LETTERS

Endogenous neurosteroids regulate $GABA_A$ receptors through two discrete transmembrane sites

Alastair M. Hosie[1], Megan E. Wilkins[1], Helena M. A. da Silva[1] & Trevor G. Smart[1]

Inhibitory neurotransmission mediated by $GABA_A$ receptors can be modulated by the endogenous neurosteroids, allopregnanolone and tetrahydro-deoxycorticosterone[1]. Neurosteroids are synthesized *de novo* in the brain during stress[2], pregnancy[3] and after ethanol consumption[4], and disrupted steroid regulation of GABAergic transmission is strongly implicated in several debilitating conditions such as panic disorder, major depression, schizophrenia, alcohol dependence and catamenial epilepsy[3,5–8]. Determining how neurosteroids interact with the $GABA_A$ receptor is a prerequisite for understanding their physiological and pathophysiological roles in the brain. Here we identify two discrete binding sites in the receptor's transmembrane domains that mediate the potentiating and direct activation effects of neurosteroids. They potentiate GABA responses from a cavity formed by the α-subunit transmembrane domains, whereas direct receptor activation is initiated by interfacial residues between α and β subunits and is enhanced by steroid binding to the potentiation site. Thus, significant receptor activation by neurosteroids relies on occupancy of both the activation and potentiation sites. These sites are highly conserved throughout the $GABA_A$ receptor family, and their identification provides a unique opportunity for the development of new therapeutic, neurosteroid-based ligands and transgenic disease models of neurosteroid dysfunction.

Endogenous neurosteroids are potent modulators of all major $GABA_A$ receptor isoforms[1,9]. At low nanomolar concentrations, observed during stress[10], alcohol intoxication[4] and oestrus[11], they potentiate GABA currents[12–14], whereas at submicromolar-to-micromolar concentrations, which occur during parturition[3], they directly activate the receptor[15]. This profile indicates that two distinct neurosteroid binding sites may exist on the $GABA_A$ receptor, but their location has remained enigmatic[1]. Previous studies have proposed that neurosteroids target the $GABA_A$ receptors' transmembrane (M) domains[16–18]. To determine how important these are, we exploited the *Drosophila melanogaster* RDL (resistance to dieldrin) GABA receptor, which has very low sensitivity to potentiation and lacks direct activation by micromolar concentrations of neurosteroids[19]. We replaced M1 through to the end of M2 in the murine α1 and β2 subunits with the corresponding sequence from the RDL subunit, forming the chimaeras αR and βR, respectively (Fig. 1a), and established their pharmacology as heterologously expressed αβγ subunit receptors. Potentiation and direct activation by tetrahydro-deoxycorticosterone (THDOC) and allopregnanolone (ALLOP) was abolished on receptors incorporating αR (Fig. 1b, and Supplementary Fig. 1), whereas receptors containing βR were indistinguishable from the wild type (data not shown). Thus, residues in the α1-subunit M1 and/or M2 domains are critical for neurosteroid action.

To regulate $GABA_A$ receptors, neurosteroids require a C3α hydroxyl group on their A-ring and a C20 ketone in the D-ring[20] (Fig. 1c). These groups are important for the binding of other steroids to a wide variety of proteins by means of hydrogen-bonding with polar or charged residues[21,22]. In particular, several polar residues in the α1 subunit M1–M2 domains are replaced by hydrophobic residues in αR (T229I, T236I, Q241W, T264V, S269M, N274A and S275A), which could account for the reduced neurosteroid sensitivity of αRβ2γ2 receptors (Fig. 1a). Sequential replacement of these polar residues in the α1 subunit with the homologous RDL variants identified Thr 236 and Gln 241 as important determinants of steroid action. Mutating Thr 236 to Ile markedly reduced receptor activation by THDOC and ALLOP without affecting their potentiation of responses to GABA (Fig. 1d, and Supplementary Table 1). By contrast, Q241W ablated the potentiation of GABA currents and markedly reduced the apparent neurosteroid agonist efficacy without affecting the concentration causing half-maximal receptor activation (Fig. 1e). This mutation did not affect the potentiation mediated by diazepam, pentobarbitone or mefenamic acid, which bind to separate sites on $GABA_A$ receptors (Fig. 1f). The distinctive functions of Thr 236 and Gln 241 in activation and potentiation by neurosteroids indicate that they may contribute to two discrete binding sites.

