

ACCELERATED COMMUNICATION

Reduced activation of the synaptic-type GABA_A receptor following prolonged exposure to low concentrations of agonists: relationship between tonic activity and desensitization

Spencer R. Pierce, Allison L. Germann, Alex S. Evers, Joe Henry Steinbach, and Gustav Akk

Department of Anesthesiology (SRP, ALG, ASE, JHS, GA) and the Taylor Family Institute for Innovative Psychiatric Research (ASE, JHS, GA), Washington University School of Medicine, St. Louis, MO 63110

Primary laboratory of origin: Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO, USA

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Correspondence: Gustav Akk, Department of Anesthesiology, Washington University School of
Medicine, Campus Box 8054, 660 S. Euclid Ave, St. Louis, MO 63110, USA. Email:
akk@morpheus.wustl.edu

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ABSTRACT

Synaptic GABA_A receptors are alternately exposed to short pulses of a high, millimolar concentration of GABA and prolonged periods of low, micromolar concentration of the transmitter. Prior work has indicated that exposure to micromolar concentrations of GABA can both activate the postsynaptic receptors generating sustained low-amplitude current, and desensitize the receptors thereby reducing the peak amplitude of subsequent synaptic response. However, the precise relationship between tonic activation and reduction of peak response is not known. Here, we have measured the effect of prolonged exposure to GABA or the combination of GABA and the neurosteroid allopregnanolone, intended to desensitize a fraction of receptors, on a subsequent response to a high concentration of agonist in human $\alpha 1\beta 3\gamma 2L$ receptors expressed in *Xenopus* oocytes. We show that the reduction in the peak amplitude of the post-exposure test response correlates with the open probability of the preceding desensitizing response. Curve-fitting of the inhibitory relationship yielded an IC₅₀ of 12.5 μ M and a Hill coefficient of -1.61. The activation and desensitization data were mechanistically analyzed in the framework of a three-state Resting-Active-Desensitized model. Using the estimated affinity, efficacy, and desensitization parameters, we calculated the amount of desensitization that would accumulate during a long (2 min) application of GABA or GABA plus allopregnanolone. The results indicate that accumulation of desensitization depends on the level of activity rather than agonist or potentiator concentration per se. We estimate that in the presence of 1 μ M GABA approximately 5% of $\alpha 1\beta 3\gamma 2L$ receptors are functionally eliminated due to desensitization.

Significance Statement: We present an analytical approach to quantify and predict the loss of activatable GABA_A receptors due to desensitization in the presence of transmitter and the steroid allopregnanolone. The findings indicate that the peak amplitude of the synaptic response is influenced by ambient GABA, and that changes in ambient concentrations of the transmitter and other GABAergic agents can modify tonically- and phasically-activated synaptic receptors in opposite directions.

INTRODUCTION

The neurotransmitter receptors in the brain are exposed to two regimes of extracellular transmitter concentration: throughout the brain an ambient, tonic concentration is present (although not necessarily constant or uniform) while near the synapse the phasic release of transmitter can result in concentrations as high as millimolar in the synaptic cleft. Extrasynaptic receptors close to the synapse experience reduced and slower transients, whereas receptors far from the synapse likely experience even slower and less extreme variations.

GABA_A receptors in the postsynaptic membrane are activated by presynaptically-released GABA, the concentration of which in the synaptic cleft reaches 1-5 mM at its peak (Clements, 1996; Mozrzymas et al., 1999; Overstreet et al., 2002). The synaptic response, reflecting activated receptors with open channels, decays with a decay time constant on the timescale of tens of milliseconds as channels close and transmitter dissociates from the receptor. The concentration transient itself is more rapid and is terminated in part by binding to the receptor and eventually by removal by membrane transporters and diffusion (Draguhn and Heinemann, 1996; Edwards et al., 1990; Jones and Westbrook, 1995; Mozrzymas et al., 1999). Between synaptic events the activity due to exposure to ambient GABA is low, although the integrated charge transfer during exposure to ambient GABA may actually exceed that during a short-lasting synaptic event within the same time period (Germann et al., 2019b).

