GABA$_A$ receptor activation in the allosteric coagonist model framework: relationship between EC$_{50}$ and basal activity

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Running title: Basal activity and activation of the GABA$_A$ receptor

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Changeux; P{open}, probability of being open.

ABSTRACT

The concerted transition model for multimeric proteins is a simple formulation for analyzing the behavior of transmitter-gated ion channels. Here, we use the model to examine the relationship between the EC_{50} for activation of the $GABA_A$ receptor by the transmitter GABA and basal activity employing concatemeric ternary $GABA_A$ receptors expressed in *Xenopus* oocytes. The basal activity reflects receptor function in the absence of transmitter, and can be changed either by mutation to increase constitutive activity, or by addition of a second agonist (acting at a different site) to increase background activity. The model predicts that either mechanism for producing a change in basal activity will result in identical effects on the EC_{50}. We examined receptor activation by GABA while changing the level of basal activity with the allosterically-acting anesthetics propofol, pentobarbital or alfaxalone, and found that the relationship between EC_{50} and basal activity was well-described by the concerted transition model. Changes in the basal activity by gain-of-function mutations also resulted in predictable changes in the EC_{50}. Finally, we altered the number of GABA-binding sites by a mutation, and again found that the relationship could be well-described by the model. Overall, the results support the idea that interactions between the transmitter GABA and the allosteric agonists propofol, pentobarbital or alfaxalone can be understood as reflecting additive and independent free energy changes, without assuming any specific interactions.

INTRODUCTION

The concerted transition model for multimeric proteins introduced by Monod, Wyman and Changeux (the "MWC model"; (Monod et al., 1965)) is an elegantly simple formulation for understanding and analyzing the behavior of transmitter-gated ion channels (Del Castillo and Katz, 1957; Karlin, 1967). The model posits that a protein exists in two interconvertible states, active and inactive. It has one or more sets of drug-binding sites. Each site in a set is equivalent to the other sites in that set, while the properties of the sites differ between the states. The model requires only 4 parameters to describe the macroscopic activation of a receptor.

On the face of it such a simple model seems unlikely to be able to account for the behavior of transmitter-gated channels. However, the behavior of ion channels in the pentameric transmitter-gated ion channel family is rather well described by this model (Auerbach, 2012; Chang and Weiss, 1999; Changeux and Edelstein, 1998; Ehlert, 2014b; Forman, 2012; Gupta et al., 2017; Jackson, 1986; Jackson, 1989; Karlin, 1967). For the γ-aminobutyric acid type A (GABA$_A$) receptor, this area of study was pioneered by Chang & Weiss (Chang and Weiss, 1999), who demonstrated that the macroscopic properties of activation of GABA$_A$ receptors by the transmitter could be well described by the MWC model. Further work by Forman and collaborators (Ruesch et al., 2012; Rusch and Forman, 2005; Rusch et al., 2004) demonstrated that the actions of several potentiating drugs on the GABA$_A$ receptor could be understood in terms of this model without additional, *ad hoc*, interactions.

We examined the application of the concerted transition model to the behavior of the GABA$_A$ receptor, in terms of the ability of allosteric agents to potentiate activation by GABA. As emphasized by Forman (Ruesch et al., 2012) the ability of a drug to potentiate can be described in

terms of its ability to decrease the concentration of agonist required to elicit a half-maximal

response (EC_{50}), as well as its ability to increase the response to a given concentration of the

agonist. We used examples of 3 types of intravenous anesthetics as potentiator (propofol, the

barbiturate pentobarbital and the anesthetic steroid alfaxalone), to determine whether the model

accurately predicts the shift in EC_{50} for GABA. We also altered the intrinsic probability of being

open for the receptor and the number of binding sites for GABA by gain-of-function mutations.

Our results indicate that the model is remarkably successful at accounting for the ability of

allosteric agonists or receptor mutations to alter activation by the transmitter.

In particular, the results suggest that the actions of these combinations of agonists do not

require any specific interactions between the agents (e.g., changes in affinity) but reflect coupling

via additive changes in the free energy as the result of interactions between the receptor and the

agonists. In more practical terms, the change in EC_{50} value for agonist A produced by any

concentration of agonist B can be predicted from the direct response to B and the parameters

describing activation by A in the absence of other activating agents. Similarly, the effects of

mutations that alter constitutive activity of the receptor or the number of sites for an agonist can be

predicted.

MATERIALS AND METHODS

Constructs and expression

The experiments were conducted on rat $\alpha 1\beta 2\gamma 2L$ GABA$_A$ receptors formed by assembly of

two concatemeric constructs comprising $\beta 2$-$\alpha 1$-$\gamma 2L$ (abbreviated as $\beta\alpha\gamma$) and $\beta 2$-$\alpha 1$ ($\beta\alpha$) subunits.

To simplify the presentation, we will refer to "wild-type" $\beta\alpha\gamma$+$\beta\alpha$ receptors when the concatemers

had no mutations present, even though the receptors *per se* are clearly not "wild-type". The use of

concatemeric receptors enabled generation of receptors containing controlled numbers and

positions of mutations. The generation and functional characterization of these constructs have

been reported previously (Bracamontes et al., 2011; Bracamontes and Steinbach, 2009). The

functional properties of the wild-type receptors comprising concatemeric constructs and free

subunits are quite similar even at the single-channel level (Akk et al., 2009), although most studies

have indicated a ~2-fold right-shift in the GABA concentration-response relationships (Akk et al.,

2009; Baumann et al., 2002; Bracamontes et al., 2011). The reason for right-shifted concentration-

response relationship is not entirely clear but a previous single-channel study found that subunit

linkage can affect receptor affinity to the transmitter (Akk et al., 2009). The concatemers are not

degraded when expressed in *Xenopus* oocytes (Bracamontes et al., 2011). Furthermore, receptors

activated by 5 μM GABA (approximately EC$_7$) were potentiated to $308 \pm 32\%$ (n = 6) of control in

the presence of 1 μM diazepam indicating that the $\gamma 2L$ subunit was incorporated, and introduction

of reporter mutations into the $\alpha 1$ subunit (see Results) indicated that the assembled receptors

incorporate both concatemers. Point mutations ($\alpha 1$(L263S), $\beta 2$(Y205S), $\beta 2$(Y143W)) were

generated using QuikChange (Agilent Technologies, Santa Clara, CA). The cDNAs were

subcloned into the pcDNA3 vector in the T7 orientation. The cRNAs were produced using

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mMessage mMachine (Ambion, Austin, TX) after linearization by digestion with Xba I (NEB

Labs, Ipswich, MA).

Oocytes from the African clawed frog (*Xenopus laevis*) were used for expression of the

receptors. Frogs were purchased from Xenopus 1 (Dexter, MI), and were housed and cared for in a

Washington University Animal Care Facility under the supervision of the Washington University

Division of Comparative Medicine. Harvesting of oocytes was conducted in accordance with the

Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National

Institutes of Health. The protocol is approved by the Animal Studies Committee of Washington

University in St. Louis (Approval No. 20170071).

Oocytes were injected with a total of 20 ng cRNA in a final volume of 30-70 nl of nuclease-

free water (Thermo Fisher Scientific, Waltham, MA) at the ratio of 1:1 ($\beta\alpha\gamma$:$\beta\alpha$). After injection,

the oocytes were incubated in ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM

$MgCl_2$, 2.5 mM Na pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml gentamycin,

5 mM HEPES; pH 7.4) at 16 ^{o}C, and used in electrophysiological recordings within 24-72 hrs.