Using the transmembrane region of the nicotinic acetylcholine receptor[23], we created a homology model to determine where in the $GABA_A$ receptor M domains Thr 236 and Gln 241 are located (Figs 2a and 3a, and Supplementary Fig. 4a). Threonine 236 lies on the receptor surface, close to the β/α subunit interface (the same interface in which GABA binds but to the amino-terminal domain) and would be accessible to hydrophobic neurosteroid molecules in the membrane (Fig. 3a). By contrast, Gln 241 lies at the base of a water-filled cavity between the α subunit's M1–M4 domains (Fig. 2a, and Supplementary Fig. 4a). After receptor activation, the depth and volume of this cavity seems to increase[24–26], which could allow neurosteroids to bind to Gln 241 and stabilize the receptor in an active state. The relative locations of Thr 236 and Gln 241 on opposite faces of the M1 helix strongly suggest that they will contribute to steroid binding at two distinct sites. We therefore determined how the hydrogen-bonding capacity of these residues affected neurosteroid potentiation and activation on $GABA_A$ receptors.

Both the Q241H and Q241N variants supported wild-type levels of potentiation by ALLOP and THDOC, whereas their potencies were slightly reduced by Q241S and Q241T mutations (Fig. 2b, and Supplementary Fig. 1b). The level of potentiation was closely correlated with the substituent's abilities to act as hydrogen-bond acceptors (by providing a lone pair of electrons), which is how histidine residues form hydrogen bonds at pH 7.4. However, hydrophobic replacement with isoleucine or leucine abolished neurosteroid potentiation, while minimal potentiation was retained with methionine (10 μM or more; Fig. 2c, and Supplementary Fig. 1b). Significantly, potentiation was also abolished with tryptophan, which acts only as a hydrogen-bond donor. Because the neurosteroid's C20 ketone can only participate as a hydrogen-bond acceptor, these data

[1]Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, UK.

