Norman G. Bowery: A Founding Father of the GABAB Receptor Research Field: Reflections on His Contribution (Second Edition)

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Chapter 15

Norman G. Bowery: A Founding Father of the $GABA_B$ Receptor Research Field – Reflections on His Contribution

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Abstract

This chapter is dedicated to the history of Norman George Bowery and his groundbreaking discovery of $GABA_B$ receptor. This chapter focused on Norman's brilliant career from the beginning, towards his retirement, and to the legacy that he left in the scientific community: that is why Norman is called the "Father of $GABA_B$ receptor." Here are reported the first evidence of $GABA_B$ receptor existence spanning to the pharmacological characterization, and the following development of Norman's own group on $GABA_B$ receptor, and the huge innovation that he brought to the scientific community, because the $GABA_B$ receptor is a major factor in the central nervous system inhibition, therefore when not working properly it is involved in many diseases. The chapter also reports about the collaboration between Norman Bowery and Alessandra Princivalle, who was so honored and fortunate to have the opportunity to work with such an inspirational and worldwide recognized scientist and is now the author of this work. This chapter consider a brief overview of the latest Noman's work, before moving to GlaxoSmithKline, Verona, Italy. This chapter concludes with the greatest recognition to Norman Bowery for being Norman and discovering the $GABA_B$ receptor.

15.1 Norman Bowery

Norman George Bowery (Fig. 15.1; Fig. 15.2) was born in London in 1944. Norman began his career at The National Institute of Medical Research. He then worked at the newly opened CIBA Research Unit (a Swiss company later bought out) at Horsham in Sussex, UK. Norman left CIBA to begin a PhD with Professor David Brown and it was this fundamental work that led to his groundbreaking discovery of the γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor. He was then invited to take the Chair of Pharmacology at the prestigious School of Pharmacy in London. He spent successful years in this position. His following appointment was at the University of Birmingham, becoming Chair of Pharmacology, and Head of Division of Neuroscience and it was here in 1998 that I had the fantastic opportunity to start to collaborate with him. Norman came to pick me up at the airport as the friendliest professor I have ever met up to that point in my life. The first impression was then confirmed

in all the following years we worked together and became friends with respective families. Norman demonstrated a very happy, easy, and warm personality, always ready for a good laugh in company. When any problem arose, work or life, he was ready to discuss it and find a solution for it. That was the inspirational man for everybody who met him: collaborators, students at all levels from undergraduates to PhD. Norman was a huge lover of Italy and Italians, and Italian wines, in fact when he left the University of Birmingham he moved to work for GlaxoSmithKline (GSK) in Verona, Italy, before going back to University of Birmingham to deliver pharmacology lectures as Honorary Professor and he did it as a hobby. He was president of the British Pharmacological Society from 1995 to 1997 and from 1999 to 2000.

Figure 15.1 Professor Norman George Bowery FBPhS (1944 - 2016) British pharmacologist.

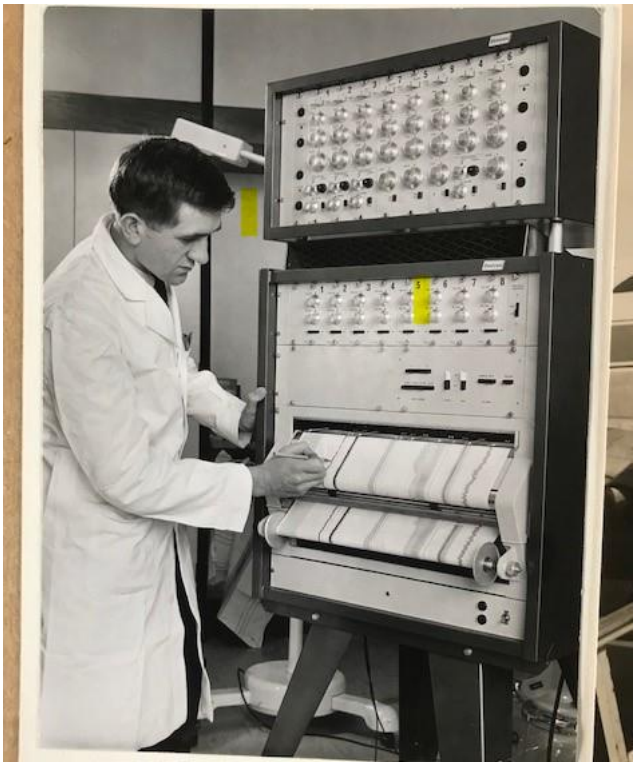

Figure 15.2 Young Norman Bowery in the laboratory.

