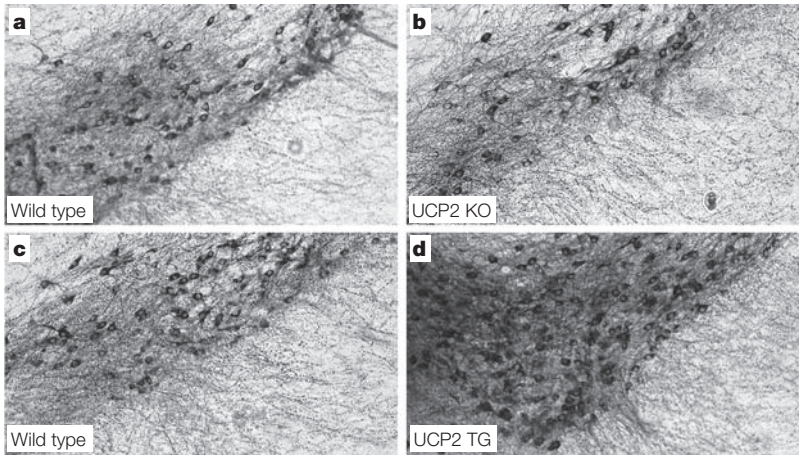


Figure 6 | **Uncoupling protein 2 prevents dopamine cell loss in the substantia nigra after MPTP treatment. a**,**b** | Uncoupling protein 2 (UCP2)-knockout mice had significantly fewer surviving dopamine neurons than their wild-type littermates after MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment. **c**,**d** | Transgenic (TG) mice that express human UCP2 had significantly more surviving neurons then their wild-type littermates after MPTP treatment. Modified, with permission, from REF. 26 © (2005) Society for Neuroscience.

impaired energy metabolism and synaptic dysfunction[41]. Indeed, UCP4 is increased in the hippocampus after 2-deoxy-D-glucose treatment, which inhibits glycolysis[33], and abnormalities in glucose regulation and insulin resistance are a risk factor for Alzheimer's disease[83,84]. Furthermore, dietary restriction, which protects neurons from dysfunction and death in models of Alzheimer's disease[41], also enhances UCP4 expression in the cortex and hippocampus[33]. Finally, neuronal UCPs are activated by ROS and oxidative stress[37–39]. These studies lead to the hypothesis that neuronal UCPs are neuroprotective against degeneration in Alzheimer's disease. It would, therefore, be of considerable interest to prove or disprove this hypothesis in future research.

***Amyotrophic lateral sclerosis.*** Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that predominantly affects upper and lower motor neurons. Spinal motor neuronal death leads to muscular atrophy and progressive respiratory muscle decay that results in death within 3–5 years[85]. ALS is epidemiologically subclassified into sporadic (90–95%) or familial (5–10%) forms, 20% of which are associated with point mutations in the gene that encodes cytosolic Cu/Zn superoxide dismutase (*SOD1*)[86,87]. Although the molecular mechanisms that are responsible for the selective loss of motor neurons remain unclear, several lines of evidence indicate that mitochondrial dysfunction, including oxidative stress and excitotoxicity, have a crucial role[87]. All three neuronal UCPs are present in the spinal cord and many UCP2 axonal processes are present in the ventral horn of the spinal cord, where motor neuron cell bodies are located[48]. Furthermore, neuronal UCPs are known to attenuate oxidative stress and mitochondrial membrane potential, and to regulate calcium homeostasis, thereby implicating UCPs in halting the pathogenesis of ALS. Initial studies are promising and indicate that uncoupling agents confer neuroprotection after spinal cord injury (SCI; a model of ALS)[66]. Mitochondrial uncouplers, such as 2,4-dinitrophenol, reduce tissue loss, mitochondrial oxidative damage and calcium loading after SCI. Moreover, trinitrophenol neither uncouples mitochondria nor offers neuroprotection. Although this is promising evidence that neuronal UCP activation could mitigate the pathogenesis of ALS, further research is required.

***Ageing.*** Mitochondrial dysfunction lies at the heart of the ageing process. This dysfunction is widely accepted to result from increased ROS production, although fundamental questions about the relationship between overall metabolic rate and the production of ROS remain unresolved[88]. The free radical theory of ageing, which was proposed almost 50 years ago[89], predicts that ROS induces oxidative stress, such as lipid peroxidation, DNA and protein oxidation. This results in progressive deterioration in the bioenergetic capacity of mitochondria, thereby compromising cell survival. This process is also thought to be integral in the pathogenesis of neurodegenerative disorders such as Parkinson's disease[90] and Alzheimer's disease[41]. Therefore, exploiting the ability of UCPs to reduce ROS production might provide a way to retard age-related mitochondrial dysfunction.

UCP2 was linked to longevity when targeted expression of human UCP2/UCP3 to mice[91] and adult fly[92] neurons extended life span without compromising fertility or physical activity. This also resulted in increased uncoupled respiration, and decreased ROS production and oxidative damage, which indicates that neuronal UCP2 regulates age-related oxidative stress. Moreover, mice with high metabolic intensities had greater resting oxygen consumption and lived longer than mice with low metabolic intensities[93]. The longer-living mice also showed greater uncoupled respiration, which implies that there is reduced ROS production in these mice despite increased oxygen consumption. These observations led to the 'uncoupling to survive' hypothesis[94]. This proposal states that increased uncoupled respiration leads to greater oxygen consumption but dissipates the proton motive force by reducing the mitochondrial membrane potential, which consequently reduces ROS production. In further support of UCP2 in longevity, UCP2 mRNA expression in the rat is progressively enhanced at 12 and 26 months of age compared with 6 months. By contrast, BMCP1 expression was no different at 12 months and significantly suppressed at 26 months compared with 6 months; UCP4 was not investigated. Further studies will be required to establish a similar pattern of expression at the protein level. Given that UCPs are activated by free radicals[37] and by-products of oxidative stress, such as 4-hydroxy-2-noneal[39], it is tempting to speculate that increased UCP2 in aged rats represents an evolutionarily adaptive mechanism to diminish oxidative stress that is associated with ageing.

## Concluding remarks and perspectives

Research into neuronal UCPs and neuroprotection is still young, and many important contributions await discovery. First, it is crucial to identify the tissue-specific effects of UCPs. For example, UCP2 negatively regulates insulin secretion in the pancreas, whereas it seems to provide neuroprotection against many models of neurodegeneration. Therefore, the next significant step forward will rely on the development of tissue-specific UCP knockouts. The ability to temporally regulate neuronal UCP suppression, through RNA interference or inducible knockouts, will also shed light on the intricacies of UCP biology.

Although studies using transfected cell lines that ectopically express neuronal UCPs might provide useful insights into biological mechanisms of function, future research should focus on *in vivo* manipulations using established models to advance the field towards clinical research. This is especially important when considering the apparently ambiguous nature of UCP function, whereby excessive uncoupling is harmful and mild uncoupling is protective. The pharmacological targeting of neuronal UCPs represents an important avenue to combat neurodegenerative disorders as well as physiological ageing. It will be crucial to establish treatments that do not have deleterious side effects, especially given the tissue-specific functions of UCP2. Early indications suggest that this might be a fruitful endeavour, as CoQ, an important cofactor in the activation of UCPs[95,96], induces nigral mitochondrial uncoupling and diminishes MPTP-induced toxicity[15], and might even slow the progression of Parkinson's disease[74]. Advances in our understanding of UCP biology are likely to deliver successful treatment strategies against neuropathological conditions.


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## Competing interests statement

The authors declare no competing financial interests.

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