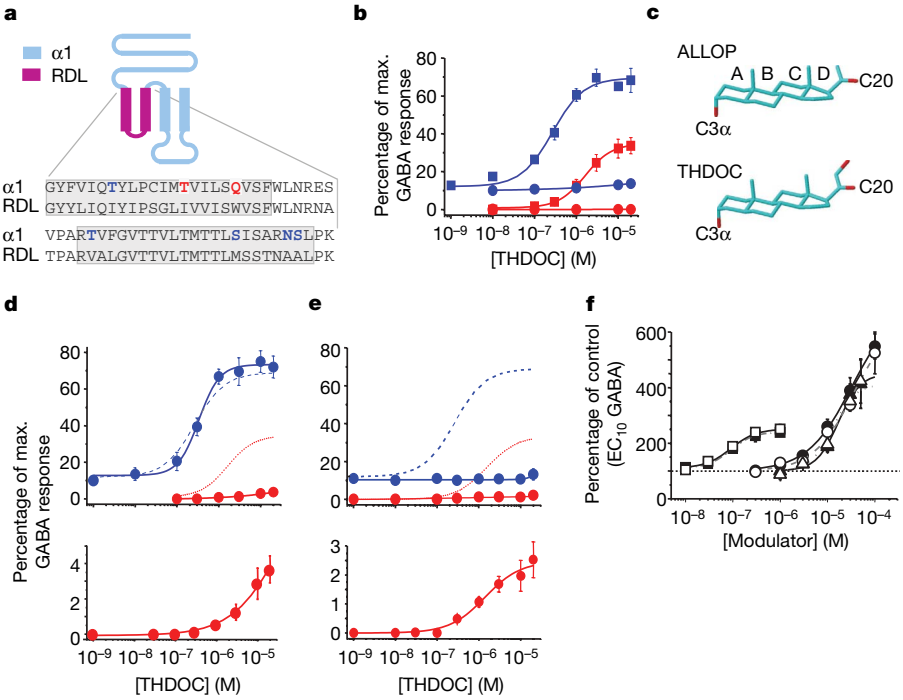

Figure 1 | Neurosteroid activity is determined by α-subunit M1 domain residues. **a**, The αR chimaera formed between α1 and RDL subunits. Polar residues present only in α1 (blue) are in bold, with Thr 236 and Gln 241 (red) highlighted. The transmembrane domains are boxed. **b**, THDOC concentration–response curves for direct activation (red) and for potentiation (blue) of EC_{10} (concentration causing 10% of maximal response) GABA currents expressed as a percentage of the maximum GABA response, at wild-type α1β2γ2 (squares) and chimaeric αRβ2γ2 (circles) receptors. **c**, Structures of ALLOP and THDOC. **d**, **e**, THDOC concentration–response curves for α1T236Iβ2γ2 (**d**) and α1Q241Wβ2γ2 (**e**) mutants. Blue points, potentiation; red points, activation. Top: the wild-type activation (dotted) and potentiation (dashed) curves are taken from **b**. Bottom: reduced potency of activation by T236I (**d**) and reduced efficacy of activation in Q241W (**e**). **f**, Concentration–response curves for the modulation of EC_{10} GABA currents by diazepam (squares), pentobarbitone (triangles) and mefenamic acid (circles). Filled symbols, wild type; open symbols, Q241W. Error bars represent s.e.m.

indicate that the C3α hydroxyl group donates a hydrogen bond to Gln 241.

By using our homology model to locate other polar residues in this cavity that might engage with the neurosteroids' C20 ketone group, we identified Asn 407 and Tyr 410 in the M4 domain of the α1 subunit. Both residues could donate a hydrogen bond to the C20 ketone and are separated from Gln 241 by about 15–18 Å (Fig. 2a), an appropriate distance to accommodate a neurosteroid molecule[21,22]. The hydrophobic substitutions N407A and Y410F reduced neurosteroid potency although not to the same extent as the mutation of Gln 241 (3–10-fold opposed to more than 100-fold; Fig. 2d, and Supplementary Table 1). Furthermore, N407V and N407D, neither of which can act as a hydrogen-bond donor, caused similar reductions in neurosteroid potency (Supplementary Table 1). The mutation N407A caused a greater reduction in the potency of THDOC (about tenfold) than in that of ALLOP (about threefold), whereas Y410F only reduced the potency of THDOC (Fig. 2d, and Supplementary Fig. 1c). This is consistent with their coordination of oxygen moieties on the neurosteroid D-ring, the only region where ALLOP and THDOC vary structurally (Fig. 1c). A combined mutation of N407A and Y410F further reduced THDOC potency (Fig. 2d), whereas mutation of other polar residues in M3 and M4 (Y293F, S298A, N407A, Y410F, T413I and Y414F) had no effect. Potentiation of GABA responses by diazepam remained unaffected by all these mutations (data not shown). Q242, N407 and Y410 are therefore ideally located for a neurosteroid-binding site that potentiates receptor function.