15.2 Norman and early evidence of a second type of GABA receptor (1970s - 1982)

Norman started his work on GABA in the seventies during his time in Professor Brown's laboratory, reporting *in vitro* observation of depolarization of sympathetic ganglia induced by gamma-aminobutyric acid (Bowery and Brown 1972). Since then, his interest in the action of GABA and its action in different compartments of the central (CNS) and peripheral (PNS) nervous system never stopped (see PubMed Bowery et al.1972-1975)

He continued his studies investigating the action of three analogues namely 4-aminotetrolic acid (4-ATA), *trans* 4-aminocrotonic acid (4-ACA), and imidazole-4-acetic acid (IAA) capable of depolarizing the ganglia in a way similar to GABA (Bowery and Jones, 1976). After these analogues he evaluated other compounds including other amino acids (Bowery et al. 1976a) bicyclic phosphorus esters as convulsant (Bowery et al.1976b). Norman and colleagues also investigated several other aspects of the GABA inhibitory system in ganglia (Bowery and Dray 1976c; Neal and Bowery 1977; Bowery and Dray 1978). In 1978 Norman and his collaborators clarified aspects of isoguvacine, and muscimol analogues acting on GABA receptors in rat ganglia (Bowery et al.1978). During the following few years Norman and colleagues advanced their studies by using baclofen which identified a new type of GABA receptor (Bowery et al.1980). In this historic paper in Nature journal, Norman and co-workers

shed light on why GABA affected and reduced the release of H^3-noradrenaline in atria, and H^3-acetylcholine in preganglionic terminals, and these events were not affected by bicuculline. So, their hypothesis was: there must be a different and separate type of GABA receptor present on nerve terminals. To test this hypothesis, they used three different *in vitro* systems with specific neurotransmitters and measured the release of evoked K^+. The first were slices from rat cerebellum, in which the release of H^3-noradrenaline was measured in three different settings incubated with (i) high KCl (25mM), (ii) baclofen 100μM, and (iii) GABA 100 μM. The same set was used to test the striatum and the release of H^3-dopamine; and the frontal cortex and the release of H^3-serotonin. All the set of experiments gave the same results: a reduction in released of the tritiated neurotransmitters (Fig. 15.3; Fig. 15.4).

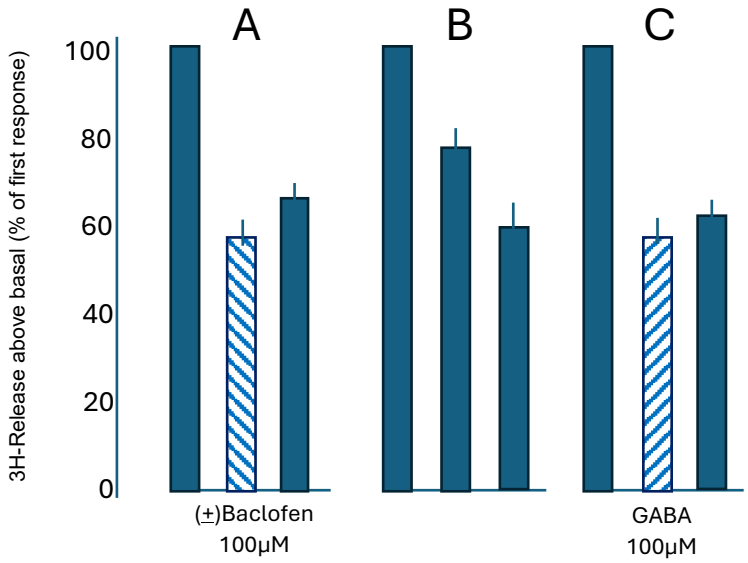

Figure 15.3. Fig. A and C, (±)baclofen (100 μM) and GABA (100 μM), respectively, added 1 min before the second period of superfusion with KCl. Fig. B shows the responses to successive additions of KCl. Figure adapted from Bowery NG, et al. 1980, with permission from Springer Nature.

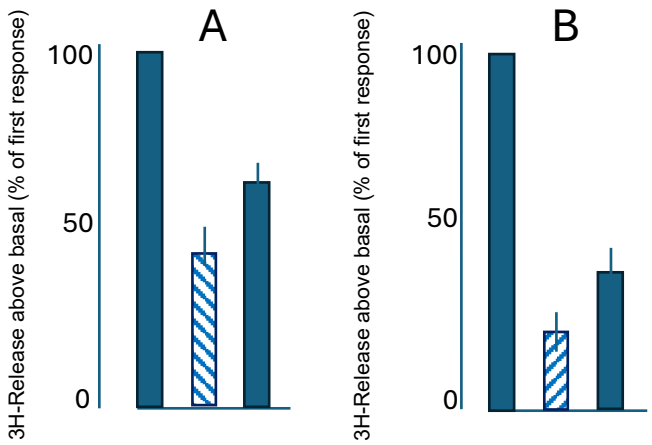

Figure 15.4. Results obtained Depression of K+ -evoked release of ^{3}H-dopamine (a) and ^{3}H-serotonin (b) by (±)baclofen. Fig. A on the evoked release of ^{3}H-dopamine from striatal slices and Fig. B ^{3}H-serotonin from cortical slices- (B) Figure adapted from Bowery NG, et al. 1980, with permission from Springer Nature.