Electrophysiology

The experiments were conducted using standard two-electrode voltage clamp. Voltage and

current electrodes were borosilicate glass capillaries (G120F-4, OD=1.20 mm, ID=0.69 mm,

Warner Instruments, Hamden, CT). When filled with 3 M KCl the pipette resistance was less than

1 MΩ. The oocytes were clamped at -60 mV. The chamber (RC-1Z, Warner Instruments) was

perfused with bath solution (92.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES; pH 7.4)

at approximately 8 ml min^{-1}. Solutions were gravity-applied from 30-ml glass syringes with glass

Luer slips via Teflon tubing to reduce adsorption. The applications of agonists were typically 20-60 s long, followed by washout in bath solution (up to 5 min) until recovery. Solutions were switched manually. Test applications, e.g., to a saturating concentration of GABA, were done at the beginning and end of recordings from each cell to verify overall stability of responses.

Currents were amplified with an OC-725C (Warner Instruments) or Axoclamp 900A amplifier (Molecular Devices, Sunnyvale, CA), filtered at 40 Hz, digitized with a Digidata 1200 or 1320 series digitizer (Molecular Devices) at 100 Hz, and recorded using pClamp software (Molecular Devices). Current traces were analyzed with Clampfit (Molecular Devices) to determine the peak amplitude of the response measured from the preceding baseline current.

Analysis of current responses

The analysis of electrophysiological recordings was aimed at determining the concentration-response properties and the level of basal activity for individual receptor-agonist combinations.

Two principal types of concentration-response experiments were conducted. In the first type, oocytes were exposed to a range of concentrations of GABA. The concentration-response curves were fitted, for each cell separately, with the following equation:

$$Y = Y_{max} \times \frac{[GABA]^{n_H}}{[GABA]^{n_H} + EC_{50}{}^{n_H}}$$

Eqn. 1

where EC_{50} is the concentration of GABA producing a half-maximal effect, n_H is the Hill slope, and Y_{max} is the high-concentration asymptote.

In the second case, oocytes were exposed to a range of concentrations of GABA in the presence of a fixed, low concentration of a secondary activator termed the "background" drug (propofol, alfaxalone, or pentobarbital). The concentration-response curves were fitted with the

following equation:

$$Y = Y_{min} + (Y_{max} - Y_{min}) \times \frac{[GABA]^{n_H}}{[GABA]^{n_H} + EC_{50}{}^{n_H}} \qquad \text{Eqn. 2}$$

where EC_{50} is the concentration of GABA producing a half-maximal effect, n_H is the Hill slope, and Y_{min} and Y_{max} are the low- and high-concentration asymptotes, respectively. The fitted Y_{min} was typically indistinguishable from the response to the background activator.

Curve-fitting was conducted using the NFIT software (The University of Texas Medical Branch at Galveston, Galveston, TX). The results are reported as mean ± S.E.M. (number of cells).

Converting membrane current to probability of being open

We adopted the procedures developed by Forman (Eaton et al., 2016; Forman, 2012; Forman and Stewart, 2012) to establish a scale for converting current responses to an estimated probability of being open (P{open}). The maximal response possible from a given cell (I_{max}) was estimated by applying a saturating concentration of GABA plus a potentiator that gave the largest measured response. In the present experiments this was accomplished using 3 mM GABA plus 100 µM pentobarbital. This response was assumed to be equivalent to a P{open} of 1 (Ziemba and Forman, 2016). The probability of being open in the absence of agonist was estimated by blocking any constitutive current using picrotoxin (300 µM for the wild-type concatemeric receptor or 500 µM for receptors containing the α1(L263S) mutation). The current level in the presence of picrotoxin was assumed to correspond to a P{open} of 0.

The GABA concentration-response relationship in the wild-type βαγ+βα receptor was converted to units of P{open} by normalization to the response to 3 mM GABA in that cell, and scaling by the estimated P{open} for the response to 3 mM GABA:

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$$P\{open,[GABA]\}=\frac{I_{GABA}-I_{PTX}}{I_{3000G}-I_{PTX}}\times\frac{I_{3000G}-I_{PTX}}{I_{max}-I_{PTX}} \qquad \text{Eqn. 3}$$

Here, P{open, [GABA]} is the probability of being open in the presence of a given concentration of GABA, I_{GABA} is the current response to that concentration of GABA, I_{3000G} is the current response to 3 mM GABA, and I_{PTX} is the amplitude of the outward current in the presence of picrotoxin. The term $(I_{3000G} - I_{PTX})/(I_{max} - I_{PTX})$, i.e., the scaling factor providing the estimated P{open} for the response to 3 mM GABA, was determined separately in 5 cells.

Materials, drugs and solutions

Most chemicals, including GABA, pentobarbital and the salts used to prepare buffers were purchased from Sigma-Aldrich (St. Louis, MO). Propofol was from MP Biomedicals (Solon, OH), and alphaxalone from Sigma-Aldrich or Tocris (Bio-Techne, Minneapolis, MN).

Stock solutions of GABA (500 mM) and pentobarbital (5 mM) were made in bath solution. Stock solutions of propofol (200 mM) and alphaxalone (10 mM) were made in DMSO. Aliquots of GABA stock were kept frozen at -20°C, and thawed and diluted as needed on the day of the experiment. Stock solutions of pentobarbital, propofol, and alphaxalone were stored at room temperature.

The maximal DMSO concentration in final working solutions was 0.5%. This concentration of DMSO is without effect on holding current or peak amplitude of the response to an EC_{50} concentration of GABA from oocytes expressing α1β3γ2L receptors (Germann et al., 2016).

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RESULTS

Description of the model

The application of the concerted transition model to the $GABA_A$ receptor has been described

previously (Chang and Weiss, 1999; Forman, 2012). We utilized this model (Fig. 1) to explore the

relationship between the concentration of GABA producing a half-maximal response (EC_{50}), the

gating equilibrium constant for unliganded receptors, L, and the number of binding sites for the

transmitter, N.

The state function for the receptor, i.e., the probability that a receptor is in the open state or on

a macroscopic level the fraction of receptors that are in the open state, is given by the following

equation:

$$P\{open\} = \frac{1}{1+L\times\left[\dfrac{1+[A]/K_A}{1+[A]/c_A K_A}\right]^{N_A}} \qquad \text{Eqn. 4}$$

where A is an agonist, K_A is the equilibrium dissociation constant for the agonist when the receptor

is in the closed state, and N_A is the number of sites for A. The parameter L is the ratio of the

fraction of channels in the closed state to the fraction of channels in the open state in the absence

of agonist, and reflects the difference in the free energies of the closed and open states in the

absence of agonist. A small value for L indicates that the channels activate readily even in the

absence of agonist. The parameter c_A is the ratio of the equilibrium dissociation constants for

binding in the open state to that in the closed state, reflecting the selectivity of the agonist between

the two states. Agonists, by definition, bind more tightly to the active state of the receptor; hence

the value for c_A is < 1. In the absence of agonist the probability of being open is P{open, min} =

1/(1+L), while the maximal probability of being active in the presence of a saturating

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concentration of A is $P\{open, max\}=1/(1+Lc_A^{N_A})$. The "stabilization energy" contributed by the

binding of agonist when all sites are occupied is $N_A RT \times ln(c_A)$, whereas the free energy difference

between the fully-liganded closed and fully-liganded open states is $N_A RT \times ln(Lc_A)$.

Interactions among agonists are mediated through change in L

If a second activator (B) is present that does not bind to the same site as A, Eqn. 4 becomes:

$$P\{open\}=\cfrac{1}{1+L\times\left[\cfrac{1+[A]/K_A}{1+[A]/c_A K_A}\right]^{N_A}\left[\cfrac{1+[B]/K_B}{1+[B]/c_B K_B}\right]^{N_B}} \qquad \text{Eqn. 5}$$

where K_B is the dissociation constant for agonist B binding to its site when the receptor is in the

closed state, c_B is the ratio of the dissociation constants for binding of B in the open to that in the

closed state, N_B is the number of sites for B, and other terms are as defined earlier. The case in

which both compounds (A and B) bind to the same site is considered by Karlin (Karlin, 1967).

