template for molecular-modelling studies aimed at discovering more compounds that bind at the different interfaces, or to any of the many other pockets present in the receptor.

The extracellular region of the receptor forms a wide chamber. Zhu *et al.* find that most of the space in this chamber is occupied by complex carbohydrate chains attached to some of the subunits, leaving relatively little space for ion permeation. If the same is true for receptors expressed in neurons, rather than in HEK cells, then this observation is mechanistically highly relevant. Ions might also enter the channel through openings found at the subunit interfaces. A structure of the α1β1γ2 $GABA_A$ receptor was recently published[6] on a preprint server, and the authors of that paper suggest that the attachment of carbohydrates to the α1 subunit has a key role in receptor assembly.

Understanding the variations between benzodiazepine binding sites found in different $GABA_A$-receptor isoforms might help researchers to find compounds that act selectively at just one isoform. This could allow researchers to realize the hope of developing drugs that produce desirable therapeutic effects (such as reducing anxiety) without the unwanted side effects (such as sedation) caused by existing drugs that target several isoforms of $GABA_A$ receptors. How diazepam — the archetypal benzodiazepine drug — binds to benzodiazepine sites is of particular interest. Computational studies[7] suggest that there are three possible binding modes (BM I–III) of benzodiazepines, of which BM I was proposed to actually occur. By contrast, an alternative approach has been used to identify the amino-acid residues in α1β2γ2 $GABA_A$ receptors that come into direct contact with diazepam[8], and this pointed instead to BM II. The information from that study was used to identify high-affinity ligands for the benzodiazepine site.

In Zhu and colleagues' structures, the position of flumazenil in the benzodiazepine site is similar to BM I. The authors suggest that docking of diazepam to the observed site would be possible through a binding mode similar to BM I, but that diazepam docking similar to BM II would lead to clashes between the molecule and the receptor. It should be noted, however, that the receptor conformations stabilized by flumazenil must be different from that stabilized by diazepam, so the idea that diazepam would dock into the reported structure in a similar way to flumazenil is questionable. A recent study[9] has shown that benzodiazepines whose structures are similar to that of flumazenil use BM I, whereas those similar to diazepam use BM II. Thus, the positioning of diazepam postulated by Zhu *et al.* will probably need to be revised.

In structural biology, there is always the possibility that the protein structure determined does not entirely correspond to a conformation adopted in nature. This is illustrated by one of Zhu and colleagues' structures (described as conformation A), in which the transmembrane part of the γ2 subunit is squeezed into the receptor's pore. This conformation is unlikely to occur in nature, and might have been caused by the type of detergent that Zhu *et al.* used to replace the natural membrane environment in their microscopy experiments. The water-soluble domain of the protein is not subject to this problem.

Proteins are dynamic structures that assume distinct conformational states. In each state, large parts of the proteins vibrate much like wobbly puddings. The binding of molecules to allosteric sites in proteins (such as the benzodiazepine site in $GABA_A$ receptors) can stabilize different conformational states. This explains how molecules can act as positive or negative allosteric modulators, or as antagonists. $GABA_A$ receptors exist in at least four conformational states: closed; ligand-bound but not open (known as the pre-activated state); open; and desensitized. Therefore, insight into allosteric modulation cannot be gained from structural studies alone, because protein structures are caught in static conformations. Structural-biology methods will need to be combined with other approaches to probe the dynamic properties of $GABA_A$ receptors.

Nevertheless, Zhu and colleagues' structures could well be the key to addressing some of the crucial issues in the study of $GABA_A$ receptors. For example, the work might help to unravel the numerous conformations of the α1β2γ2 $GABA_A$ receptor, and the differences in the benzodiazepine binding site between different isoforms of $GABA_A$ receptors, as well as providing information on all the possible modulatory sites. It is to be hoped that this will, in turn, lead to the development of improved drugs that target these receptors. ■ SEE EDITORIAL P.5

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NEUROSCIENCE

Social isolation's molecular signature

Extended social isolation causes debilitating effects in social mammals such as humans. A study in mice shows that the gene *Tac2* is upregulated throughout the brains of socially isolated animals, driving massive behavioural changes.