Prolonged exposure to low concentrations of GABA can also desensitize the GABA_A receptor (Orser et al., 1994; Overstreet et al., 2000), even when the amplitude of the response is small. This manifests as reduction in the peak amplitude of inhibitory postsynaptic currents (IPSCs) or a decrease in the peak response to subsequent test application of agonist. The effect is observed at concentrations of GABA that are close to the estimated ambient tonic level. However, there is no clear theoretical framework for understanding the relationship between tonic levels of activity and phasic responses. This relationship is particularly interesting in the

case of GABA_A receptors because their activity is known to be modulated by both endogenous and exogenous chemicals, that could affect one or both of the tonic and phasic responses.

Here, we have measured receptor desensitization by exposing cells expressing the human $\alpha 1\beta 3\gamma 2L$ receptor to GABA or the combination of GABA and the neurosteroid allopregnanolone (3 α 5 α P), and then comparing pre- and post-exposure responses to a high concentration of agonist to characterize the phasic response. We were motivated to understand how increases in ambient concentrations of endogenous GABAergic agents increase tonic activity but may, somewhat counterintuitively, reduce the amplitude of the phasic response. Employing a three-state cyclic Resting-Active-Desensitized model, we show that the loss of activatable receptors can be satisfactorily predicted using the activation and desensitization parameters of the receptor estimated in separate experiments. The data indicate that the reduction in available activatable receptors correlates with the open probability of the preceding desensitizing response.

METHODS

The human $\alpha 1\beta 3\gamma 2L$ GABA_AR were expressed in *Xenopus laevis* oocytes purchased from Xenocyte (Dexter, MI) as quarter ovaries. Oocytes were digested in 2% w/v (mg/ml) collagenase A solubilized in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES; pH 7.4) plus 100 U/ml penicillin and 100 μ g/ml streptomycin at 37 °C with shaking at 250 RPM for 30-40 minutes. Following digestion, the oocytes were rinsed clean of collagenase A in ND96, and stored in ND96 with supplements (2.5 mM Na pyruvate, 100 U/ml penicillin, 100 μ g/ml streptomycin, 50 μ g/ml gentamycin) at 15°C for at least 4 hours before injection.

The cDNAs for human $\alpha 1$ (GenBank accession no NM_000806), $\beta 3$ (NM_000814), and $\gamma 2L$ (NM_198904) subunits in the pcDNA3 vector were linearized with XbaI (NEB Labs, Ipswich, MA), and the cRNAs were generated using mMessage mMachine (Ambion, Austin, TX). The oocytes were injected with a total of 3.5 ng of cRNA in the ratio of 1:1:5 (α : β : γ) per oocyte, and incubated in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES; pH 7.4) with supplements at 15°C for 1-2 days prior to conducting electrophysiological recordings.

The electrophysiological recordings were conducted at room temperature using standard two-electrode voltage clamp. The oocytes were clamped at -60 mV. Bath and drug solutions were gravity-applied from glass syringes equipped with glass luer slips via Teflon tubing to the recording chamber (RC-1Z, Warner Instruments, Hamden, CT) at the rate of 6-8 ml/min.

The current responses were amplified with an OC-725C amplifier (Warner Instruments, Hamden, CT), filtered at 30 Hz with a low-pass Bessel filter (Model 900, Frequency Devices, Ottawa, IL), digitized at 100 Hz with a Digidata 1200 series digitizer (Molecular Devices, San Jose, CA), and stored using pClamp (Molecular Devices). Analysis of the current traces with respect to peak and steady-state amplitudes was done with Clampfit (Molecular Devices).

