Electrophysiology

GABAA Receptors

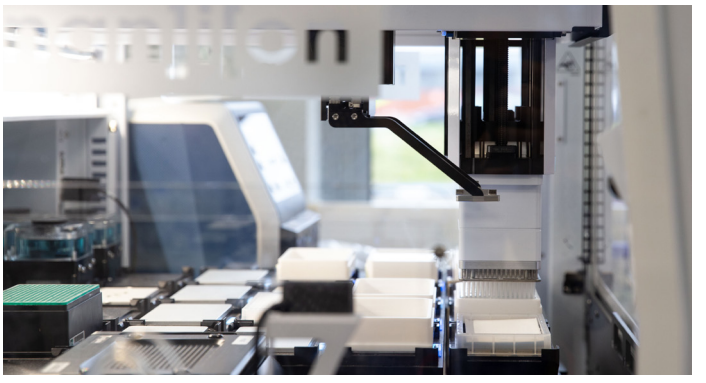
SB DRUG DISCOVERY
A SYGNATURE DISCOVERY BUSINESS

The majority of inhibitory neurotransmission in the brain is mediated by the binding of γ-aminobutyric acid (GABA) to the $GABA_A$ receptor. These receptors mainly consist of two α subunits, two β subunits, and either a γ or δ subunit with the varying subunit compositions determining the biophysical and pharmacological properties of the receptor. Dysregulation of GABAergic neurotransmission is associated with many neurological diseases and psychiatric disorders, making these receptors attractive targets for drug discovery programs.

Developments in automated patch clamp technologies have allowed for rapid testing of large numbers of compounds against ion channel targets, however until now, the lack of an expansive panel of $GABA_A$ cell lines has limited the identification and characterization of subtype-selective $GABA_A$ compounds. To help overcome this, SB has developed a $GABA_A$ receptor discovery platform comprising a comprehensive panel of 20 human $GABA_A$ recombinant cell lines stably expressing each α subunit in combination with one of three β subunits and γ2L. In addition this panel also includes the extra-synaptic α4β3δ and α6β3δ receptor subtypes, creating opportunities to improve subtype-specific drug efficacy, safety and tolerability.

$GABA_A$ Receptor Discovery Platform

Utilizing the 384-well SyncroPatch (Nanion Technologies), SB has designed a comprehensive suite of flexible electrophysiology assays capable of assessing agonists, modulators and inhibitors of $GABA_A$ receptors. This rapid, high-throughput assay format has been rigorously optimized to ensure consistent high quality data generation, supporting you throughout your discovery campaign.



Methods

HEK-293 cells stably expressing $GABA_A$ subunit combinations were generated at SB Drug Discovery. Whole-cell patch-clamp experiments were carried out at room temperature using multi-hole chips on the SyncroPatch automated electrophysiology platform. To monitor currents, a steady state voltage pulse of -80 mV was applied in combination with the stacked addition method for rapid compound application and removal.

Using these optimized conditions and proprietary processes, GABA elicits a reproducible, concentration-dependent activation of the $GABA_A$ receptor, with consistently comparable potencies shown across all receptor subtypes (Figure 1).

Figure 1: Effect of agonist GABA on GABAA α1β2γ2L subtype

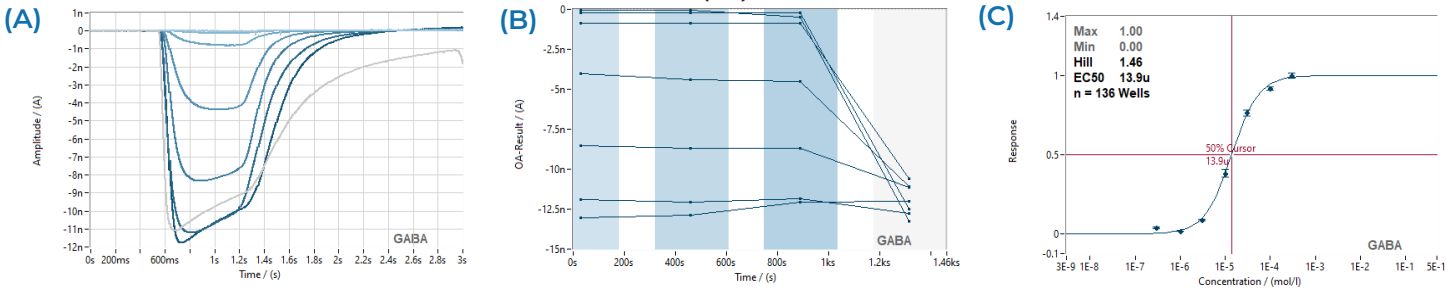


Figure 1: Example of current traces (A), time-courses (B) and EC50 plots (C) for the GABAA α1β2γ2L cell line in the presence of increasing concentrations of agonist GABA (0.3-300 μM; blue), followed by application of a saturating concentration of GABA (1 mM; grey). The normalised concentration-response curve shows a calculated value of approximately 14 μM. Mean ± S.E.M is shown (n≥3 cells per concentration).