NOGA ZILKHA & TALI KIMCHI

Even the toughest prisoners fear solitary confinement. There is a growing awareness across the globe that we are facing an epidemic of loneliness. Prolonged social isolation and loneliness can lead to many profound physiological and neuropsychiatric conditions, including depression and heart disease, and to increased mortality rates[1]. In the United States, more than 50% of people over the age of 60 experience loneliness[2], and the United Kingdom has appointed a government minister to tackle the issue of loneliness. But the biological mechanisms underlying the effects of social isolation are poorly understood. Writing in *Cell*, Zelikowsky *et al.*[3] reveal a signalling mechanism that acts in several brain regions in mice to drive some of the harmful effects of the stress caused by chronic social isolation.

The authors examined the effects of two weeks of social isolation on the brains and behaviour of male mice (equivalent to more than a year in these conditions for humans[4]). First, the researchers used an array of behavioural tests to compare mice kept in isolation with control mice that had been housed in groups. These assays revealed widespread effects. Compared to control animals, isolated mice showed enhanced aggression and hypersensitivity to diverse stressful stimuli. For example, the socially isolated mice responded more aggressively to an unfamiliar mouse placed in their cage. In another assay, the researchers presented mice with a dark circle that loomed overhead, simulating an approaching predator. Control animals froze in response to the threat, but moved normally after the stressful stimulus was removed, whereas isolated mice remained frozen long after the apparent threat was removed.

Next, Zelikowsky *et al.* investigated the brain mechanisms underlying this behaviour. In a

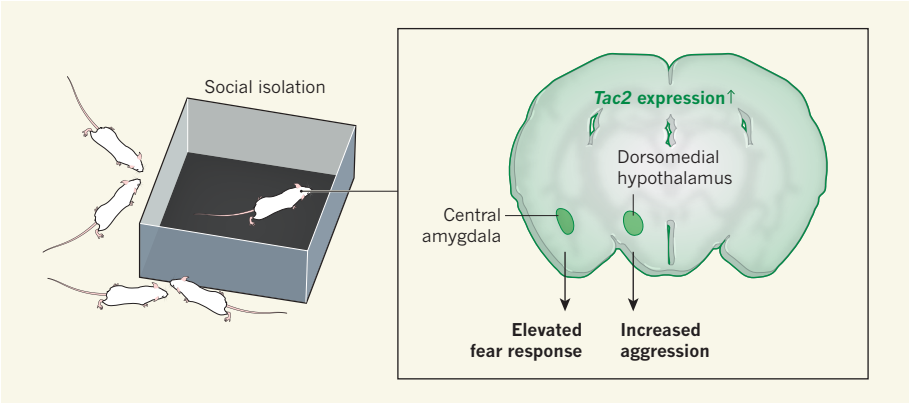

Figure 1 | The gene *Tac2* mediates various effects of social isolation in mice. Zelikowsky *et al.*[3] investigated how two weeks of isolation affected the brains and behaviour of male mice. They found that *Tac2* expression is upregulated throughout the brain, and that the gene's upregulation in particular areas — including the central amygdala and dorsomedial hypothalamus — led to specific changes in the animals' social behaviour and in their response to various stressful stimuli.

previous study of fruit flies, the same group had identified the gene *Tac* as essential for the regulation of aggression induced by social isolation[5]. Rodents have two versions of *Tac*, which are expressed in various brain regions, including regions associated with social behaviour, anxiety and emotions. Using several independent methods, Zelikowsky and colleagues now found a massive increase in the expression of *Tac2* throughout the brain following social isolation.

The gene *Tac2* encodes a protein called neurokinin B (NkB), which binds specifically to the receptor Nk3R. The researchers performed a series of experiments to alter NkB signalling in the brain. First, they systemically inhibited NkB signalling in isolated male mice using a drug called osanetant, which inhibits the activity of Nk3R. Administration of osanetant, either throughout the social-isolation period or 20 minutes before behavioural testing, substantially reduced the effects of social isolation on behaviour. Next, the authors genetically upregulated *Tac2* expression and simultaneously activated *Tac2*-expressing neurons in group-housed animals, using specially designed viruses that were injected intravenously but could cross the blood–brain barrier to reach the brain. They found that this genetic manipulation led to group-housed mice behaving in a similar way to those that had been isolated.

Finally, Zelikowsky *et al.* locally manipulated *Tac2* expression and NkB signalling, by injecting either osanetant or viruses to downregulate *Tac2* expression or inhibit the activity of *Tac2*-expressing neurons, into particular locations in the brain. These experiments enabled the authors to attribute specific behaviours to regulation of *Tac2* in specific brain regions. The main social effect of isolation — enhanced aggression towards an intruder — was controlled by *Tac2* in the dorsomedial hypothalamus. By contrast, acute and persistent stress responses were regulated primarily by *Tac2* in the central amygdala (Fig. 1).