Inspection of Eqn. 5 indicates that in the presence of a constant concentration of agonist B the

effects of B on responses to agonist A can be understood simply as a change in the apparent value

of L:

$$L^*=L\times\left[\cfrac{1+[B]/K_B}{1+[B]/c_B K_B}\right]^{N_B} \qquad \text{Eqn. 6}$$

where L* is the modified L reflecting a change due to the presence of agonist B. This equation

explains the relationship between activation by one compound and that compound's ability to

potentiate responses to another. This relationship has been investigated and supported for the

GABA$_A$ receptor in a series of papers by Forman and collaborators (Ruesch et al., 2012; Rusch et

al., 2004).

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The relationship between the EC_{50} for an agonist and L

The EC_{50} is the concentration of agonist that produces a response half-way between the baseline current in the absence of the agonist and the maximal response the agonist can generate. In the MWC model, the EC_{50} for activation of a receptor by an agonist normalized to its affinity to the compound is given by (Karlin, 1967):

$$EC_{50}/(cK)=\frac{(2+L+Lc^N)^{1/N}-(1+c^N+2Lc^N)^{1/N}}{(1+c^N+2Lc^N)^{1/N}-c(2+L+Lc^N)^{1/N}} \qquad \text{Eqn. 7}$$

The relationship between EC_{50} and L for values of N of 1, 2 and 5 (with $c = 0.01$) is shown in Fig. 2A. The predictions cover a large range of values for L, to illustrate that the value for EC_{50} shows asymptotes at both low and high values of L. Chang and Weiss (Chang and Weiss, 1999) were the first to demonstrate experimentally for $GABA_A$ receptors that the relationship shows an asymptote when $L < 1$. Edelstein and Changeux (Edelstein and Changeux, 1996) provided expressions for the values of these asymptotes. As L approaches 0, EC_{50} approaches $cK(2^{1/N}-1)$, and as L approaches infinity, EC_{50} approaches $K/(2^{1/N}-1)$. Inspection of the relationship in Fig. 2A indicates that the curvature towards the asymptotes becomes marked when $L < 1$ and $L > 1/c^N$. When $1/c^N$ is much larger than 1, the asymptotes are well separated and the slope of the relationship between $\log(EC_{50})$ and $\log(L)$ approaches 1/N, as first pointed out by Karlin (Karlin, 1967). The approximation is good over a particularly wide range for $N = 2$.

Figure 2B shows plots of the predicted EC_{50} against L for N values of 1, 2 and 5, to indicate the nature of the relationships that might be experimentally obtained for an intermediate value of c ($c = 0.01$) and values of L from 1 to 100,000. Figures 2C and 2D shows similar plots for N values

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of 2 and 5, now calculated with 3 values for c that cover most of the range of values reported for

agonists acting on the $GABA_A$ receptor (c = 0.1, 0.01 and 0.001). In these plots the slopes of the

predicted relationships are shown, estimated from the linear regression of $\log(EC_{50})$ on $\log(L)$ over

the range of values of L where the relationship appeared linear (indicated in the figures). The fitted

slope is larger than 1/N when N is greater than 1 and the deviation increases as N increases. The

reason for this difference can be seen in Fig. 2A and arises because the predicted values for the

EC_{50} initially lie below the line indicating $L^{1/N}$, so the slope for L values not that much larger than

1 is actually greater than 1/N.

The logarithmic regression slopes fitted to the predicted values are shown in Table 1. The

slopes were fitted over the linear portion of the relationship (indicated in the table) and

demonstrate that the 1/N relationship is most accurate in the case that N = 2, but in general for N >

1 the actual slopes are larger than 1/N.

The values of L for wild-type $GABA_A$ receptors reported in the literature are in the range of

1000 to 100,000 (Chang and Weiss, 1999; Ziemba and Forman, 2016), and for receptors with gain-

of-function mutations L can be less than 1 (Chang and Weiss, 1999). The reported values of c

range from about 0.001 for a strong agonist such as GABA (see below) to about 0.5 for a weaker

agonist such as diazepam (Chang and Weiss, 1999; Rusch and Forman, 2005; Rusch et al., 2004).

Summary

In summary, there are 2 major points from this introduction.

(1) Interactions between activators in the context of the concerted transition model (Fig. 1) can be

understood as resulting from modification of L. An important caveat is that the compounds do not

bind to the same site(s) on the receptor.

(2) For a given value of L, the EC_{50} value, and in fact the whole concentration-response
relationship, for agonist X can be predicted given values for c_X, K_X and N_X.

Relating the model to experimental data: activation of the wild-type βαγ+βα receptor

Oocytes expressing wild-type βαγ+βα $GABA_A$ receptors were exposed to 1-1000 μM GABA.
The concentration-response curves were fitted to Eqn. 1. The mean values of the fit were:
$EC_{50,GABA} = 34 \pm 8$ μM (n = 5 cells) and $n_{H,GABA} = 1.38 \pm 0.07$ (Fig. 3A).

The peak response in the presence of 3 mM GABA plus 100 μM pentobarbital was 1.08 ± 0.02
times the response to 3 mM GABA alone (n = 7, P = 0.01 that the responses are equal), indicating
that P{open, GABA max} = 0.93 ± 0.02. This estimate is in agreement with previous P{open,
GABA max} estimates in single-channel and whole-cell studies (Hernandez et al., 2017; Lema and
Auerbach, 2006; Ruesch et al., 2012; Steinbach and Akk, 2001). Application of 300 μM picrotoxin
resulted in outward current, reflecting block of constitutively active receptors (Fig. 3B). We
estimated L for the wild-type βαγ+βα receptor from the relationship (1- P{open, constitutive})/
P{open, constitutive} yielding 8101 ± 1238 (n = 15). This value is close to the geometric middle of
the range of estimates for L (1000 to 100,000) for $GABA_A$ receptors composed of free wild-type
subunits (Chang and Weiss, 1999; Ziemba and Forman, 2016). We assumed for the rest of this
analysis that the best estimate for the value of L was close to the directly measured value and set
$L_{WT} = 9000$ for receptors composed of wild-type concatemers in the absence of agonist.

The concentration-response relationship for membrane currents was converted into units of
P{open} (Fig. 3C) as described in the Methods. From fitting the curve to Eqn. 4, with L_{WT} set to

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9000 and N_{GABA} to 2, we obtained a K_{GABA} of 72 µM and c_{GABA} of 0.0033. The fitted efficacy

measure indicates that GABA binds 300-fold (c^{-1}) more tightly to the open than the closed

receptor, and that the binding of a GABA molecule contributes -3.37 kcal/mol of free energy to

stabilization of the open state.

Changing L by allosteric potentiators or gain-of-function mutations alters the EC_{50} for GABA

in a predictable way

We examined the relationship between the EC_{50} for GABA and the value for L. We altered L

by addition of allosteric drugs propofol, alfaxalone and pentobarbital. Fig. 4A shows the log-log

plot of measured EC_{50} for GABA against the L value for wild-type βαγ+βα receptors for activity

elicited by GABA in the absence or presence of the 3 allosteric agonists: propofol at

concentrations of 5, 10 and 20 µM, pentobarbital at concentrations of 100 and 200 µM, and

alfaxalone at 1 µM. The presence of the allosteric agonists shifts the value for L up to 1000-fold

from the value for L in the absence of drugs. The corresponding shift in the observed EC_{50} for

GABA is well-described by the measured change in L coupled with the parameters estimated for

activation by GABA (c_{GABA} and K_{GABA}) in the absence of any drugs. That is, no information about

the properties of the combination of drugs was used in predicting the relationship. The slope of a

linear regression of $\log(EC_{50})$ on $\log(L)$ is 0.63 ± 0.05 (mean $\pm$ S.E.M). This is greater than

$1/N_{GABA}$ (1/2), but not significantly so (P = 0.06 by a two-tailed t-test).