They also tested other analogues of the GABA, and different concentrations of K^{+} support these findings. The huge breakthrough of a second GABA binding site, or as they call it first "a novel GABA receptor", was made… YES!!! Hurray!!! This was the birth of $GABA_B$ receptor and Norman Bowery was its absolute father. Norman's work on this fascinating and exciting novelty continued by further corroboration for the existence of the second GABA binding site. Norman with David Hill performed classical homogenized crude synaptic membranes radioligand binding experiments on rat brain first using H^3-baclofen, with different experimental conditions adding different cations to the buffer to check saturability of baclofen. They first set the best conditions for the investigation of the second GABA binding site, and then they used (-) baclofen and GABA as non labelled displacing compounds. They showed that the displacement curves for GABA and (-) baclofen were the same in the same experimental conditions whereas (+) baclofen did not show any displacement (Fig. 15.5). They also tested other GABA type-A ($GABA_A$) receptor agonists (isoguvacine) and antagonists (bicuculline), and they did not have any effect on the displacement of H^3-baclofen (Fig. 15.5).

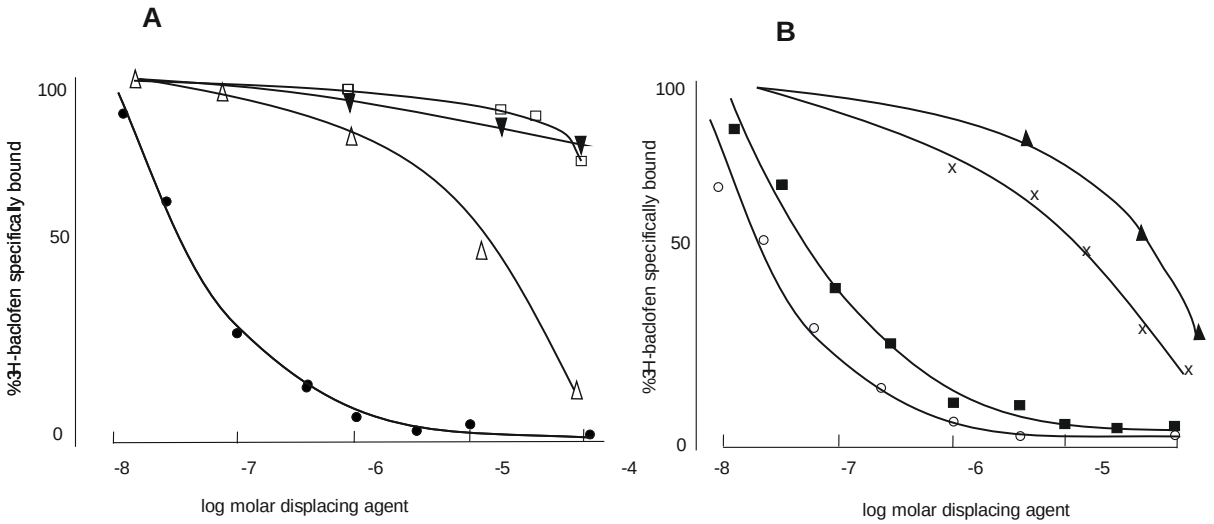

Figure 15.5. Inhibition of (±)^{3}H-baclofen binding by GABA-related compounds. Unlabelled analogues at final concentrations between 0.01 and 100 μM were added to each incubation mixture together with ^{3}H-baclofen (20 nM final concentration). Specific binding was taken as the amount of label displaced by (±)baclofen (100 μM). The amount displaced by individual concentrations of each analogue (log molar, abscissa) is expressed as a percentage of the total displaced by 100 μM (±)baclofen within the same experiment. The data have been separated for clarity. A Shows the effect of GABA (●), muscimol (△), isoguvacine (▼) and bicuculline methobromide (□). B Shows the effects of (-)baclofen (○), (±)baclofen (■), 3-aminopropanesul-phonic acid (x) and (+)baclofen (▲). Figure and legend adapted from Hill and Bowery 1981, with permissi0n from Springer Nature.

Finally, to distinguish the two different binding sites Norman and David named $GABA_A$ receptor the original binding site and $GABA_B$ receptor the newly discovered and confirmed binding site. (Hill and Bowery 1981).

Norman and collaborators did it and continue their research on the $GABA_B$ receptor, and a lot of new knowledge emerged quickly (Bowery et al. 1981a; Doble et al. 1981).

Other papers were based on pharmacological techniques which led to the breakthrough of the existence of a GABA receptor which was not the ionotropic one but a metabotropic (Bowery et al. 1981b; Wilkin et al. 1981).

15.3 Norman's studies on the "novel GABA receptor" (1982 - 1992)

Norman and various collaborators continue their studies to localize this novel GABA receptor which they named $GABA_B$ to be distinguished from the benzodiazepine activated ionotropic receptor named $GABA_A$ (Bowery et al. 1983). Norman and his team went on with their studies to gather and advance knowledge about this new receptor: there were a lot of new questions opening at the horizon of this discovery: such as the localization in different nuclei and parts, and physiological pathways of CNS and PNS; as well as the specific cellular and subcellular localization (Bowery et al.1984: Price et al.1984; Bristow et al.1986; Price et

al.1987; Bowery et al.1987). Norman also did other studies on the heterogeneity of $GABA_B$ receptors. Sort of “a preconception” to the molecular characterization of the whole $GABA_B$ multidimer (Bowery et al. 1990), and they were also able to pharmacologically differentiate “subtypes” of $GABA_B$ receptors. Additional studies in Norman’s group demonstrated that neuronal degeneration could be induced by tetanus toxin accompanied by a reduction of $GABA_A$ and not $GABA_B$ sites in rat hippocampus (Bagetta et al. 1990), a further corroboration for the two different GABA receptors. Most importantly from the whole body of evidence emerged that a new versatile drug target was available (Bowery and Pratt 1992).