The effect of exposure to a desensitizing agonist was determined as follows. First, the cells were repeatedly activated by brief (10-15 s) applications of a test agonist (1 mM GABA in

experiments employing GABA as the desensitizing agonist, and 1 mM GABA + 1 μ M 3 α 5 α P in experiments involving the combination of GABA + 3 α 5 α P as the desensitizing agonist) until a stable peak response (less than 10% change between consecutive responses) was observed. The washout time between successive applications of the test agonist was 3 min. It took between 3 and 8 applications to obtain a stable response. If a stable response to test agonist was not attained by the eighth application the cell was discarded. Each cell was then exposed to a 1-min wash in bath followed by a 2-min desensitizing application of GABA or the combination of GABA + 3 α 5 α P. This was immediately followed by another application of the test agonist. The fraction of receptors desensitized during exposure to the desensitizing agonist was determined as $1 - \frac{\text{peak amplitude of post-exposure}}{\text{peak amplitude of last pre-exposure}}$. Pilot experiments with varying exposure and washout times indicated that a 2 min exposure to the desensitizing agonist is sufficient to reach steady-state.

Receptor activation by GABA was recorded by exposing the cells to 90-300 s applications of 0.3 μ M to 1 mM GABA. To minimize measurement error associated with long-duration recordings, each cell was exposed to only 1-2 concentrations of GABA rather than to a full range of agonist concentrations. Each cell was also tested with 1 mM GABA + 50 μ M propofol that was considered to activate all receptors (Shin et al., 2018). Peak and steady-state open probabilities ($P_{A,\text{peak}}$ and $P_{A,\text{s.s.}}$, respectively) were estimated by normalizing peak and steady-state amplitudes in the presence of GABA to the peak response to GABA + propofol. The peak open probability and the ratio of steady-state to peak responses in the presence of saturating GABA are similar to those previously estimated in whole-cell and single-channel recordings from HEK cells (Bianchi and Macdonald, 2002; Feng et al., 2004; Steinbach and Akk, 2001). The data from different cells were pooled and averaged for analysis. Curve fitting was done using Origin 2020 (OriginLab Corp., Northampton, MA). Parameters estimated by fitting models to the data are presented as best fitting value \pm estimated S.D. of the fit.

The findings are reported according to the guidelines detailed in (Michel et al., 2020). The

sample sizes ranged from 5 to 7 cells for individual experiments. All data for each individual experiment were collected before conducting data analysis. All data are included in analysis, and are presented as mean \pm S.D. The study is exploratory by nature.

RESULTS

Prolonged exposure to GABA reduces the number of activatable receptors: description of the phenomenon

Sample traces in Fig. 1 show the loss of activatable receptors due to desensitization. Peak responses to brief test applications of 1 mM GABA were compared before and after a 2 min desensitizing application of 1 μ M (Fig. 1A) or 300 μ M GABA (Fig. 1B). The response to a test application following exposure to 1 μ M GABA was 0.94 ± 0.04 (mean \pm S.D.; $n = 6$ cells) of the test response before exposure to 1 μ M GABA. Following exposure to 300 μ M GABA, the fractional response to the test application was 0.29 ± 0.11 ($n = 5$ cells) of control.

The ratio of amplitudes of responses to the test application as a function of the concentration of GABA in the desensitizing application is shown in Fig. 1C. Fitting the Hill equation to the data yielded a Y_{MAX} of 0.94 ± 0.02 (best-fit parameter \pm S.D. of the fit), a Y_{MIN} of 0.30 ± 0.02 , an IC_{50} of 12.5 ± 1.4 μ M, and a n_H of -1.61 ± 0.26 . The non-zero Y_{MIN} closely corresponds to the steady-state response to saturating GABA in the related $\alpha 1\beta 2\gamma 2L$ receptor (Germann et al., 2019b).

The Resting-Active-Desensitized model predicts accumulation of receptors in the desensitized state during exposure to agonist

We postulate that the decrease in the amplitude of the test response following prolonged exposure to GABA, as illustrated in Fig. 1, is due to accumulation of a fraction of receptors in the desensitized state. These receptors are neither active nor activatable following exposure to GABA, and thus do not contribute to the second test response.

