sites and conformational changes initiated by ligand binding.

The study by Laverty *et al.* has three new methodological aspects. First, the authors used full-length $GABA_A$ subunits, not the truncated subunits used in previous studies[6,7]. Second, they reconstituted the receptors in discoidal membranes (nanodiscs) composed of a double layer of lipid molecules girdled by scaffold proteins, an environment similar to their natural cell-membrane surroundings (Fig. 1). This contrasts with the detergent environment that has been used in the vast majority of previous studies of this group of receptors. Third, the receptors were coupled to a synthetically enlarged antibody (called a megabody) to aid receptor orientation and alignment during cryo-EM imaging. Using this methodology, Laverty *et al.* solved the structure of the receptor bound to GABA, and Masiulis *et al.* solved five additional structures with different ligands or ligand combinations: picrotoxin (an agent that blocks the open channel of the receptor); picrotoxin and GABA; bicuculline (a drug that induces epilepsy symptoms); alprazolam (a benzodiazepine) and GABA; and diazepam (another benzodiazepine) and GABA.

$GABA_A$ receptors are notorious for requiring a particular lipid environment to undergo functional conformational transitions. Recently, two breakthrough studies[6,7] determined the first heterotrimeric $GABA_A$-receptor structures. In both studies, the transmembrane domain of the receptor was in an unnatural conformation, which might have been caused by the replacement of the natural membrane environment with a detergent. Given the allosteric nature of the $GABA_A$ receptor, any conformational alterations of the structure of the transmembrane domain of one or a few subunits might affect the transmembrane domains of other subunits, and potentially also the extracellular domains. Therefore, the new structures obtained in a membrane environment might be a closer representation of the natural conformations of the binding sites of the receptor. Notably, Laverty *et al.* demonstrated that their method of receptor reconstitution preserved the physiological long-distance crosstalk between different ligand-binding sites: varying the amount of a given ligand bound to the receptor modulated the binding of a different, radioactively marked ligand to a distant site (Fig. 1f and Extended Data Fig. 2d of ref. 1).

The authors of both studies incubated the $GABA_A$-receptor-nanodisc samples with the megabody to orient the $GABA_A$-megabody complexes during cryo-EM imaging in such a way that snapshots from different angles could be obtained. The use of megabodies might also be a technological advance because it can increase the size of protein complexes that would otherwise be too small for their structures to be determined using cryo-EM.

The findings show how the binding of a given ligand to its site leads to conformational and functional changes throughout the $GABA_A$ receptor, including changes to the binding sites of other ligands. For example, one structure shows that binding of GABA to both of its sites in the extracellular domain causes a conformational change that contracts and closes these sites (Fig. 3 of ref. 1). Another structure shows that picrotoxin, which binds to the transmembrane domain, stabilizes the receptor in a closed-channel conformation (Fig. 1 of ref. 2). However, a third structure reveals that when GABA and picrotoxin are both present, one of the two GABA-binding sites is incompletely closed (Fig. 2 of ref. 2), indicating that picrotoxin binding influences the GABA-binding sites. Intriguingly, structures obtained when both GABA and a benzodiazepine are present indicate that these compounds strengthen the interactions between the otherwise weakly associated extracellular portions of the α and γ subunits (Figs 5 and 6 of ref. 2). This might explain how benzodiazepines promote the activity of the receptor when GABA is present.

The studies also give clues about the cytosolic parts of the receptor. Only short fragments of the large intracellular domains could be resolved, even though full-length $GABA_A$ subunits were imaged. This is in stark contrast to the well-resolved structures of the intracellular segments of full-length cation-conducting nicotinic acetylcholine- and serotonin-receptor channels, which are members of the same superfamily of pentameric neurotransmitter channels as the $GABA_A$ receptor[8–11]. The present findings might be the first experimental hint that the intracellular segments of $GABA_A$ and other anion-conducting pentameric channels might not have a defined secondary structure, whereas the intracellular domains of cation-conducting pentameric channels are structured even when expressed in the absence of the extracellular and transmembrane domains[12]. The diversity in length and amino-acid composition of the intracellular domain remains a challenge in the structural and functional characterization of $GABA_A$ and related receptors.

"Binding of ligands to non-GABA sites can alter communication between different binding sites."

In theory, the involvement of specific combinations of $GABA_A$-receptor subtypes in particular normal and disease processes holds promise for precise pharmacological interventions. In practice, the large number of different $GABA_A$ subunits (19 subunits) that have substantially similar amino-acid sequences renders subunit-specific drug targeting tedious, if not impossible. The detailed structural insights reported in the two papers discussed here, as well as a

50 Years Ago

While he was working on the various biological problems that obsessed him, Charles Darwin relentlessly bombarded friends and acquaintances with requests for information and specimens. In a correspondence that lasted more than twenty years, W. B. Tegetmeier, one of his most valuable contacts, was continually questioned about such topics as breeds of fowls, length of cats' teeth, sex ratio at birth and race horse records … Sometimes Darwin reached a wider public by publishing his requests in journals … One of these, *Questions about the Breeding of Animals*, has been reprinted in facsimile … The questions are concerned largely with the outcome of crosses involving wild and domesticated animals, and the likeness of the hybrid progeny to parents and grandparents.
From *Nature* 25 January 1969

100 Years Ago

In an interesting essay on "Camouflage" … Mr Abbott H. Thayer illustrates his well-known conclusions in regard to the cryptic coloration of animals that hunt or are hunted. In their "superhuman perfection" the concealing coats of wild animals have become the models for the camouflage corps of armies … What is practically universal is background–imitation … Mr. Thayer illustrates this by interesting views of brook-scenes and wood-scenes photographed through a stencil of bird or beast. The creature has the garment of invisibility because its "costume is pure scenery". "All the patterns and brilliant colours on the animal kingdom, instead of making their wearers conspicuous, are, on the contrary, *pure concealing coloration*, being the *actual colour notes of the scene in which the wearer lives*, so that he really is Nature's utmost *picture* of his background."
From *Nature* 23 January 1919