15.4 Norman’s pharmacological studies on the $GABA_B$ receptor (1993 – 1997)

Norman went on, with his amazing and contagious curiosity, to inspire collaborators and they tested several compounds such as pertussis toxin in CNS (Knott et al. 1993a; 1993b) or forskolin in spinal cord (Malcangio and Bowery 1993a) and Substance P in rat cortex (Malcangio and Bowery 1993b) and desipramine (Pratt and Bowery 1993) on the recently isolated $GABA_B$.

Then Norman and Meret Facklam isolated the $GABA_B$ receptor from pigs’ brain and found the receptor had the same pharmacological characteristics as those isolated from rats’ brain (Facklam and Bowery, 1993). However, these $GABA_B$ receptors were present in homogenates derived from CNS or PNS, and cells membrane, and up to this point there was no evidence of the gene or protein for this pharmacologically characterized receptor. After three more years of further studies combining neurotransmitters, their actions and responses in different compartment of the nervous system, Norman and coworkers make another step forward describing that once $GABA_B$ receptors are activated it can determine opposite effects of up- or down regulation, therefore activation or inhibitions of adenylyl cyclase (Malcangio et al.1995; Knight and Bowery, 1996). Furthermore, Norman with Hill and Hudson start to describe the $GABA_B$ receptors binding site in rat brain (Bowery at al., 1997a).

At the end of 1990s, the application of molecular biology techniques gave a huge burst to the pharmacology field, due to the increasing number of genes cloned for receptors, receptors subunits, and neurotransmitters, and it was the case also for the $GABA_B$ receptor.

Kaupmann et al. (1997) published a paper in Nature reporting the molecular cloning of the gene for $GABA_B$ receptor and the sequence of the protein, including a predicted structure similar to that of the metabotropic glutamate receptors. Norman and David Brown analyzed

the latest scientific development concerning the $GABA_B$ (Bowery 1997; Bowery and Brown 1997).

15.5 When Norman met Alessandra: localization and expression studies of the $GABA_B$ receptor in brain and spinal cord (1998 - 2003)

I met Norman in person at the very end of January 1998, as mentioned in part 15.1. However, at the annual American Society for Neuroscience (SfN) meeting, in New Orleans, LA, in 1997, I already heard scientists referring to Norman as **"$GABA_B$ man"**, which says all about him. Been called "$GABA_B$ man" meant that he was internationally, worldwide, recognized as the guru, and the father of the $GABA_B$ receptor.

Our collaboration and friendship started that long ago, I cannot believe it is over 25 years ago (scary), but better refocus attention on what we did together concerning the $GABA_B$ receptor research.

In 1998 I began to collaborate with Norman in his laboratory with a fellowship from a European project, at The Medical School in Birmingham, UK. Here we did immunohistochemistry on rat brains to initially localize the $GABA_B$ protein, finding specific patterns of expression in the piriform cortex (Princivalle et al. 2000a). While I was working with Norman, I deepen my knowledge in all what he did scientifically, and how much he had contributed to the knowledge in the field of $GABA_B$ receptor, no wonder why he was referred to as "$GABA_B$ man" (very American way) and what an honor to be there doing research with him!

The European project was based on previous evidence showing a decrease inhibition in the thalamus and somatosensory cortex of non-epileptic rat and Genetics Absence Epilepsy Rats from Strasburg (GAERS), the rat model of *petit mal* or absence seizures. This was a validated model of human absence epilepsy based on neurological, behavioral, and pharmacological findings (Vergnes et al. 1982, Marescaux et al. 1992). So, we started investigating the localization of the $GABA_B$ protein in rat somatosensory cortex and thalamus in adult and developing non GAERS rat brains (Princivalle et al. 2000b). Then we moved forward to investigate the semi-quantitative expression of $GABA_B$ receptor in control rats and GAERS (Princivalle et al. 2003). At the end of 1998 Norman organized a $GABA_B$ Satellite Symposium to the 28th Annual Meeting of the SfN in Los Angeles, CA, of course who else!!! On that occasion Kaupmann and Bettler (1998) unveiled the finer structure of the fully functional $GABA_B$ receptor, demonstrating for the first time that the $GABA_B$ receptor is a metabotropic heterodimeric receptor… no way!!! Norman and I listened to this exciting piece