We next determined whether αThr 236 contributes to a second binding site associated with receptor activation. Replacing αThr 236 with non-hydrogen-bonding isoleucine or valine markedly reduced the agonist potency of ALLOP and THDOC (Fig. 3b). By contrast, ALLOP and THDOC had similar potencies but lower apparent efficacies on αT236Sβγ than on wild-type receptors (Fig. 3b), indicating that the presence and orientation of a hydroxyl group at position 236 might be critical for wild-type neurosteroid activation (Fig. 3b). If αThr 236 contributes to a neurosteroid-binding site, it should lie about 15 Å from a second surface-exposed residue capable of hydrogen-bonding with the neurosteroid molecule. In our model, Tyr 284 in the β2 subunit's M3 domain fulfilled these criteria (Fig. 3a). Consistent with a role in binding, the replacement of βTyr 284 with phenylalanine (removing the hydrogen-bonding hydroxyl group) markedly reduced the potencies of both THDOC and ALLOP as direct agonists (Fig. 3b) but had no effect on their potentiation of GABA currents (Supplementary Fig. 1i). The effects of mutating Thr 236 and Tyr 284 on receptor activation by neurosteroids were quite specific because they did not markedly affect activation by piperidine-4-sulphonic acid, a partial agonist of the $GABA_A$ receptor, or by the allosteric activator pentobarbitone

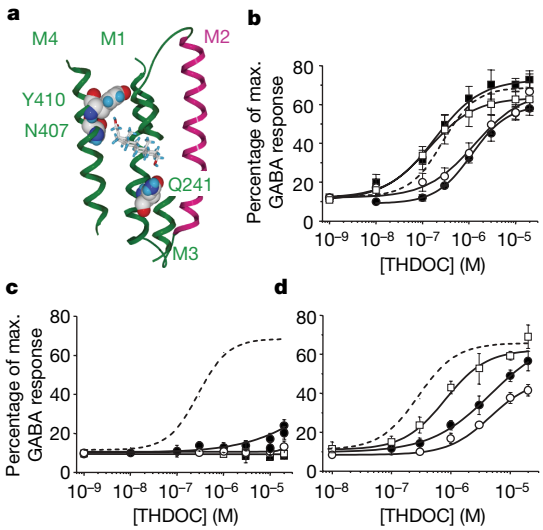

Figure 2 | Neurosteroid potentiation requires α-subunit M1 and M4 residues. **a**, Ribbon structure of α subunit M1–M4, showing αGln 241, αAsn 407 and αTyr 410 docking with a THDOC molecule, viewed from the lipid bilayer. The channel lining the M2 domain is shown in purple, and a section of M3 is omitted for clarity. **b**, **c**, THDOC concentration–response relationships for potentiation of EC_{10} GABA responses on wild-type (α1β2γ2, dashed line) and α1 mutant receptors where Gln 241 is replaced with polar (**b**; filled squares, His; open squares; Asn; filled circles, Ser; open circles, Thr) or hydrophobic (**c**; filled squares, Ile; open squares, Leu; filled circles, Met; open circles, Trp) residues. **d**, Effects of mutating αAsn 407 and αTyr 410 on THDOC potentiation. Dashed line, wild type; open circles, N407A Y410F; filled circles, Y410F; open squares, N407A. Error bars represent s.e.m.

(Supplementary Fig. 2a, c; Supplementary Table 2). This indicated that these residues might form a second neurosteroid-binding site, distinct from that mediating potentiation.

Although our data supported the existence of two distinct neurosteroid-binding sites on the GABA receptor, the mutation αQ241W not only abolished potentiation but also disrupted neurosteroid-induced activation. The apparent agonist efficacies for neurosteroids were reduced by αQ241W without affecting their potencies (Figs 1e and 3d, Supplementary Table 1). This raises the possibility that binding to αThr 236–βTyr 284 elicits only low-efficacy activation and that the high-efficacy activation, characteristic of wild-type receptors, depends on additional neurosteroid binding to αGln 241–αAsn 407, thereby potentiating neurosteroid-induced activation. Disruption of the potentiation site should therefore attenuate, but not abolish, the efficacy of neurosteroid activation. Indeed, substitutions that significantly reduced the potentiation of GABA responses (for example αQ241S, αN407A and αQ241M) caused proportionate reductions in the apparent agonist efficacy of neurosteroids, whereas larger activation efficacies were observed for mutants (Q241H and Q241N) that supported greater levels of potentiation (Fig. 3c, d, and Supplementary Table 1). Overall, high-efficacy activation by neurosteroids seems to require occupancy of both the activation (αThr 236, βTyr 284) and potentiation (αGln 241, αAsn 407) sites. Further evidence for two sites was provided by using the stereoisomer of ALLOP, 5β-pregnan-3α-ol-20-one (5β3α), which is as efficacious as ALLOP and THDOC as a potentiator but is considerably less efficacious as an agonist (Supplementary Fig. 3). Because mutating αGln 241–αAsn 407 and αThr 236–βTyr 284 reduced potentiation and activation by 5β3α, it must bind to the same residues as ALLOP and THDOC. However, whereas the potentiating site cannot distinguish between 5α3α and 5β3α isomers, the reduced agonist efficacy for 5β3α indicates that the activating site might have a different pharmacophore.