We explored desensitization in the framework of the three-state Resting-Active-Desensitized ("RAD") model (Fig. 2). The model is based on the Monod-Wyman-Changeux two-state cyclic

model originally used to describe enzyme function (Monod et al., 1965). In the RAD model, an additional inactive state is placed beyond the active state. This enables analysis of equilibrium activity from transmitter-gated channels that undergo transition to a non-conducting, high-affinity state upon prolonged exposure to agonist (“desensitization”). The critical aspects of the RAD model are: i) a ligand can have different affinities to different states; ii) the individual sites for a ligand in a given state have identical binding properties; and iii) the receptor undergoes a concerted state transition in which all sites change properties simultaneously. We have previously employed the RAD model to analyze steady-state activity in $\alpha 1\beta 2\gamma 2L$ and $\alpha 4\beta 2\delta$ GABA_A receptors (Germann et al., 2019a; Germann et al., 2019b; Pierce et al., 2019).

In the RAD model, the fraction of receptors in the D-state at a given agonist concentration depends on the parameters L (the ratio of resting to active receptors; R/A in Fig. 2) and Q (the ratio of active to desensitized; A/D in Fig. 2). A lower value of L (more active vs. resting) and a lower value of Q (more desensitized vs. active) increase the fraction of receptors in the D-state.

Formally, the steady-state probability of being in the active state at a given concentration of agonist X is:

$$P_{A,s.s.,[X]} = \frac{1}{1 + \frac{1}{Q} \left[\frac{1}{\left(\frac{1+[X]/(K_X c_X)}{1+[X]/(K_X d_X)} \right)^{N_X}} \right] + L \left[\frac{1+[X]/K_X}{1+[X]/(K_X c_X)} \right]^{N_X}} = \frac{1}{1 + \frac{1}{Q\Delta_X} + L\Gamma_X} \quad 1$$

where [X] is the concentration of agonist X, K_X is the equilibrium dissociation constant for X in the resting state, c_X is the ratio of the equilibrium dissociation constants in the active and resting states, d_X is the ratio of the equilibrium dissociation constants in the desensitized and active states, and N_X is the number of binding sites for X. The parameters L and Q are as described earlier. Δ_X and Γ_X are defined as:

$$\Delta_X = \left[\frac{1+[X]/(K_X c_X)}{1+[X]/(K_X c_X d_X)} \right]^{N_X} \quad 2$$

and

$$\Gamma_X = \left[\frac{1+[X]/K_X}{1+[X]/(K_X c_X)} \right]^{N_X} \quad 3$$

We have assumed that $d_{GABA} = 1$, so $\Delta_{GABA} = 1$.

The steady-state probability of being in the desensitized state is:

$$P_{D,s.s.,[X]} = \frac{1}{1+Q\Delta_X+Q\Delta_X L\Gamma_X} \quad 4$$

$P_{R,s.s.}$, the steady-state probability of being in the resting state, can be calculated as $1 - P_{A,s.s.} - P_{D,s.s.}$.

Fig. 3 illustrates simulated probability distributions in resting, active, and desensitized states at a range of concentrations of agonist X. For the calculations, K_X was held at 10 μ M, c_X was 0.005, d_X was 1, and N_X was 2. In the absence of X, the vast majority (>99%) of receptors are in the resting state, i.e., $P_{R,s.s.} > 0.99$. As the concentration of X is increased, $P_{R,s.s.}$ decreases (Fig. 3A). This is accompanied by increases in the peak and steady-state probabilities of being active ($P_{A,peak}$ and $P_{A,s.s.}$, respectively) as well as desensitized ($P_{D,s.s.}$).

This study is focused on the fraction of receptors that is active or available for activation by agonist, as a function of tonic activity. This relationship is shown in Fig. 3B for different values of Q reflecting the ratio of active to desensitized receptors. The calculations indicate that as $P_{A,s.s.}$ increases, the fraction of available receptors decreases steadily. In the absence of agonist, $P_{A,s.s.} = 1/(1+1/Q+L)$ and $P_{D,s.s.} = (1/Q) \times P_{A,s.s.}$ while in the presence of a saturating concentration $P_{A,s.s.} = 1/(1+1/(Qd^N)+Lc^N)$ and $P_{D,s.s.} = (1/(Qd^N)) \times P_A$.