of news and we both were almost incredulous. This was opening the door to a new and immense field for pharmacological research and treatment of countless diseases.
The following year I went back again to Norman's laboratory, and we started a three-year collaboration to finish some of the previous European project to put together the first few papers of our collaboration. Norman offered me a position to work with him on a newer project on $GABA_B$. I must admit that the idea of working with **"$GABA_B$ man"** was overly exciting and of course how could I say no to such an amazing offer?! So later that year, I started this new position continuing in parallel the previous European project, and other collaborations (Towers et al. 2000; Princivalle et al. 2001) and starting the new project focused on the expression of $GABA_B$ receptor in human sclerotic hippocampi from temporal lobe epilepsy (TLE) affected patients not responding to drug therapies. There was already indirect evidence showing a decreased inhibition in animal model of pharmaco-resistant TLE associated with hippocampal sclerosis (HS). Therefore, our hypothesis was that $GABA_B$ receptor might play a role in this specific category of epileptic patients, and likely to show a lower expression in the hippocampal regions of TLE-HS. From the first part of this project, we used a classical quantitative imaging technique, the autoradiographic binding, using a newly synthesized radioligand with higher affinity for the $GABA_B$ receptor and published these results demonstrating a decreased expression of the $GABA_B$ receptor in the CA3, dentate gyrus, and hilus of the hippocampal region. We found an increased expression of $GABA_B$ receptors per surviving neuron in CA3 of TLE-HS patients compared to post-mortem controls (Princivalle et al.2002). Since with the imaging quantitative technique we investigated the protein receptor and found downregulation of the $GABA_B$ receptor, we wanted to also check if that was due to quantitative differential expression of the transcripts for the $GABA_B$ receptor. We used three different probes to check the three different transcripts for the two splice variants called $GABA_{B1}$ subunit ($GABA_{B1a}$ and $GABA_{B1b}$) and the second subunit called $GABA_{B2}$ (Kaupmann et al.1998). That research was done using quantitative imaging with *in situ* hybridization technique (Princivalle et al. 2003a). We also investigated the plasticity of $GABA_B$ receptor in heterosynaptic connections at mossy fibers (Chandler et al. 2003).

Norman's 2004-2008

I then moved to a Senior Lecturer position in Sheffield, UK, but that did not mean that our friendship and collaboration were over. In 2004, Norman moved to GSK, Verona, Italy, continuing collaborations with other groups. Norman worked on furtherly expanding knowledge on the $GABA_B$ receptor focusing on physiological aspects and pharmacological properties, within his group (Amantea and Bowery 2004a, Amantea et al.2004b; Smith et al.2007), and in collaboration with others (Dang et al.2004; Meza-Toledo and Bowery 2008).

Norman also wrote reviews about the diverse topics involving $GABA_B$ (Enna and Bowery 2004; Bowery 2006; 2010). His last work before passing away was a chapter in a book edited by the same editor of this book, Giancarlo Colombo, and it was an historical overview of the $GABA_B$ receptor from Norman's initial studies on modelling presynaptic inhibition, passing to the pharmacological characterization, agonist and antagonist, the physiological role, a quick consideration of the distribution in the CNS, the structure, modulation, and clinical aspects targeting the receptor with baclofen as muscle relaxant (Bowery 2016). Part of the work I started in Norman's lab was concluded and published posthumous his dead, so much so that the article was dedicated to him (Sheilabi et al. 2017), even if some of the authors never met him but knew about his greatest discovery.

Conclusions

To collaborate with the father of $GABA_B$ receptor and the most inspirational scientist I had met so far, and still, was awesome!!! One other most important quality of Norman was to leave total freedom to the collaborators in technical terms, in cogitating, trying new way for research, in expanding and expressing themselves as researchers and scientists, and the enthusiasm that Norman was capable to spread around to everyone, anywhere, and in any context.

From our collaborations papers were published, but the major lesson by Norman was never give up: if there is a problem we need to find the solutions. Last but not least, taught way of doing research, which I use even now with my own younger collaborators and students, when observing and/or interpreting data was/is: "Do I see what I believe, or do I believe what I see?". This is what every scientist must ask themselves always when they look at their own data. That question represents the essence of an intellectually honest, inspirational, and great scientist like Norman Bowery.

Acknowledgement

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Bibliography

1. Bowery NG, Brown DA. -Aminobutyric acid uptake by sympathetic ganglia. Nat New Biol. 1972 Jul 19;238(81):89-91. doi: 10.1038/newbio238089a0. PMID: 4505419.
2. Bowery NG, Jones GP. A comparison of gamma-aminobutyric acid and the semi-rigid analogues 4-aminotetrolic acid, 4-aminocrotonic acid and imidazole-4-acetic acid on the isolated superior cervical ganglion of the rat. Br J Pharmacol. 1976 Mar;56(3):323-30. doi: 10.1111/j.1476-5381.1976.tb07646.x. PMID: 1260178; PMCID: PMC1666980.
3. Bowery NG, Brown DA, Collins GG, Galvan M, Marsh S, Yamini G. Indirect effects of amino-acids on sympathetic ganglion cells mediated through the release of gamma-aminobutyric acid from glial cells. Br J Pharmacol. 1976 May;57(1):73-91. doi: 10.1111/j.1476-5381.1976.tb07658.x. PMID: 1276543; PMCID: PMC1667007.
4. Bowery NG, Collins JF, Hill RG. Bicyclic phosphorus esters that are potent convulsants and GABA antagonists. Nature. 1976 Jun 17;261(5561):601-3. doi: 10.1038/261601a0. PMID: 934303.
5. Bowery NG, Dray A. Barbiturate reversal of amino acid antagonism produced by convulsant agents. Nature. 1976 Nov 18;264(5583):276-8. doi: 10.1038/264276a0. PMID: 187949.
6. Neal MJ, Bowery NG. Cis-3-aminocyclohexanecarboxylic acid: a substrate for the neuronal GABA transport system. Brain Res. 1977 Dec 9;138(1):169-74. doi: 10.1016/0006-8993(77)90793-4. PMID: 589464.
7. Bowery NG, Dray A. Reversal of the action of amino acid antagonists by barbiturates and other hypnotic drugs. Br J Pharmacol. 1978 May;63(1):197-215. doi: 10.1111/j.1476-5381.1978.tb07790.x. PMID: 206305; PMCID: PMC1668297.
8. Bowery NG, Collins JF, Hudson AL, Neal MJ. Isoguvacine, isonipecotic acid, muscimol and N-methyl isoguvacine on the GABA receptor in rat sympathetic ganglia. Experientia. 1978 Sep 15;34(9):1193-5. doi: 10.1007/BF01922953. PMID: 214333.
9. Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, Turnbull M. (-) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature. 1980 Jan 3;283(5742):92-4. doi: 10.1038/283092a0. PMID: 6243177.
10. Hill DR, Bowery NG. 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. Nature. 1981 Mar 12;290(5802):149-52. doi: 10.1038/290149a0. PMID: 6259535.