Positive Allosteric Modulator Studies

In positive allosteric modulator (PAM) mode, receptor activity is initially measured in the presences of GABA (EC_{10}). GABA alone is applied three times to verify the reproducibility of the response. After pre-incubation of the compound, re-application of agonist (GABA EC_{10}) in the presence of the compound enables measurement of modulatory activity. This is followed by an application of a saturating concentration of agonist (GABA EC_{100}) to elicit maximal response. Using this approach, the effect of test compound can be calculated as a fold increase over GABA alone or normalised to maximum GABA response, enabling assessment of potency, efficacy and kinetics (Figure 2).

Figure 2: Effect of neurosteroid Allopregnanolone on GABAA α1β2γ2L subtype

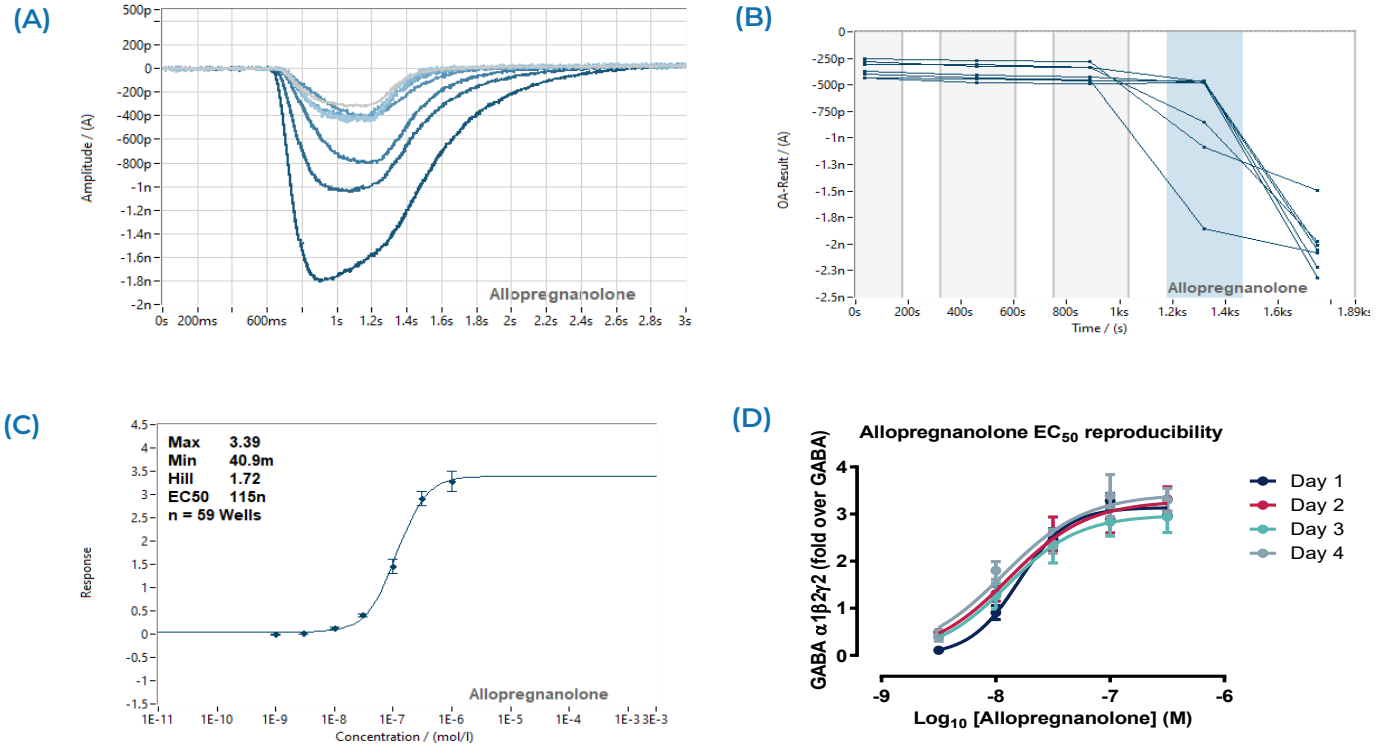


Figure 2: Example of current traces (A) and corresponding time-courses plots (B) for the GABAA α1β2γ2L cell line in the presence of increasing concentrations of neurosteroid Allopregnanolone (0.01-1 μM; blue), followed by application of a saturating concentration of agonist GABA (1 mM; grey). The concentration-response curve of Allopregnanolone is shown (C) as fold increase above GABA alone with a calculated EC_{50} value of approximately 115 nM. Mean ± S.E.M is shown (n≥3 cells per concentration). Superimposed concentration-response curves of Allopregnanolone (D) verify robust day-to-day reproducibility with EC_{50} values within 2-fold.

SB has used its GABAA receptor discovery platform to characterize a range of GABAA receptor subtype-selective compounds, including α2/3 selective anxiolytics and compounds selectively modulating α5, α6 and δ-subunits (Figure 3).