We then used our homology model to locate residues that line each site whose substitution might disrupt binding. We predicted that when bound to the potentiation site (αGln 241 and αAsn 407), the hydrophobic A-ring of the neurosteroid is juxtaposed to the hydrophobic side chain of αIle 238 (Fig. 4a). Introducing a similar-sized but polar side chain at residue 238 should cause electrostatic repulsion, reducing neurosteroid potency. As predicted, mutant I238N caused a rightward shift in the THDOC concentration–response curve for potentiation (Fig. 4c) without affecting the potentiation by diazepam (not shown). The apparent efficacy of neurosteroid activation was also reduced, which is consistent with high-efficacy neurosteroid activation requiring occupancy of both the activation and potentiation sites (Fig. 4d). Mutation of other residues lining this cavity, but not expected to disrupt neurosteroid binding, did not diminish potentiation (for example L231E and M235K; data not shown).

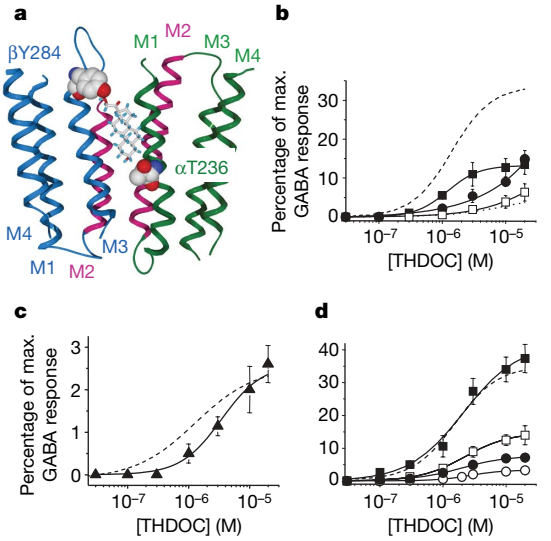

Figure 3 | Neurosteroid activation binding site spans the β/α-subunit interface. **a**, View through the lipid bilayer of model $GABA_A$ receptor transmembrane region (extracellular and cytoplasmic domains removed) with a bound THDOC molecule. αThr 236 and βTyr 284 are predicted to face the surrounding lipid. **b**, THDOC concentration–response relationships for α1β2γ2 receptor activation in the absence of GABA for the αT236 and βY284 mutants. The wild-type (dashed) and αT236I (dotted) curves are taken from Fig. 1d. Filled squares, αT236S; open squares, αT236V; filled circles, βY284F. **c**, Hydrophobic mutations of αGln 241 reduce the apparent agonist efficacy of THDOC. Note that the concentration giving half-maximal response is unchanged from the wild type (dashed line). Dashed line, Q241W; triangles, Q241M. **d**, Mutations of αGln 241 and αAsn 407 support varying degrees of direct receptor activation of α1β2γ2 receptors by THDOC. Dashed line, wild type; filled squares, Q241H; open squares, Q241N; filled circles, N407A; open circles, Q241S. Error bars represent s.e.m.