This work opens a gateway to much future research. First and foremost, it will be interesting to determine whether *TAC3*, the human equivalent of *Tac2*, is involved in mediating the effects of loneliness and social isolation in people. To our knowledge, *TAC3* has not yet been directly associated with sociality or social behaviour of any kind in humans. However, it is expressed in the human brain and has shown abnormal gene-expression levels in children with autism-spectrum disorder[6], which profoundly affects social interaction. The systemic manipulations presented in Zelikowsky and colleagues' paper could be rapidly applied to humans, because osanetant and other NkB inhibitors have already been tested in clinical trials. These drugs could potentially treat antisocial disorders induced by isolation, as well as mood and anxiety disorders.

Although most of their experiments focused on male mice, Zelikowsky *et al.* found upregulation of *Tac2* in response to social isolation in both males and females. Sex differences in response to stress and isolation are well documented, and are usually conserved across species[7]. It will therefore be interesting to test whether the roles of *Tac2* in mediating the effects of social isolation in females are similar to or different from those in males.

The need for social interactions and the response to social isolation can differ enormously between and within species. Mice and humans, for example, are typically considered to be highly social creatures[8]. When their social needs are not filled, they can experience debilitating outcomes[1,9]. Some species (and individuals within a species), however, are more solitary, or even avoid social interactions[10]. Such species or individuals might harbour neuronal mechanisms that are adapted to the lack of social interaction. Whether or not members of the *Tac* gene family act differently in solitary individuals or species compared to how they do in more-social individuals or species remains to be determined.

Finally, one has to wonder: to what extent

50 Years Ago

Thirty years ago, those of us who argued that hunger and malnutrition existed and would get worse unless research on agriculture and contraception got very much more support, were variously labelled atheists, communists, dreamers or idealists according to the prejudices of the critic. But facts are stubborn, and awareness of the need for more food is now widespread. Consequently, we are deluged with books and symposia on what could and should be done to balance food and population … Optimists tend to overlook the fact that, unless a population control policy depends on a dictatorial act along Herodian lines, it will not be effective until people have learnt the rudiments of biology and hygiene.
From *Nature* 6 July 1968

100 Years Ago

Anthrax is an acute, infective disease of man and animals and is caused by the anthrax bacillus … In order to prevent the disease in dangerous trades working with possibly infected animal material it would, at first sight, appear to be a simple thing to disinfect the infected material. In practice, however, this is found to be exceedingly difficult on account of the truly enormous powers of resistance of the spore … complete success can now be attained without risk to the workers and without damage to the material disinfected. The main feature of the process is a preliminary treatment in which material is submitted to the action of a warm solution of soap and water containing alkali … This causes softening and disintegration of any infected blood-clots, and the spores are laid bare for the subsequent destroying process … ten million pounds of infected wool can be effectively disinfected for something less than 0 824 penny per pound weight.
From *Nature* 4 July 1918

can we rely on a mouse model of social isolation to truly examine the underlying mechanisms of human loneliness? After all, loneliness and mental isolation are subjective, and a person might feel alone even when surrounded by other people. The traits exhibited by mice under prolonged social isolation greatly resemble those found in humans experiencing solitary confinement, so these animals do provide a good model for studying this process. What we currently lack are relevant animal models for other forms of human loneliness, such as social withdrawal or antisocial personality disorder. Expanding our research toolbox — for example, by studying various species, including non-social and community-living animals, as well as humans — might bring us closer to understanding the biology of human loneliness. ■

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ASTRONOMY

General relativity passes another test

Einstein's theory of gravity — the general theory of relativity — is based on the principle that all objects accelerate identically in an external gravitational field. A triple-star system provides a stringent test of this principle. SEE LETTER P.73

CLIFFORD M. WILL

All bodies in a given gravitational field are thought to fall with the same acceleration. This idea, known as the equivalence principle, is central to our understanding of gravitational physics. It was promoted by thinkers ranging from the sixth-century scholar John Philoponus to Galileo; it is the founding principle of Albert Einstein's general theory of relativity, and was famously demonstrated when Apollo astronaut David Scott dropped a hammer and a feather on the Moon and saw that they hit the lunar surface at the same time. For decades, experimentalists have verified the equivalence principle using exquisitely delicate instruments. Now, on page 73, Archibald *et al.*[1] report the results of a remarkable test of the principle, in which the falling objects are two stellar remnants: a neutron star and a white dwarf.