11. Bowery NG, Doble A, Hill DR, Hudson AL, Shaw JS, Turnbull MJ, Warrington R. Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. Eur J Pharmacol. 1981 Apr 24;71(1):53-70. doi: 10.1016/0014-2999(81)90386-1. PMID: 6263651.
12. Doble A, Iversen LL, Bowery NG, Hill DR, Hudson AL. 6 -Hydroxydopamine decreases benzodiazepine but not GABA receptor binding in rat cerebellum. Neurosci Lett. 1981 Dec 11;27(2):199-204. doi: 10.1016/0304-3940(81)90268-8. PMID: 6119659.
13. Bowery NG, Doble A, Hill DR, Hudson AL, Turnbull MJ, Warrington R. Structure/activity studies at a baclofen-sensitive, bicuculline-insensitive GABA receptor. Adv Biochem Psychopharmacol. 1981;29:333-41. PMID: 6266218.
14. Wilkin GP, Hudson AL, Hill DR, Bowery NG. Autoradiographic localization of GABAB receptors in rat cerebellum. Nature. 1981 Dec 10;294(5841):584-7. doi: 10.1038/294584a0. PMID: 6273750.
15. Bowery NG, Hill DR, Hudson AL. Characteristics of GABAB receptor binding sites on rat whole brain synaptic membranes. Br J Pharmacol. 1983 Jan;78(1):191-206. doi: 10.1111/j.1476-5381.1983.tb09380.x. PMID: 6297646; PMCID: PMC2044790.
16. Bowery NG, Price GW, Hudson AL, Hill DR, Wilkin GP, Turnbull MJ. GABA receptor multiplicity. Visualization of different receptor types in the mammalian CNS. Neuropharmacology. 1984 Feb;23(2B):219-31. doi: 10.1016/0028-3908(84)90063-7. PMID: 6324016.
17. Price GW, Wilkin GP, Turnbull MJ, Bowery NG. Are baclofen-sensitive GABAB receptors present on primary afferent terminals of the spinal cord? Nature. 1984 Jan 5-11;307(5946):71-4. doi: 10.1038/307071a0. PMID: 6318120.
18. Bowery NG, Hill DR, Hudson AL. [3H](-)Baclofen: an improved ligand for GABAB sites. Neuropharmacology. 1985 Mar;24(3):207-10. doi: 10.1016/0028-3908(85)90075-9. PMID: 2986036.
19. Bristow DR, Bowery NG, Woodruff GN. Light microscopic autoradiographic localisation of [3H]glycine and [3H]strychnine binding sites in rat brain. Eur J Pharmacol. 1986 Jul 31;126(3):303-7. doi: 10.1016/0014-2999(86)90062-2. PMID: 3019717.
20. Price GW, Kelly JS, Bowery NG. The location of GABAB receptor binding sites in mammalian spinal cord. Synapse. 1987;1(6):530-8. doi: 10.1002/syn.890010605. PMID: 2843995.