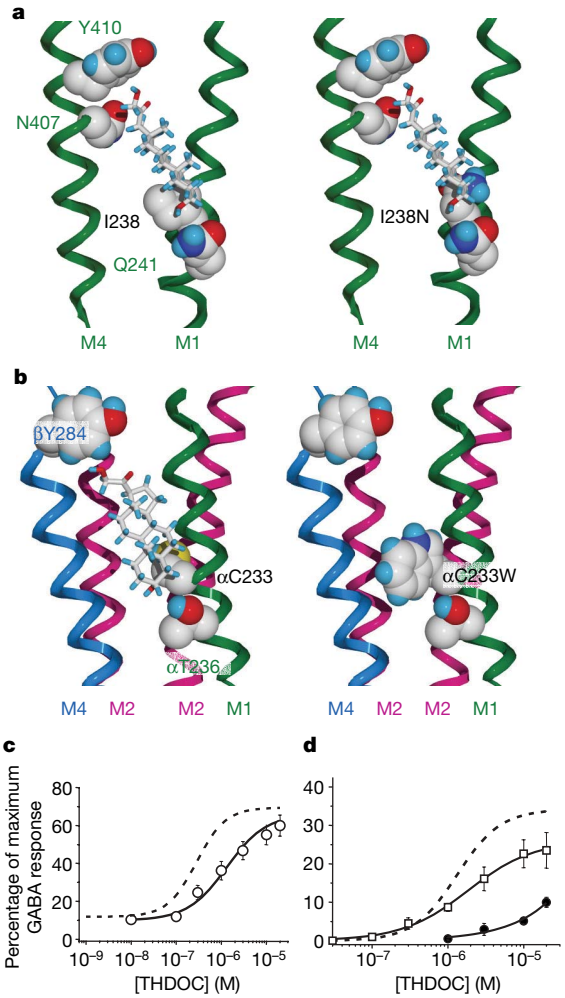

Figure 4 | Steric and dipolar disruption to the neurosteroid-binding sites. **a**, Homology model of THDOC bound to the potentiation site between M1 and M4 of the α subunit (M3 domain removed for clarity). Ile 238 is predicted to lie close to the A-ring of THDOC (left) and replacement with asparagine should repel the steroid (right). **b**, Cys 233 (left) is predicted to lie on the surface of the receptor; its replacement with tryptophan (right) increases the steric hindrance for THDOC binding to βTyr 284 and αThr 236. **c**, Effect of αI238N (circles) on the potency of THDOC potentiation of EC_{10} GABA responses. Dashed line, wild type. **d**, Effect of C233W (filled circles) on the agonist potency of THDOC. Disruption of the potentiation site (αI238N; open squares) reduces THDOC apparent direct agonist efficacy. Dashed line, wild type. Error bars represent s.e.m.

The model also predicted that replacing C233 with tryptophan should sterically hinder neurosteroid binding to the activation site delineated by αThr 236 and βTyr 284 (Fig. 4b). Indeed, C233W substantially reduced the potency of neurosteroid activation (Fig. 4d) without affecting either the potentiation of GABA responses or receptor activation by GABA, piperidine-4-sulphonic acid or pentobarbitone (Supplementary Fig. 2a–c). This further indicates that αThr 236–βTyr 284 might form a second binding site from which neurosteroids initiate receptor activation. Substitution of vicinal residues (for example, αPro 232), predicted not to line the putative binding site, had no effect on neurosteroid activation (data not shown). The mutations αI238N and αC233W therefore have specific effects on neurosteroid actions, each consistent with the disruption of distinct binding sites.

Our study shows that the activation and potentiation of $GABA_A$ receptors by neurosteroids are mediated by two discrete groups of residues in the $GABA_A$ receptor transmembrane domains: αThr 236 and βTyr 284 initiate activation, whereas αGln 241 and αAsn 407 mediate the potentiation of responses to GABA or neurosteroids. It is also evident that significant receptor activation by neurosteroids relies on occupancy of both sites (Supplementary Fig. 3). The requirement for suitably spaced hydrogen-bonding residues at each site, and their specific disruption by mutating residues predicted to line the binding pockets, indicates that these residues might participate directly in neurosteroid binding. Given the receptor stoichiometry of 2α:2β:1γ, there will be two copies of these sites per receptor molecule (Supplementary Fig. 3). Moreover, these residues are conserved throughout the α and β subunit families and thus are ideally placed to mediate neurosteroid regulation at all $GABA_A$ receptor subtypes. The identification of neurosteroid-binding sites on the $GABA_A$ receptor will now enable the pathophysiological roles of these potent endogenous modulators of inhibitory neurotransmission to be clearly defined.