Figure 3: Effect of selective PAMs against GABAA alpha subtypes

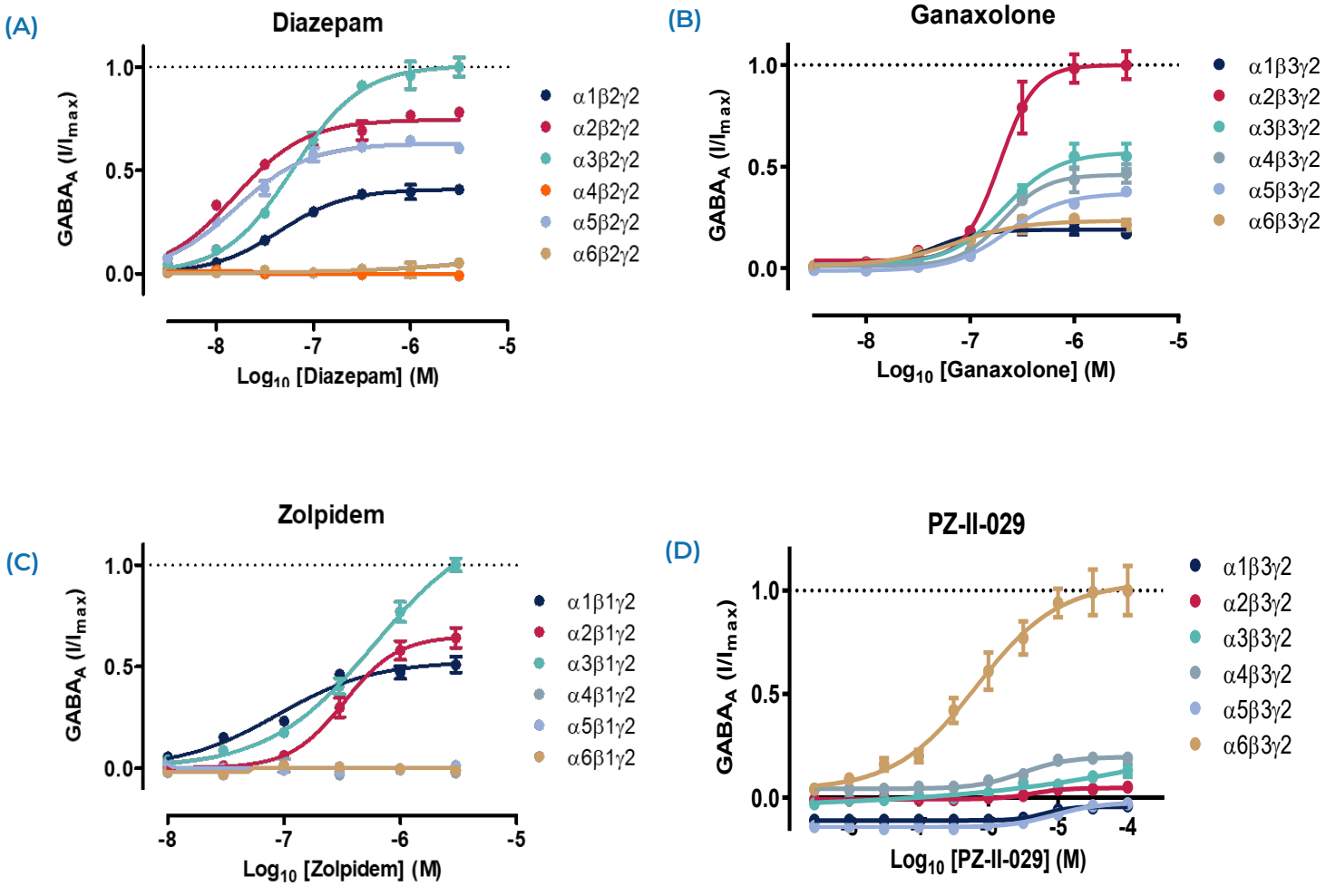


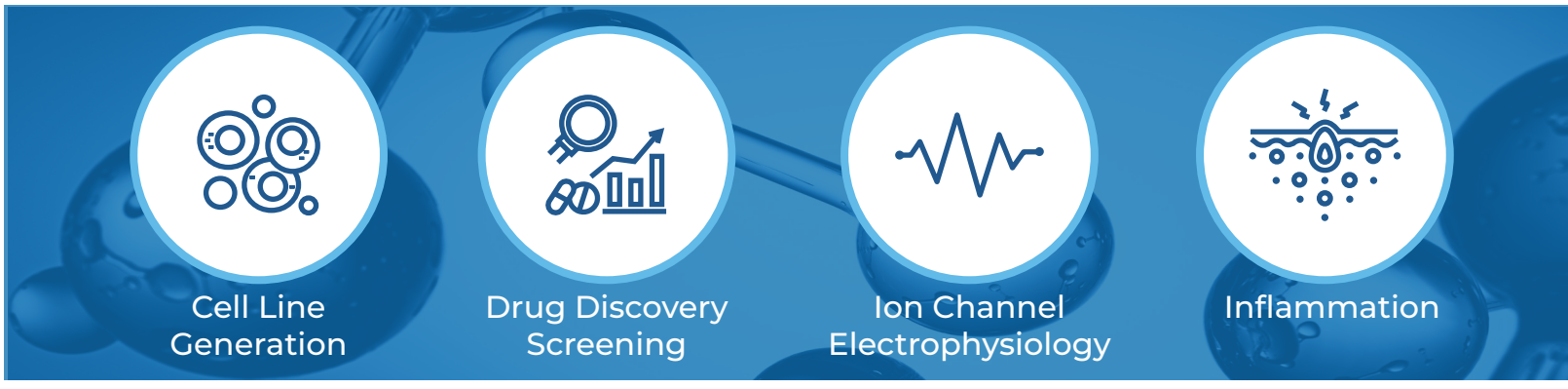
Figure 3: Normalised concentration response curves of of benzodiapine Diazepam (A), neurosteroid Ganaxolone (B), sedative-hypnotic Zolpidem (C) and null benzodiazepine agonist PZ-II-029 (D) are shown. The plots have been normalised to highlight the maximum response observed against any of the alpha subunits (α1- α6).

The advent of 384-well automated electrophysiology platforms has significantly increased the possibilities for $GABA_A$ receptor drug discovery, generating a wealth of high quality electrophysiology data and guiding researchers on compound potency and subtype selection. In combination with robust, high quality cell line reagents, we have the potential to speed-up the search for lead candidates in the $GABA_A$ receptor drug discovery process and, for the first time, offer the possibility to quickly assess the molecular mechanisms of these potential drug candidates. In the future, the aim is to achieve more selective $GABA_A$ receptor-targeted drugs in order to improve their efficacy, safety and tolerability for a range of therapeutic purposes. As our understanding of $GABA_A$ receptor subunit composition and molecular pharmacology improves, there is great hope for the discovery of more effective CNS therapeutics.

SB Drug Discovery's Commitment to Ion Channel Research

SB Drug Discovery is proud to play a role in these discoveries through our comprehensive suite of $GABA_A$ receptor tools and years of experience. With state-of-the-art automated patch clamp, high-throughput screening and lead optimisation capabilities, SB Drug Discovery provides the ideal platform for in-depth analysis of $GABA_A$ receptor modulation.

SB Drug Discovery's expertise and cutting-edge resources make us the perfect partner for collaborative research on $GABA_A$ receptor modulation.Reach out to us today to explore the exciting possibilities of working together to advance your research goals in this promising field.



SB Drug Discovery
Telford Pavilion, Todd Campus
West of Scotland Science Park
Glasgow
G20 0XA
United Kingdom

T: +44 (0)141 587 6100
enquiries@sbdrugdiscovery.com
sbdrugdiscovery.com

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