At Q (=A/D) of 1000, the equilibrium between active and desensitized states is strongly biased towards active and there is minimal desensitization. Accordingly, the sum of fractions of

active + resting receptors (A+R) remains near 1 at all values of $P_{A,s.s.}$. When Q is constrained to 1, in the presence of saturating agonist X, ~45% of receptors are in the active and desensitized states, each, and ~9% are in the resting state. When Q is 0.2, the $P_{A,s.s.}$ in the presence of saturating [agonist] is 0.16, 81% of receptors are in the desensitized state, and ~3% in the resting state. The reported experimentally-determined values of Q for the GABA_A receptor range from ~0.2 to 0.8 (Germann et al., 2019b; Pierce et al., 2019).

Activation and desensitization properties of the $\alpha 1\beta 3\gamma 2L$ GABA_A receptor

We tested the ability of the model to account for accumulation of $\alpha 1\beta 3\gamma 2L$ GABA_A receptors in the desensitized state. We began by estimating the activation and desensitization properties of the $\alpha 1\beta 3\gamma 2L$ receptor in the presence of GABA. Receptor activation by GABA was examined by measuring peak and steady-state current responses to 0.3 μ M - 1 mM GABA. The P_A of the responses was estimated by comparing the raw amplitudes of the peak and steady-state responses to the peak response to 1 mM GABA + 50 μ M propofol recorded in the same cell. The latter was considered to have a P_A indistinguishable from 1 (Ruesch et al., 2012; Shin et al., 2018).

Receptor activation parameters were determined as described in detail previously (e.g., (Akk et al., 2018)). The peak currents, in P_A units, were fitted with the following equation (Forman, 2012; Steinbach and Akk, 2019):

$$P_{A,peak,[GABA]} = \frac{1}{1+L \left[\frac{1+[GABA]/K_{GABA}}{1+[GABA]/(K_{GABA} c_{GABA})} \right]^{N_{GABA}}} \quad 5$$

The number of binding sites for GABA (N_{GABA}) was set to 2, and L (=R/A) was constrained to 8000 (Akk et al., 2018; Shin et al., 2017). The analyses yielded a K_{GABA} of 39.0 ± 7.6 (best-fit parameter \pm S.D. of the fit) and a c_{GABA} of 0.0027 ± 0.0004 (n = 5-6 cells per concentration). The

steady-state currents from the same set of cells were analyzed using Eq. 1, with K_{GABA} and c_{GABA} constrained to the values determined in the analysis of peak responses and d_{GABA} held at 1. The fitted value of Q was 0.24 ± 0.01 . Sample current traces are shown in Fig. 4A, and the concentration-response relationships for peak and steady-state currents are given in Fig. 4B.

Observed and predicted desensitization of the $\alpha 1\beta 3\gamma 2\text{L}$ receptor in the presence of GABA or the combination of GABA and the steroid $3\alpha 5\alpha\text{P}$

Next, we employed the estimated activation and desensitization parameters to predict the degree of desensitization of the $\alpha 1\beta 3\gamma 2\text{L}$ receptor following tonic exposure to agonist, and to compare the RAD model-based predictions with the observed data. The RAD model predicts that the reduction in the response to a saturating concentration of agonist (that is, the amount of desensitization) is determined by the steady-state level of activation whether elicited by GABA alone or by GABA in the presence of a potentiation agent.

The experiments were conducted as shown in Fig. 1, and the probability of being desensitized at the end of the long pulse ($P_{\text{D,s.s.}}$) was calculated as (1-ratio of the response to the second application to the response to the first). The data are expressed as steady-state probability of being desensitized at different levels of steady-state probability of being active at the end of the long pulse (Fig. 5). We analyzed two data sets: one in which exposure to GABA was used to desensitize the receptor, and the other in which the response to GABA was potentiated by the addition of 0.1-1 μM of the neurosteroid $3\alpha 5\alpha\text{P}$.