We also altered L by mutation of the receptor. Figure 4B shows data for receptors containing

the α1(L263S) and/or β2(Y143W) mutation. Both mutations have been reported to enhance

constitutive activity in the $GABA_A$ receptor without altering K_{GABA} (Chang et al., 1996; Chang and

Weiss, 1999; Eaton et al., 2016). Introduction of the mutation in either construct shifted the value for L, indicating that both concatemers incorporated in the receptor. The shifts in EC_{50} with the different L values are close to the predicted values, and scatter around the regression for the data for the relationship between EC_{50} and L seen in Fig. 4A. Furthermore, the shift in GABA EC_{50} for a mutant receptor (β(Y143W)$\alpha\gamma$+$\beta\alpha$) in the presence of a background drug (propofol) is close to the predicted value. The slope of a linear regression log (EC_{50}) on log (L) is 0.60 ± 0.11, that did not differ from the slope for wild-type $\beta\alpha\gamma$+$\beta\alpha$ receptors shown in Fig. 4A. Accordingly, changes in basal activity by mutation or addition of a background agonist can have similar effects on the EC_{50} for activation by GABA.

Changing N_{GABA}: effect of the β2(Y205S) mutation on activation by GABA

Previous work has found that the β2(Y205S) mutation greatly increases $EC_{50,GABA}$ (Amin and Weiss, 1993; Baumann et al., 2003), effectively removing the GABA-binding site. If placed in a single β2 subunit it should therefore reduce the value of N_{GABA} from 2 to 1. We expressed receptors composed of the $\beta\alpha\gamma$ concatemer and the β(Y205S)α concatemer, and determined values for the EC_{50} for GABA in the absence and presence of propofol or the α(L263S) mutation. The results are shown in Fig. 5. The solid line shows the predicted values for the EC_{50} assuming that values obtained for the wild-type receptor apply ($L_{WT} = 9000$, $K_{GABA} = 72$ μM and $c_{GABA} = 0.0033$), while $N_{GABA} = 1$. The predicted curve, with no free parameters, goes remarkably close to the data. The predictions clearly demonstrate that the consequences of a reduced value for N are apparent in the relationship between EC_{50} and L. Not only is the degree of curvature altered over this range of values for L but the slope for $L < 500$ is increased when N is decreased. The linear

regression for L < 500, shown by the dashed line in Fig. 5, has a slope of 0.74 ± 0.1. The predicted

results for the mutated receptor show the asymptotic saturation at high L, supported by the

experimental data from the mutant receptors.

Quality of the description provided by the model

The predictions in the preceding experimental section were made using parameters derived

from fitting the experimental concentration-response data for wild-type concatemeric receptors

activated by GABA (see Fig. 3). The experimental values for EC_{50}s for GABA were estimated

from fitting concentration-response curves. L was estimated from the response to the background

drug alone or from inhibition of constitutive activity by picrotoxin. The predictions were then

compared to the experimentally determined values for various combinations of drugs or mutations.

The agreement is qualitatively acceptable, considering that the predictions covered a range of

about 1000-fold in L and 100-fold in EC_{50}. Perhaps the ability to describe the altered relationship

between EC_{50} and L when the number of transmitter binding-sites is altered is most striking, as the

only parameter derived from data using the mutated subunit was L.

The first step in applying the model was to extract parameter estimates for activation of wild-

type receptors by GABA in the absence of other drugs. In fitting the concentration-response curves

we restricted the value for N to integer values, and treated N and L as constants with K and *c* as

fitting parameters. All of the predictions shown in the experimental section were generated with

parameters derived using $L_{WT} = 9000$ and $N_{GABA} = 2$ for the wild-type receptors.

To compare the abilities of different estimates to describe the concentration-response data we

also fit with values of $L_{WT} = 27,000$ and $L_{WT} = 3000$. These values are near the middle of the range

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of published values for wild-type α1β2γ2L GABA$_A$ receptors formed from free subunits (L = 1000 to 100,000 (Chang and Weiss, 1999; Ziemba and Forman, 2016)). N$_{GABA}$ values of 1 to 5 were used. To examine the quality of the description we computed the mean squared deviation (MSD) between the fit and the data. Figure 6 shows results for fits of the concentration-response data for GABA. As can be seen, fitting with different values for L$_{WT}$ gave similar MSD values. There is a minimum at N$_{GABA}$ = 2, although an F test on the variance ratios shows no or minor statistical significance.

It may be surprising that a wide range of values for L$_{WT}$ can be used to describe the data for activation of wild-type receptors. This is more understandable when the relationship between the concentration-response data and the model is examined: in the data the two asymptotes are P{open, basal} and P{open, max}. The model defines P{open, basal} as $1/(1 + L)$ and P{open, max} as $1/(1+Lc^N)$. When P{open, basal} is very low then both L and c are determined largely by P{open, max}, and for a given P{open, max} pairs of L and c are possible as long as $Lc^N = L'c'^N$. For example, fitting the wild-type data with Eqn. 4 for activation by GABA with N$_{GABA}$ = 2 and L$_{WT}$ = 27,000 gave a value for c_{GABA} of 0.0019 (P{open, max} = 0.91), while L$_{WT}$ = 9000 gave c_{GABA} = 0.0033 (0.91) and L$_{WT}$ = 3000 gave c_{GABA} = 0.0056 (0.91). These findings are in agreement with previous theoretical studies on the relationships between constitutive activity and MWC parameters (Ehlert, 2014a). Similarly, the value for N can vary. Again, if L is set then pairs of values for c and N are possible provided $c'^{N'} = c^N$. However, the value for N is additionally constrained by the shape of the concentration-response curve, as indicated by the fact that different values of N affect the MSD to a larger extent (Fig. 6).

Different values for L$_{WT}$ and N result in altered relationships between EC$_{50}$ and L. To assess the ability of the parameters to describe the relationship between EC$_{50}$ and L we predicted the EC$_{50}$

values for data for the data in Fig. 4A and 4B (wild-type receptors in the absence or presence of

background drugs and receptors containing mutations). The wild-type receptor concentration-

response data were fitted with Eqn. 4 using different assumed values for L_{WT} (3000, 9000, or

27,000) and N_{GABA} (1, 2, or 3) to obtain estimates for K_{GABA} and c_{GABA}. These parameters were

used to predict the relationship between $EC_{50,GABA}$ and L (Fig. 7). The differing values for L_{WT}

shifted the predicted values along the abscissa (Fig. 7A), whereas a change in N_{GABA} led to a

change in slope (Fig. 7B).

We then computed the error from the logarithms of the ratio of the experimental to the

predicted EC_{50} values, to reduce the consequences of the large range in observed EC_{50} values that

would skew the simple squared difference estimate. Figures 7C-D show the ratio $EC_{50,exp}$ / $EC_{50,pred}$

plotted logarithmically against L. The calculations indicate that an L_{WT} of 9000 and N_{GABA} of 2

provide predictions that are relatively close to the observed EC_{50}.

The results of a statistical analysis of the data are shown in Table 3. Inspection of the table

indicates that for wild-type βαγ+βα the closest description of those tested was obtained with L_{WT} =

9000 and N_{GABA} = 2, as also suggested by inspection of Fig. 7.

Overall these analyses indicate that it can be difficult to accurately estimate the activation

parameters from concentration-response curves alone, especially when basal activity is low.

However the relationship between EC_{50} and L can allow us to determine whether some values

provide better descriptions than others.