METHODS

Further details on the methods used in this study can be found in Supplementary Information.

Complementary DNA constructs and transfection. Murine α1, β2 and γ2S subunits, chimaeras with RDL subunits, and all point mutants, were cloned into the plasmid pRK5. Functional GABA receptor expression was achieved by calcium phosphate transfection in human embryonic kidney cells.

Patch-clamp electrophysiology and data analysis. Whole-cell currents were recorded from single human embryonic kidney cells voltage-clamped at −40 mV, with an Axopatch 200B amplifier. Membrane currents were normalized to the maximal GABA response amplitude in each cell. Concentration–response curves were generated with the Hill equation. For studies of potentiation, neurosteroids and GABA were applied together without preincubation.

Homology modelling. The $GABA_A$ receptor α1 and β2 subunit transmembrane domains were modelled on a cryo-electron microscopic image of the homologous nicotinic acetylcholine receptor M1–M4 domains (Protein Data Bank accession number 1OED)[23] with the use of Deep View[27]. The $GABA_A$ receptor transmembrane domains were aligned such that residues believed to contribute to volatile-anaesthetic-binding sites faced into the cavity between the four transmembrane domains where these compounds are thought to bind[28]. Residue Gln 241 lies on the same side of the M1 helix as Leu 231, a known member of this site. The models were energy-minimized with the GROMOS 43B1 force field and the positions of side chains after mutations were assessed for conformational integrity with the use of Ramachandran plot analysis. For illustrative purposes, ALLOP and THDOC molecules were docked by eye and are not intended to predict the molecules' absolute orientations within the proposed binding cavities.

Received 16 May; accepted 9 October 2006.
Published online 15 November 2006.