A spinning neutron star that emits a beam of electromagnetic radiation is known as a pulsar. The emission seems to pulse because it can be seen only when the beam is pointing towards Earth. The pulses are so regular that variations in their observed period can be readily interpreted as being due to the gravitational tug of another astronomical body on the pulsar. Such variations have been used to discover more than 220 binary systems containing a neutron star, and a handful of pulsars that have associated planets (go.nature.com/2mslfj4).

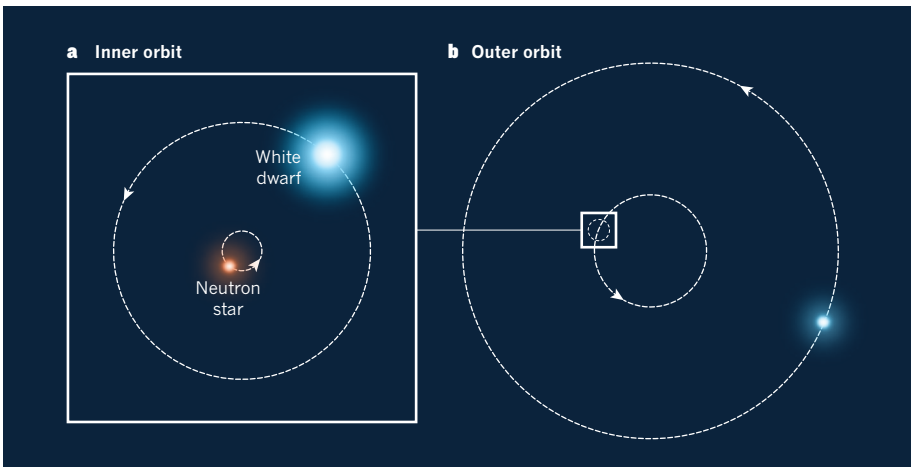

Figure 1 | Triple-star system. In 2014, astronomers reported a system that contains three stellar remnants: a neutron star and two white dwarfs[2]. **a**, The neutron star is in a close 1.6-day orbit with one of the white dwarfs. **b**, This pair of objects is itself in a 327-day orbit with the other white dwarf. Archibald *et al.*[1] report no evidence of a deformation of the inner orbit, which would be expected if there were a difference between the accelerations of the neutron star and the inner white dwarf towards the outer white dwarf. The results provide support for Einstein's theory of gravity — the general theory of relativity.

In 2014, astronomers reported a pulsar that is unusual because it has two stellar companions[2] (Fig. 1). The neutron star, weighing 1.4 solar masses, is in a close 1.6-day orbit with a 0.2-solar-mass white dwarf. This pair of objects is itself in a 327-day orbit with a 0.4-solar-mass white dwarf. The inner and outer orbits are nearly circular and exist in almost exactly the same plane.

If the neutron star and the inner white dwarf were to fall with different accelerations towards the outer white dwarf, there would be a tiny deformation of the inner orbit. Archibald and colleagues report an analysis of approximately six years of data showing no evidence of such a deformation. The accelerations of the two bodies differ by no more than 2.6 parts per million, in agreement with the equivalence principle.

Tests of this principle have a long heritage. In the late nineteenth century, the Hungarian physicist Roland von Eötvös devoted years to verifying that the accelerations of various laboratory materials in Earth's gravitational field differ by less than a few parts per billion[3]. His modern-day successors, the Eöt-Wash group[4] in Seattle, Washington, pushed this bound to parts per 10^{13}. And, in 2017, data from the French space mission MICROSCOPE[5] moved the goalpost by a further factor of ten.

Given that a typical object in a physics lab consists of a swarm of elementary particles and their associated fields and energies, it is quite extraordinary that the responses of different materials to gravity should be so similar. In Einstein's unique imagination, there was a reason: gravity is not a force that acts on all of these particles in some fantastically fine-tuned manner, but is simply an effect of space-time geometry. The constituents of matter follow universal paths in a space-time that is curved by massive bodies, such as Earth or the Sun.

But does gravitational energy act in the same way as matter? The small objects used in lab experiments do not contain enough