DISCUSSION

The results in this study indicate that the concerted transition model is able to accurately predict the relationship between the EC_{50} for activation by GABA and basal activity. The agreement is remarkable, considering that the parameters for activation by GABA were established by analysis of a single data set: the concentration-response relationship for GABA acting on wild-type $\beta\alpha\gamma+\beta\alpha$ receptors in the absence of any other GABAergic agent. We perturbed the system by altering L_{WT} by addition of a background drug or introducing a gain-of-function mutation. We altered N_{GABA} (the number of binding sites for GABA) by mutation. For all cases, the new relationship between EC_{50} and L was predictable by the model.

It is particularly interesting that predicting the effects of a combination of drugs (e.g., propofol as the background drug in combination with GABA) did not require any data from experiments involving the combination: the effect of the background drug was assessed from modified L measured in the absence of GABA while the activation parameters for GABA were obtained in the absence of the background drug. Removal of one GABA-binding site produced the expected changes in the relationship between EC_{50} and L for a change in N_{GABA} from 2 to 1, as would be expected from the ample data supporting the idea that the wild-type receptor has 2 transmitter binding sites (Amin and Weiss, 1993; Baumann et al., 2003).

The predicted $EC_{50, GABA}$ values for receptors containing the gain-of-function $\alpha1(L263S)$ or $\beta2(Y143W)$ mutations were calculated using the value for basal activity (L) from the mutant receptor and the estimate for affinity to the transmitter (K_{GABA}) in the wild-type receptor. Similarities in the predicted and experimental $EC_{50, GABA}$ indicated that the effects of the mutations could be accounted for by assuming that the mutations modified L with no effect on K_{GABA}. There

is no *a priori* reason to expect that a mutation that affects receptor activation by the transmitter acts

indirectly, solely through changes in basal activity. These two mutations were selected based on

prior reports that had indicated minimal effect on K_{GABA} (Chang and Weiss, 1999; Eaton et al.,

2016). Mutations that additionally modify K_{GABA} would be expected to exhibit deviations from the

predicted, model-based relationship between EC_{50} and L. Therefore, compliance with the predicted

relationship can serve as indicator of the mechanism of action for a mutation.

The $GABA_A$ receptor is a target for many drugs, including sedatives, tranquilizers and

anesthetics. These drugs can both directly activate the receptor and potentiate the responses to

GABA. It will be quite useful if our present results can be extended to more complex combinations

of drugs, since the use of the MWC model greatly simplifies the task of characterizing the

properties of combinations of drugs.

The particular kinetic model we used is a simplified version of a cyclic model, in which

thermodynamic cycles are closed. A cyclic model was first proposed to underlie aspects of

transmitter-gated channel function in the context of desensitization of nicotinic receptors (Katz and

Thesleff, 1957). The particular scheme we used simplified a cyclic model significantly. The basic

assumption is that the receptor exists in only two states: active (open channel) and inactive (closed

channel), in varying degrees of ligation. That is, there are no intervening states in which the rate

constants for one or another transition depend on the degree of ligation of the receptor (e.g.,

association of drug A in the absence of bound drug B versus in the presence of bound drug B).

This assumption reduces the number of free parameters greatly, as can be seen when the model is

compared to a full allosteric model (for example, the "binary elements" model used by

(Goldschen-Ohm et al., 2014). The two-state assumption also leads to the idea that the receptor

undergoes the gating transition as a unit – all subunits undergo a concerted transition since

otherwise a variety of combinations of subunits in various conformational states would ensue. The

second assumption is that all sites for a given ligand are identical. The concerted nature of the

activation necessitates that the sites change affinity (if they do at all) in a concerted fashion when

the receptor as a whole changes state between active and inactive.

These assumptions result in the kinetic scheme shown in Figure 1. Many alternative possible

schemes exist, as laid out initially by Koshland et al. (Koshland et al., 1966). In terms of full cyclic

schemes, MWC analyses have been applied to GABA$_A$ (Chang and Weiss, 1999; Ruesch et al.,

2012; Rusch et al., 2004) and muscle nicotinic (Auerbach, 2012; Jackson, 1989) receptors.

Partially cyclic schemes ("flip" and "prime") have been used for data from glycine (Burzomato et

al., 2004; Plested et al., 2007) and nicotinic receptors (Lape et al., 2008; Mukhtasimova et al.,

2016). Finally, a "binary element" analysis has been applied to studies of GABA$_A$ receptors, which

allows for all possible pairwise interactions between functional elements in a receptor (Goldschen-

Ohm et al., 2014).

The peak responses studied here are pseudo-steady state measurements, reflecting both open

and brief duration desensitized states while slow desensitization is explicitly excluded from the

two-state cyclic model we used. Desensitization of the GABA$_A$ receptor is not fully understood but

it seems likely that there are at least two phases distinguishable by kinetics - a faster and a slower

phase (Bianchi and Macdonald, 2002; Jones and Westbrook, 1995; Steinbach and Akk, 2001). In

terms of single-channel kinetics, a cluster at high [GABA] has a mean duration of 2 to 3 sec (Akk

et al., 2001), producing slow desensitization that would be observed in oocyte recordings. There

are also intracluster closed time components that have been associated with receptor

desensitization (Haas and Macdonald, 1999; Lema and Auerbach, 2006; Steinbach and Akk,

2001). In single-channel activity from concatemeric GABA$_A$ receptors these closed states have

mean durations ranging from 0.5 to 25 ms (Akk et al., 2009). Our simulations (not shown) indicate

that omission of these states in the model (Fig. 1) has a relatively small effect (less than 10%) on

the estimated open probability of the peak response and the predicted EC_{50}. We did not analyze

responses at the overall steady-state, reflecting long-lived desensitization.

The major strength of the MWC model is its simplicity. There is no pairwise specific

interaction among agents; all interactions are mediated through the overall energy available to

activate the receptor. The fact that the scheme consists of closed cycles means that at equilibrium

the state function for a single agonist can be fully described with only 4 parameters. The energetic

contribution of an agonist is defined in a single parameter (c) scaled by the number of sites for the

agent (N). Activation is enhanced by the increased affinity of drug for its sites in the open state,

reflected in the stabilization energy $\Delta G_A = N_A RT \times (\ln((1+[A]/K_A) / (1+[A]/(c_A K_A)))$ which

offsets the free energy difference between the unliganded closed and open states, $\Delta G_{int} = RT\ln(L)$.

Interactions between drugs are predicted to occur because the application of a background drug

reduces the free energy difference to be overcome by agonist A ($\Delta G_{int+B} = RT\ln(L) + \Delta G_B$) rather

than as a result of a change in any of the parameters for activation by A.

The three drugs used to potentiate the responses to GABA (propofol, pentobarbital, and

alfaxalone) produced similar shifts in the EC_{50} for GABA for similar shifts in L. These drugs

belong to chemically distinct classes of anesthetic and have distinct (although possibly

overlapping) binding sites on the receptor (Jayakar et al., 2014). The observation that they produce

similar shifts gives circumstantial and model-independent support to the idea that the shifts reflect

a non-specific effect on receptor activation. Our analysis does not address the validity of the

MWC model as a detailed description of activation of the $GABA_A$ receptor by a single agonist, but

rather explores the mechanism by which interactions between the transmitter and an allosteric

agonist might take place. Although the MWC model has been shown to be able to describe the

macroscopic activation of GABA receptors and interactions between several anesthetics and

GABA (Chang and Weiss, 1999; Ruesch et al., 2012; Rusch et al., 2004), it is not clear that it is as

successful at accounting for responses to combinations of diazepam with GABA ((Gielen et al.,

2012; Goldschen-Ohm et al., 2014), however see (Campo-Soria et al., 2006; Rusch and Forman,

2005)). In particular, there may be a requirement for an additional activated-but-closed state

between inactive and active-open (Gielen et al., 2012; Goldschen-Ohm et al., 2014).

We have emphasized the ability of the model to predict the relationship between EC_{50} and L.