1. Belelli, D. & Lambert, J. J. Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nature Rev. Neurosci.* **6,** 565–575 (2005).
2. Reddy, D. S. Is there a physiological role for the neurosteroid THDOC in stress-sensitive conditions? *Trends Pharmacol. Sci.* **24,** 103–106 (2003).
3. Stoffel-Wagner, B. Neurosteroid biosynthesis in the human brain and its clinical implications. *Ann. NY Acad. Sci.* **1007,** 64–78 (2003).
4. Kumar, S., Fleming, R. L. & Morrow, A. L. Ethanol regulation of gamma-aminobutyric acid A receptors: genomic and nongenomic mechanisms. *Pharmacol. Ther.* **101,** 211–226 (2004).
5. Backstrom, T. *et al.* Pathogenesis in menstrual cycle-linked CNS disorders. *Ann. NY Acad. Sci.* **1007,** 42–53 (2003).
6. Finn, D. A., Ford, M. M., Wiren, K. M., Roselli, C. E. & Crabbe, J. C. The role of pregnane neurosteroids in ethanol withdrawal: behavioral genetic approaches. *Pharmacol. Ther.* **101,** 91–112 (2004).
7. Eser, D. *et al.* Neuroactive steroids as modulators of depression and anxiety. *Neuroscience* **138,** 1041–1048 (2006).
8. Marx, C. E. *et al.* Neuroactive steroids are altered in schizophrenia and bipolar disorder: relevance to pathophysiology and therapeutics. *Neuropsychopharmacology* **31,** 1249–1263 (2006).
9. Fritschy, J. M. & Brunig, I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol. Ther.* **98,** 299–323 (2003).
10. Purdy, R. H., Morrow, A. L., Moore, P. H. Jr & Paul, S. M. Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc. Natl Acad. Sci. USA* **88,** 4553–4557 (1991).
11. Barbaccia, M. L. *et al.* The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations. *Br. J. Pharmacol.* **120,** 1582–1588 (1997).
12. Zhu, W. J. & Vicini, S. Neurosteroid prolongs $GABA_A$ channel deactivation by altering kinetics of desensitized states. *J. Neurosci.* **17,** 4022–4031 (1997).
13. Stell, B. M., Brickley, S. G., Tang, C. Y., Farrant, M. & Mody, I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors. *Proc. Natl Acad. Sci. USA* **100,** 14439–14444 (2003).
14. Belelli, D. & Herd, M. B. The contraceptive agent Provera enhances $GABA_A$ receptor-mediated inhibitory neurotransmission in the rat hippocampus: evidence for endogenous neurosteroids? *J. Neurosci.* **23,** 10013–10020 (2003).
15. Majewska, M. D., Harrison, N. L., Schwartz, R. D., Barker, J. L. & Paul, S. M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* **232,** 1004–1007 (1986).
16. Rick, C. E., Ye, Q., Finn, S. E. & Harrison, N. L. Neurosteroids act on the $GABA_A$ receptor at sites on the N-terminal side of the middle of TM2. *Neuroreport* **9,** 379–383 (1998).
17. Akk, G. *et al.* Neurosteroid access to the $GABA_A$ receptor. *J. Neurosci.* **25,** 11605–11613 (2005).
18. Ueno, S., Tsutsui, M., Toyohira, Y., Minami, K. & Yanagihara, N. Sites of positive allosteric modulation by neurosteroids on ionotropic gamma-aminobutyric acid receptor subunits. *FEBS Lett.* **566,** 213–217 (2004).
19. Chen, R. *et al.* Cloning and functional expression of a *Drosophila* γ-aminobutyric acid receptor. *Proc. Natl Acad. Sci. USA* **91,** 6069–6073 (1994).
20. Harrison, N. L., Majewska, M. D., Harrington, J. W. & Barker, J. L. Structure-activity relationships for steroid interaction with the gamma-aminobutyric acid-A receptor complex. *J. Pharmacol. Exp. Ther.* **241,** 346–353 (1987).
21. Grishkovskaya, I. *et al.* Crystal structure of human sex hormone-binding globulin: steroid transport by a laminin G-like domain. *EMBO J.* **19,** 504–512 (2000).
22. Brzozowski, A. M. *et al.* Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389,** 753–758 (1997).
23. Miyazawa, A., Fujiyoshi, Y. & Unwin, N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* **423,** 949–955 (2003).
24. Williams, D. B. & Akabas, M. H. γ-Aminobutyric acid increases the water accessibility of M3 membrane-spanning segment residues in γ-aminobutyric acid type A receptors. *Biophys. J.* **77,** 2563–2574 (1999).
25. Lobo, I. A., Mascia, M. P., Trudell, J. R. & Harris, R. A. Channel gating of the glycine receptor changes accessibility to residues implicated in receptor potentiation by alcohols and anesthetics. *J. Biol. Chem.* **279,** 33919–33927 (2004).
26. Jung, S., Akabas, M. H. & Harris, R. A. Functional and structural analysis of the $GABA_A$ receptor α1 subunit during channel gating and alcohol modulation. *J. Biol. Chem.* **280,** 308–316 (2005).
27. Schwede, T., Kopp, J., Guex, N. & Peitsch, M. C. SWISS-MODEL: An automated protein homology-modeling server. *Nucleic Acids Res.* **31,** 3381–3385 (2003).
28. Trudell, J. R. & Bertaccini, E. Comparative modeling of a $GABA_A$ α1 receptor using three crystal structures as templates. *J. Mol. Graph. Model.* **23,** 39–49 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank I. Duguid, P. Miller and P. Thomas for comments on the manuscript. This work was supported by the Medical Research Council and The Wellcome Trust.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to T.G.S. (t.smart@ucl.ac.uk) and A.M.H. (alastair.hosie@ucl.ac.uk).