21. Bowery NG, Hudson AL, Price GW. GABAA and GABAB receptor site distribution in the rat central nervous system. Neuroscience. 1987 Feb;20(2):365-83. doi: 10.1016/0306-4522(87)90098-4. PMID: 3035421.
22. Bowery NG, Knott C, Moratalla R, Pratt GD. GABAB receptors and their heterogeneity. Adv Biochem Psychopharmacol. 1990;46:127-39. PMID: 1963262.
23. Bagetta G, Knott C, Nisticó G, Bowery NG. Tetanus toxin produces neuronal loss and a reduction in GABAA but not GABAB binding sites in rat hippocampus. Neurosci Lett. 1990 Feb 5;109(1-2):7-12. doi: 10.1016/0304-3940(90)90529-i. PMID: 2156194.
24. Bowery, N. G., & Pratt, G. D. (1992). GABAB receptors as targets for drug action. *Arzneimittel-Forschung*, *42*(2A), 215–223.
25. Knott C, Maguire JJ, Moratalla R, Bowery NG. Regional effects of pertussis toxin in vivo and in vitro on GABAB receptor binding in rat brain. Neuroscience. 1993 Jan;52(1):73-81. doi: 10.1016/0306-4522(93)90183-g. PMID: 8381927.
26. Knott C, Maguire JJ, Bowery NG. Age-related regional sensitivity to pertussis toxin-mediated reduction in GABAB receptor binding in rat brain. Brain Res Mol Brain Res. 1993 Jun;18(4):353-7. doi: 10.1016/0169-328x(93)90102-u. PMID: 8392134.
27. Malcangio, M., & Bowery, N. G. (1993a). GABAB receptor-mediated inhibition of forskolin-stimulated cyclic AMP accumulation in rat spinal cord. *Neuroscience letters*, *158*(2), 189–192. https://doi.org/10.1016/0304-3940(93)90261-i
28. Malcangio, M., & Bowery, N. G. (1993b). Gamma-aminobutyric acidB, but not gamma-aminobutyric acidA receptor activation, inhibits electrically evoked substance P-like immunoreactivity release from the rat spinal cord in vitro. *The Journal of pharmacology and experimental therapeutics*, *266*(3), 1490–1496.
29. Pratt, G. D., & Bowery, N. G. (1993). Repeated administration of desipramine and a GABAB receptor antagonist, CGP 36742, discretely up-regulates GABAB receptor binding sites in rat frontal cortex. *British journal of pharmacology*, *110*(2), 724–735. https://doi.org/10.1111/j.1476-5381.1993.tb13872.x
30. Facklam, M., & Bowery, N. G. (1993). Solubilization and characterization of GABAB receptor binding sites from porcine brain synaptic membranes. *British journal of pharmacology*, *110*(4), 1291–1296. https://doi.org/10.1111/j.1476-5381.1993.tb13958.x
31. Malcangio M, Libri V, Teoh H, Constanti A, Bowery NG. Chronic (-)baclofen or CGP 36742 alters GABAB receptor sensitivity in rat brain and spinal cord. *Neuroreport*. 1995; 6(2):399-403. doi:10.1097/00001756-199501000-00042

32. Knight AR, Bowery NG. The pharmacology of adenylyl cyclase modulation by GABAB receptors in rat brain slices. *Neuropharmacology*. 1996;35(6):703-712. doi:10.1016/0028-3908(96)84642-9
33. Bowery NG, Hill DR, Hudson AL. Characteristics of GABAB receptor binding sites on rat whole brain synaptic membranes. 1983. *Br J Pharmacol*. 1997;120(4 Suppl):452-467. doi:10.1111/j.1476-5381.1997.tb06835.x
34. Kaupmann K, Huggel K, Heid J, et al. Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature*. 1997;386(6622):239-246. doi:10.1038/386239a0
35. Bowery NG. Metabotropic GABA(B) receptors cloned at last. *Trends Pharmacol Sci*. 1997;18(4):103.
36. Bowery NG, Brown DA. The cloning of GABA(B) receptors. *Nature*. 1997;386(6622):223-224. doi:10.1038/386223a0
37. Vergnes M, Marescaux C, Micheletti G, Reis J, Depaulis A, Rumbach L, Warter JM. Spontaneous paroxysmal electroclinical patterns in rat: a model of generalized non-convulsive epilepsy. Neurosci Lett. 1982 Nov 16;33(1):97-101. doi: 10.1016/0304-3940(82)90136-7. PMID: 6818498.
38. Marescaux C, Vergnes M, Depaulis A. Genetic absence epilepsy in rats from Strasbourg--a review. J Neural Transm Suppl. 1992;35:37-69. doi: 10.1007/978-3-7091-9206-1_4. PMID: 1512594.
39. Princivalle A, Spreafico R, Bowery N, De Curtis M. Layer-specific immunocytochemical localization of GABA(B)R1a and GABA(B)R1b receptors in the rat piriform cortex. Eur J Neurosci. 2000a Apr;12(4):1516-20. doi: 10.1046/j.1460-9568.2000.01060.x. PMID: 10762380.
40. Princivalle A, Regondi MC, Frassoni C, Bowery NG, Spreafico R. Distribution of GABA(B) receptor protein in somatosensory cortex and thalamus of adult rats and during postnatal development. Brain Res Bull. 2000b Jul 15;52(5):397-405. doi: 10.1016/s0361-9230(00)00256-2. PMID: 10922519.
41. Towers S, Princivalle A, Billinton A, Edmunds M, Bettler B, Urban L, Castro-Lopes J, Bowery NG. GABAB receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia. Eur J Neurosci. 2000 Sep;12(9):3201-10. doi: 10.1046/j.1460-9568.2000.00237.x. PMID: 10998104.
42. Princivalle AP, Pangalos MN, Bowery NG, Spreafico R. Distribution of GABA(B(1a)), GABA(B(1b)) and GABA(B2) receptor protein in cerebral cortex and thalamus of adult rats. Neuroreport. 2001 Mar 5;12(3):591-5. doi: 10.1097/00001756-200103050-00032. PMID: 11234770.