However the predictions are not exact; there is some scatter of observed values around the

predicted line. The reasons for this disagreement are not known at present. One clear possibility is

the presence of variability in the experimental values. Other possibilities are that the MWC model

is overly restrictive in its assumptions, for example that there are no specific interactions among

drugs or that conformational changes are strictly concerted.

AUTHORSHIP CONTRIBUTIONS:

Participated in research design: Akk, Steinbach.

Conducted experiments: Shin, Germann.

Contributed new reagents or analytical tools: N/A

Performed data analysis: Akk, Shin, Germann, Steinbach.

Wrote or contributed to the writing of the manuscript: Akk, Shin, Germann, Steinbach.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. The figure shows a partial state diagram for two drugs (A and B, each with two sites) acting on a receptor following the concerted transition scheme. Closed receptor states (R) occupy the plane at the bottom (note that some states are obscured (e.g., B_2RA)) while open states (R^*) occupy the plane at the top. The diagram is distorted, to show states with only agonist A bound (solid line box at front), only agonist B bound (long dashed line box at left) and some states with both agonists bound (short dash box at right). In the absence of both A and B the receptor activates constitutively with $L = R/R^*$. The value of the parameter L is a property of the receptor, not of any agonist. Agonist A binds to its site with dissociation constant K_A on the closed receptor and K_A^* on the open receptor, with $c_A = K_A^*/K_A$. Note that the presence of bound B does not affect binding of A nor *vice versa*. The equilibrium between R and R^* states is determined by the respective values for c, as dictated by detailed mass action in the coupled cycles (e.g. $B_2R / B_2R^* = c_B^2L$ and $B_2RA_2 / B_2R^*A_2 = c_B^2c_A^2L$).

Figure 2. The theoretical relationship between normalized EC_{50} and L. The figure shows the predicted EC_{50} plotted logarithmically against the value for L. The values for EC_{50} are normalized to the dissociation constant for the open state. Panel **A** shows the relationships for N values of 1 (circles), 2 (squares) and 5 (triangles) for $c = 0.01$ over a wide range of values for L (10^{-2} to 10^{12}). The filled symbols show the predicted asymptotic values (see Results). The lines show slopes of 1/N. Panel **B** shows the same as A but over a range of L values previously reported for the $GABA_A$ receptor to illustrate results that might be obtained experimentally. The dashed lines show the linear regression on the logarithmically transformed data; the slopes of the regression lines are 0.75 for N = 1, 0.58 for N = 2 and 0.37 for N = 5. The solid lines show slopes of 1/N. Panels **C** and **D**

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show the relationships between predicted EC_{50} and L for N = 2 (C) and N = 5 (D). Three values for

c were used, $c = 0.1$, $c = 0.01$ and $c = 0.001$, to cover the range appropriate for the $GABA_A$

receptor (L = 1 to 100,000). The solid lines show lines with slope of 1/N. Table 1 provides slopes

for the linear regression on the logarithmically transformed data for a number of combinations for

N, L and c.

Figure 3. Properties of the wild-type βαγ+βα concatemeric receptor. Panel **A** shows mean current

responses elicited by GABA (circles) normalized to the maximal fitted response. The line shows

predictions of Eqn. 1 to the data. The mean values of the fit were $EC_{50, GABA} = 34 \pm 8$ μM and

$n_{H,GABA} = 1.38 \pm 0.07$ (n = 5 cells). Panel **B** shows responses to 300 μM picrotoxin and 1000 μM

GABA. The current traces are from the same cell. Panel **C** shows the concentration-response data

expressed as the estimated probability of being open plotted against the agonist concentration. The

lines show the predictions of the MWC model (Eqn. 4) fitted to the data with the parameters $L_{WT} =$

9000, $N_{GABA} = 2$, $K_{GABA} = 72 \pm 15$ μM and $c_{GABA} = 0.0033 \pm 0.0004$.

Figure 4. Effect of change in L on activation by GABA. The measured EC_{50} for GABA is plotted

logarithmically against the value for L. Panel **A** shows data for receptors composed of wild-type

concatemers in the absence of any other drug ($L_{WT;}$ filled diamond), or in the presence of propofol

(hollow diamonds; concentrations of 20, 10 and 5 μM from lower to higher L), pentobarbital

(circles; 200 and 100 μM) or alfaxalone (square, 1 μM). The symbols show mean values, while the

error bars indicate ±1 S.E.M. The solid line shows the predicted relationship between the EC_{50} and

L using the values estimated for the wild-type: $L_{WT} = 9000$, $N_{GABA} = 2$, $K_{GABA} = 72$ μM and c_{GABA}

= 0.0033. The dashed line shows the linear regression of $\log(EC_{50})$ on $\log(L)$ (slope = 0.63 ± 0.05).

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Panel **B** shows data for receptors containing the α1(L263S) mutation (hollow triangles; from lower

L to higher L mutation is in both constructs, in βαγ, or in βα), the β2(Y143W) mutation (filled

triangle; mutation is in both constructs), or the combination of β2(Y143W) in βαγ and α1(L263S)

in βα (inverted filled triangle). The plot also shows data for the β(Y143W)αγ+βα receptor in the

presence of 25 μM propofol (filled square). The solid line shows the predicted relationship using

the values from fits of data from wild-type receptor. The dashed line shows the linear regression of

$\log(EC_{50})$ on $\log(L)$ (slope $= 0.60 \pm 0.11$). The data are summarized in Table 2. Panels **C** and **D**

show the corresponding concentration-response relationships. The symbols show mean $\pm$ S.E.M.

The symbols are as in A and B. The curves show fits to the Hill equation incorporating a low-

concentration offset. The EC_{50}s are given in Table 2.

Figure 5. Effect of change in N_{GABA} on activation by GABA. The figure shows data for receptors

composed of a βαγ concatemer and a β(Y205S)α concatemer in the absence of propofol (filled

triangle) and in the presence of 4 different concentrations of propofol (hollow triangles, 40, 20, 10,

and 5 μM from lower to higher L). The filled circle shows data from a receptor containing 2

mutations: βα(L263S)γ and β(Y205S)α. The solid line shows the predicted values for the EC_{50}

assuming that $L_{WT} = 9000$, $K_{GABA} = 72$ μM and $c_{GABA} = 0.0033$ (unchanged from wild-type

receptor values), while $N_{GABA} = 1$. The dashed line shows the logarithmic regression from the

linear region of the predicted line (slope $= 0.74 \pm 0.1$).

Figure 6. Quality of description of concentration-response data with different assumed values for

L_{WT} and N_{GABA}. The mean squared differences (MSD) between the predicted and measured

concentration-response data are shown for different values of L_{WT} and N_{GABA}. The concentration-

response data (see Fig. 3B) were fit with various values for N_{GABA} as indicated on the abscissa and

3 values for L_{WT} (9000 filled diamonds, 27000 hollow squares, and 3000 crosses). An F test on the ratio of MSD values indicated that the difference between $N_{GABA} = 2$ and $N_{GABA} = 4$ or 5 was marginally significant ($P < 0.04$ for all L values, uncorrected for multiple comparisons).