43. Kaupmann K, Bettler B. Heteromerization of $GABA_B$ Receptors: A New Principle for G Protein-Coupled Receptors. Satellite Symposium to the 28th Annual Meeting of the Society for Neuroscience Los Angeles, CA, November 5-7, 1998. CNS Drug Rev. 1998 Dec;4(4):376-379. doi: 10.1111/j.1527-3458.1998.tb00077.x. PMID: 29200228.
44. Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B. GABA(B)-receptor subtypes assemble into functional heteromeric complexes. Nature. 1998 Dec 17;396(6712):683-7. doi: 10.1038/25360. PMID: 9872317.
45. Princivalle AP, Duncan JS, Thom M, Bowery NG. Studies of GABA(B) receptors labelled with [(3)H]-CGP62349 in hippocampus resected from patients with temporal lobe epilepsy. Br J Pharmacol. 2002 Aug;136(8):1099-106. doi: 10.1038/sj.bjp.0704812. PMID: 12163342; PMCID: PMC1573440.
46. Princivalle AP, Duncan JS, Thom M, Bowery NG. GABA(B1a), GABA(B1b) AND GABA(B2) mRNA variants expression in hippocampus resected from patients with temporal lobe epilepsy. Neuroscience. 2003;122(4):975-84. doi: 10.1016/j.neuroscience.2003.08.044. PMID: 14643764.
47. Chandler KE, Princivalle AP, Fabian-Fine R, Bowery NG, Kullmann DM, Walker MC. Plasticity of GABA(B) receptor-mediated heterosynaptic interactions at mossy fibers after status epilepticus. J Neurosci. 2003 Dec 10;23(36):11382-91. doi: 10.1523/JNEUROSCI.23-36-11382.2003. PMID: 14673002; PMCID: PMC6740526.
48. Princivalle AP, Richards DA, Duncan JS, Spreafico R, Bowery NG. Modification of GABA(B1) and GABA(B2) receptor subunits in the somatosensory cerebral cortex and thalamus of rats with absence seizures (GAERS). Epilepsy Res. 2003 Jun-Jul;55(1-2):39-51. doi: 10.1016/s0920-1211(03)00090-1. PMID: 12948615.
49. Amantea D, Tessari M, Bowery NG. Reduced G-protein coupling to the GABAB receptor in the nucleus accumbens and the medial prefrontal cortex of the rat after chronic treatment with nicotine. Neurosci Lett. 2004a Jan 30;355(3):161-4. doi: 10.1016/j.neulet.2003.10.060. PMID: 14732456.
50. Amantea D, Bowery NG. Reduced inhibitory action of a GABAB receptor agonist on [3H]-dopamine release from rat ventral tegmental area in vitro after chronic nicotine administration. BMC Pharmacol. 2004b Oct 20;4:24. doi: 10.1186/1471-2210-4-24. PMID: 15494079; PMCID: PMC526276.
51. Smith CG, Bowery NG, Whitehead KJ. GABA transporter type 1 (GAT-1) uptake inhibition reduces stimulated aspartate and glutamate release in the dorsal spinal cord in vivo via different GABAergic mechanisms. Neuropharmacology. 2007

Dec;53(8):975-81. doi: 10.1016/j.neuropharm.2007.09.008. Epub 2007 Sep 29. PMID: 17981306.

52. Dang K, Bowery NG, Urban L. Interaction of gamma-aminobutyric acid receptor type B receptors and calcium channels in nociceptive transmission studied in the mouse hemisected spinal cord in vitro: withdrawal symptoms related to baclofen treatment. Neurosci Lett. 2004 May 6;361(1-3):72-5. doi: 10.1016/j.neulet.2003.12.009. PMID: 15135896.
53. Meza-Toledo SE, Bowery NG. Reversal of GABA-mediated inhibition of the electrically and potassium chloride evoked [3H]-GABA release from rat substantia nigra slices by DL-3-hydroxy-3-phenyl pentanamide. Arzneimittelforschung. 2008;58(2):53-61. doi: 10.1055/s-0031-1296469. PMID: 18412018.
54. Enna SJ, Bowery NG. GABA(B) receptor alterations as indicators of physiological and pharmacological function. Biochem Pharmacol. 2004 Oct 15;68(8):1541-8. doi: 10.1016/j.bcp.2004.06.037. PMID: 15451397.
55. Bowery NG. GABAB receptor: a site of therapeutic benefit. Curr Opin Pharmacol. 2006 Feb;6(1):37-43. doi: 10.1016/j.coph.2005.10.002. Epub 2005 Dec 19. PMID: 16361115.
56. Bowery NG. Historical perspective and emergence of the GABAB receptor. Adv Pharmacol. 2010;58:1-18. doi: 10.1016/S1054-3589(10)58001-3. PMID: 20655476.
57. Bowery NG. A Brief History of the GABA B Receptor. 2016 In Colombo (Eds.) GAGAB Receptor (2nd Ed., pp. 1-13). Humana Press, Springer Nature
58. Sheilabi MA, Battacharyya D, Caetano L, Thom M, Reuber M, Duncan JS, Princivalle AP. Quantitative expression and localization of $GABA_B$ receptor protein subunits in hippocampi from patients with refractory temporal lobe epilepsy. Neuropharmacology. 2018 Jul 1;136(Pt A):117-128. doi: 10.1016/j.neuropharm.2017.08.001. Epub 2017 Aug 3. PMID: 28782512.