Figure 7. Predictions of EC_{50} values made using parameters generated with different values for L and N. Panels **A** and **B** show data for $EC_{50,GABA}$ and L replotted from Fig. 4A and B. In Panel A the lines show the predicted relationships for 3 different assumed values of L_{WT} with $N_{GABA} = 2$ while in Panel B the lines show predicted relationships for 3 assumed values for N_{GABA} with $L_{WT} = 9000$. The open triangles show data for wild-type receptors in the absence of other agonists. Note that 3 open triangles are shown in Panel A, corresponding to the assumed values for L_{WT}. Hollow diamonds show values in the presence of a background drug while filled diamonds show values in the presence of an α1(L263S) mutation. Panels **C** and **D** show the quality of the descriptions assessed by calculating the ratio of the experimental $EC_{50,GABA}$ to the predicted $EC_{50,GABA}$, that is plotted against L. Panel C shows the data when the value of L_{WT} was changed while $N_{GABA} = 2$, and panel D shows changes in N_{GABA}, with $L_{WT} = 9000$. The lines identified by the symbols at the ends of the lines show the linear regression of log(ratio) on log(L). Comparison of Panels A to C indicates that an L_{WT} value of 3000 ($N_{GABA} = 2$) provides predictions that consistently lie above the observed values (ratio < 1), for $L_{WT} = 27,000$ consistently lie below, while predictions for $L_{WT} = 9000$ are relatively close to the observed EC_{50}. Similarly, the predictions for $N_{GABA} = 1$ or 3 (Panels B and D) demonstrate increasing large inaccuracies relative to the experimental EC_{50} values as L departs further from the value for wild-type receptors in the absence of agonists, while the predictions for $N_{GABA} = 2$ have a relatively constant relationship to the observed EC_{50}. The results are summarized in Table 3.

Table 1. Slope of the relationship between log(L) and predicted log(EC$_{50}$).

N	c	slope	1/N	Range of L
1	0.1	0.42	1.00	1-10
1	0.01	0.75	1.00	1-40
1	0.001	0.86	1.00	1-200
2	0.1	0.59	0.50	1-100
2	0.01	0.58	0.50	1-1000
2	0.001	0.55	0.50	1-1000
3	0.1	0.55	0.33	1-400
3	0.01	0.45	0.33	1-2000
3	0.001	0.44	0.33	1-2000
4	0.1	0.48	0.25	1-2000
4	0.01	0.39	0.25	1-2000
4	0.001	0.39	0.25	1-2000
5	0.1	0.42	0.20	1-1000

| 5 | 0.01 | 0.37 | 0.20 | 1-1000 |
| 5 | 0.001 | 0.37 | 0.20 | 1-1000 |

The table shows the results of linear regression of the value for $\log(EC_{50})$ on $\log(L)$ (third column), for various values of N (first column) and c (second column). The values were chosen to cover the range of proposed number of sites, and reported values for c for agonists acting on the $GABA_A$ receptor. The predictions were fit over a range of values for L that by eye appeared largely linear, as indicated in the fifth column. The fourth column gives the value of 1/N.

Table 2. Experimental observations on GABA EC$_{50}$ and L.

Receptor	Background drug	EC$_{50,GABA}$ (µM)	L	note
βαγ+βα	none	34 ± 8	9000	a
βαγ+βα	5 µM Pro	11 ± 1	1826 ± 904	c
βαγ+βα	10 µM Pro	1.2 ± 0.1	37 ± 4	b
βαγ+βα	20 µM Pro	0.50 ± 0.09	11 ± 4	b
βαγ+βα	1 µM ALF	3.3 ± 0.4	606 ± 161	b
βαγ+βα	100 µM PEB	1.1 ± 0.3	59 ± 16	b
βαγ+βα	200 µM PEB	0.43 ± 0.04	23 ± 6	b
βαγ+βα(L263S)	none	3.0 ± 0.1	74 ± 9	a
βα(L263S)γ+βα	none	2.0 ± 0.1	19 ± 2	a

βα(L263S)γ+βα(L263S)	none	0.28 ± 0.04	7.3 ± 0.4	a
β(Y143W)αγ+β(Y143W)α	none	2.4 ± 0.2	141 ± 20	a
β(Y143W)αγ+βα(L263S)	none	0.70 ± 0.06	67 ± 5	a
β(Y143W)αγ+βα	25 μM Pro	0.46 ± 0.06	7.4 ± 1.5	b
βαγ+β(Y205S)α	none	129 ± 16	9000	d
βαγ+β(Y205S)α	5 μM Pro	88 ± 9	539 ± 119	b
βαγ+β(Y205S)α	10 μM Pro	24 ± 2	315 ± 58	b
βαγ+β(Y205S)α	20 μM Pro	8.2 ± 2.0	42 ± 5	b
βαγ+β(Y205S)α	40 μM Pro	3.9 ± 0.5	11 ± 2	b
βα(L263S)γ+β(Y205S)α	none	6.1 ± 1.4	35 ± 1	a

The table shows values for $EC_{50, GABA}$ and L for the various receptors studied. The 1st column shows the composition of the receptor and the 2nd shows the background drug used to modify L (propofol: Pro, pentobarbital: PEB, alfaxalone: ALF). The 3rd shows the $EC_{50, GABA}$ and the 4th the value of L. The note column indicates how L was determined. (a) L was based on the effect of picrotoxin on constitutive activity. (b) L was calculated from the current response in the presence of the background drug, assuming no constitutive activity. (c) L was calculated from the current response in the presence of the background drug corrected for constitutive activity. (d) L was considered to equal that of the wild-type receptor. Values are mean ± S.E.M. for data from 5 or more oocytes.

Table 3. Quality of description with different values for N and L_{WT}.

L_{WT}	N_{GABA}	$\log(EC_{50,exp}/EC_{50,pred})$	P to 0
3000	2	-0.32 ± 0.07	0.001
9000	2	-0.08 ± 0.06	0.22
27000	2	0.15 ± 0.06	0.04
9000	1	0.69 ± 0.13	<0.001
9000	2	-0.08 ± 0.06	0.22
9000	3	-0.26 ± 0.07	0.005

The experimental values for EC_{50} for GABA were compared to values predicted with different assumed values for N_{GABA} and L_{WT}, using the logarithm of the ratio ($EC_{50,exp}$ / $EC_{50,pred}$) to assess the quality. The first and second columns give the assumed value for L_{WT} and N_{GABA}. The third column gives mean $\pm$ S.E.M. for the parameter. The fourth column gives the P value that the mean differs significantly from 0 (where a value of 0 indicates identity of $EC_{50,exp}$ and $EC_{50,pred}$; one sample two-tailed t-test). The data sets analyzed are shown in Fig. 4A and 4B, 13 pairs of values for L and $EC_{50,GABA}$.
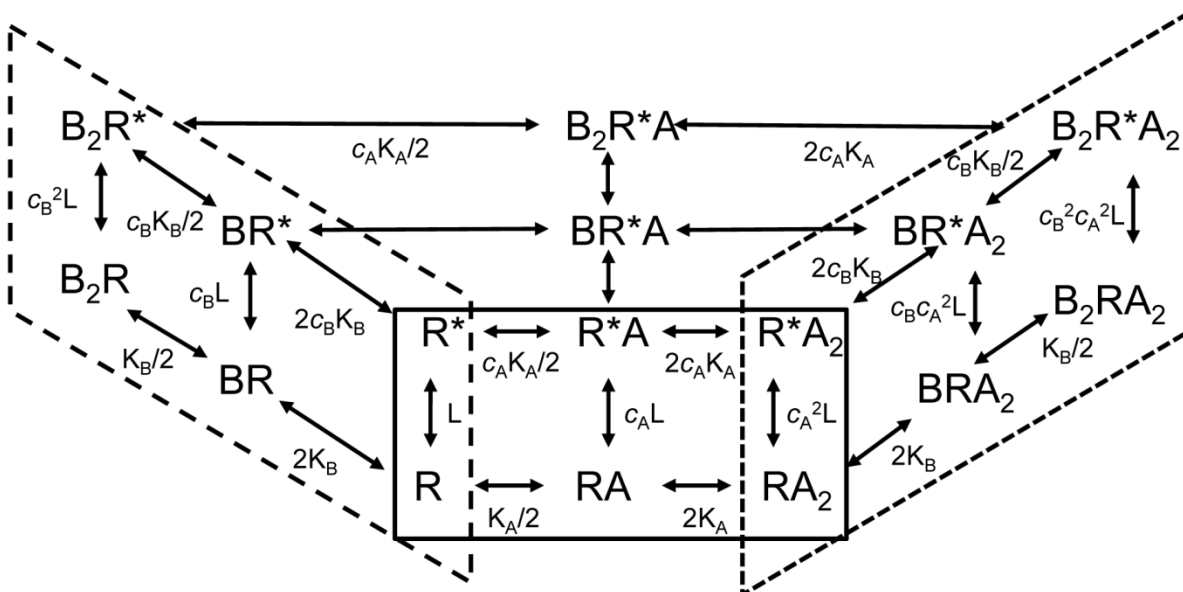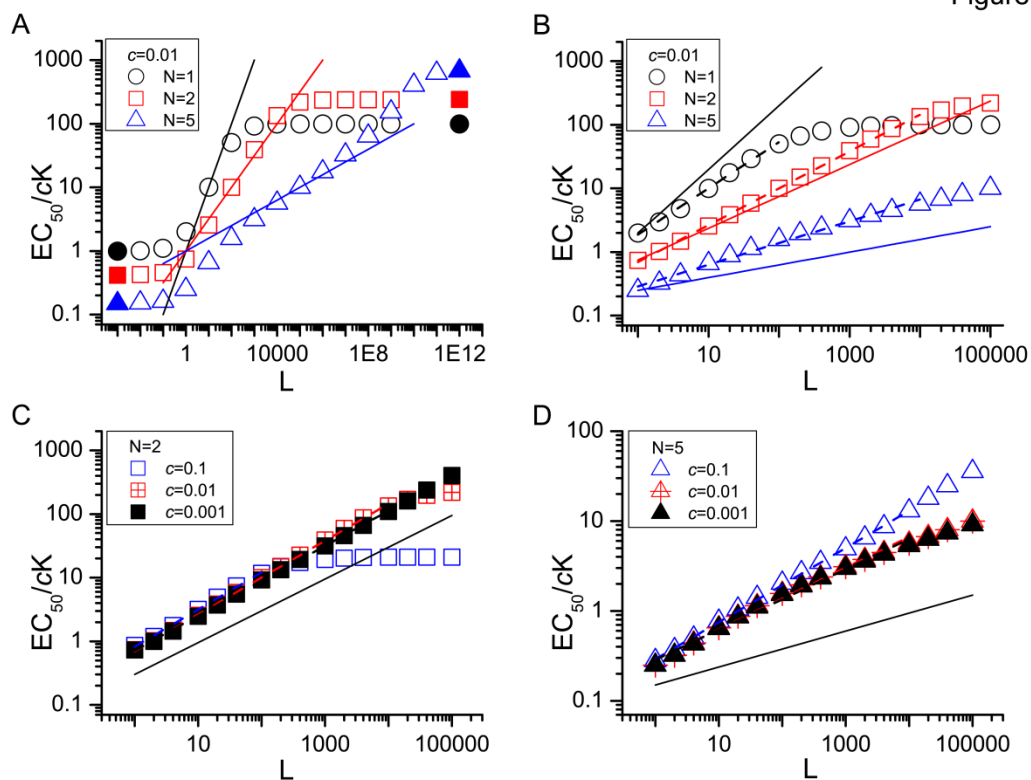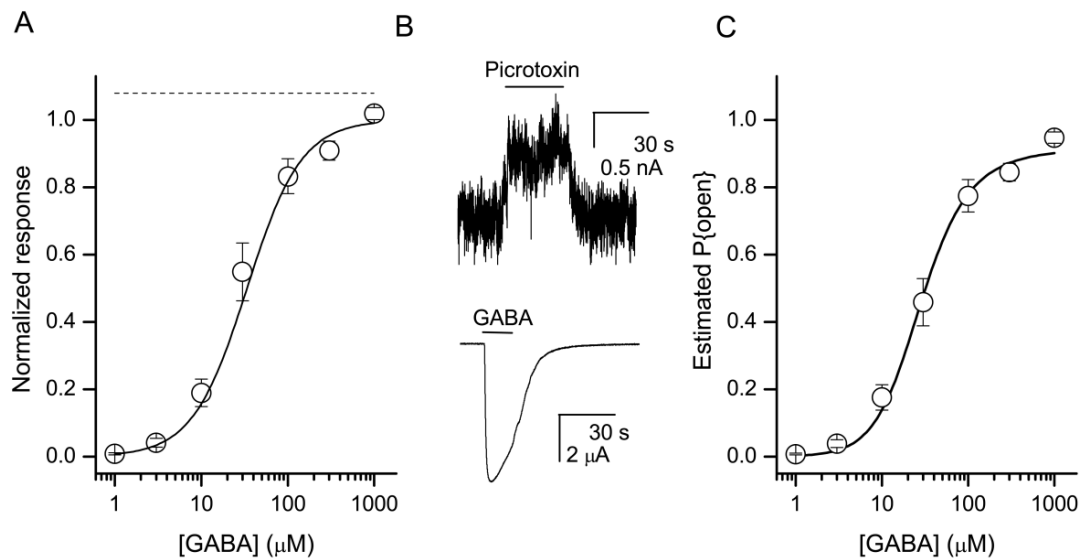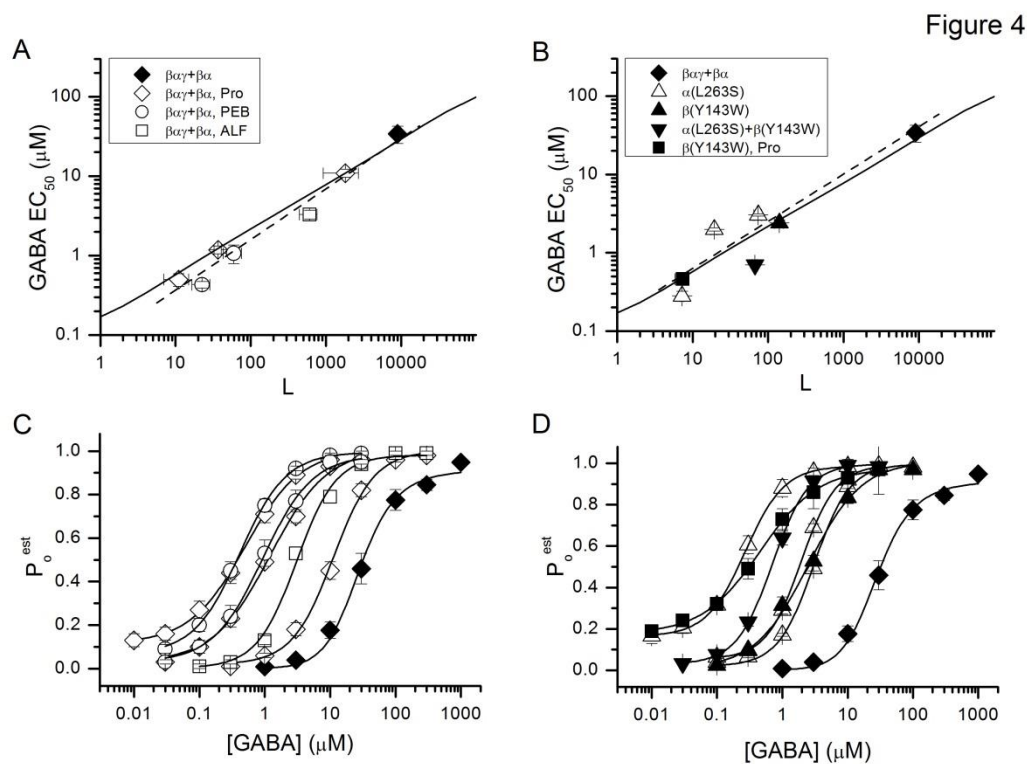
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7