The data in Fig. 5 demonstrate that $P_{\text{D,s.s.}}$ increases with the $P_{\text{A,s.s.}}$ of the desensitizing response. Following exposure to 1 μM GABA, that generated a steady-state response with P_{A} of 0.014, the P_{D} was 0.064, indicating a loss of 6.4% of theoretically available receptors. At a concentration producing a maximal $P_{\text{A,s.s.}}$ (~ 0.2), the estimated P_{D} was ~ 0.8 . In other words, at

saturating GABA, the vast majority (~98%) of receptors are either active or desensitized. There was no apparent difference for data recorded in the absence or presence of the steroid. Linear regression analysis provided estimates for the intercept and slope for data with GABA alone of -0.02 ± 0.05 and 3.15 ± 0.34 and in the presence of $3\alpha 5\alpha P$ of -0.04 ± 0.06 and 3.36 ± 0.42 . The slope of the linear regression is predicted to be $1/Q$ when $d = 1$, so these values for the slope correspond to values of Q of 0.32 and 0.30.

The predicted $P_{D,s.s.}$ at various $P_{A,s.s.}$ was calculated using Eq. 4, and is shown as solid line in Fig. 5. The experimental observations qualitatively agree with the predictions of the RAD model in two critical respects. First, there is a linear relationship between steady-state activation and desensitization ($R^2 = 0.94$). Activation is a nonlinear function of [GABA] so this indicates that desensitization involves the same number of GABA-binding sites as activation; in the RAD model the same sites are used for both physiological responses. Furthermore, the linearity indicates that the affinities of the sites for activation and desensitization are the same, i.e., d_{GABA} is unity. Second, enhancement by $3\alpha 5\alpha P$ of the steady state response does not shift the relationship, indicating that activation rather than the concentration of agonist is the critical parameter connecting activation to desensitization.

DISCUSSION

Synaptic events initiated by presynaptic release of GABA are interspersed with intervals when the concentration of the transmitter drops to low, background concentration. While the level of tonic activity in synaptic receptors is low compared to the amplitude of a synaptic event, it has been suggested that the integrated charge transfer during the prolonged periods of tonic activity can exceed that during the short-lived IPSC occurring during the same period (Germann et al., 2019b). Furthermore, receptors exposed to ambient GABA undergo desensitization that reduces the number of receptors available for subsequent synaptic activation (Overstreet et al., 2000).

Here, we recorded the loss of receptor responsiveness following prolonged exposure to GABA or GABA + 3 α 5 α P. The major findings are summarized in Fig. 5 that illustrates the relationship between the level of steady-state activity ($P_{A,s.s.}$) and the fraction of desensitized receptors ($P_{D,s.s.}$), i.e, the relative number of receptors that are neither already active nor available for activation (resting). The level of steady-state activity indicates the fraction of receptors that are already active in the presence of the given agonist. The fraction of resting receptors can be calculated as $1 - P_{D,s.s.} - P_{A,s.s.}$.

An increase in $P_{A,s.s.}$ is associated with an increase in $P_{D,s.s.}$, the extent of which is determined by the parameter Q. When Q is high (there is little desensitization), most receptors remain either active or activatable while in contrast when Q is low the majority become desensitized (Fig. 3B). We emphasize that for combinations of GABA and 3 α 5 α P it is the overall level of activity that matters. Indeed, a decrease in [GABA] accompanied by an increase in [3 α 5 α P] that results in the same $P_{A,s.s.}$ results in the same observed $P_{D,s.s.}$ (Fig. 5).

In the absence of a GABAergic agonist, the α 1 β 3 γ 2-type synaptic receptors are minimally active (~1/8000) or desensitized (~4/8000), and the vast majority of receptors are available to respond to presynaptically released transmitter. The precise extent of desensitization of

synaptic GABA_A receptors is unclear, because we do not know the true background concentration of GABA in the cleft. The concentration is likely strongly influenced by transmitter release frequency and the geometry of the synapse, as well as expression of GABA transporters (Scimemi, 2014; Scimemi and Beato, 2009). Using 3D Monte Carlo reaction-diffusion simulations, Scimemi (Scimemi, 2014) estimated a steady-state GABA concentration of 0.1-1 μM in the cleft. This is similar to the concentration of measured in the cerebrospinal fluid (de Groote and Linthorst, 2007; Lerma et al., 1986; Zandy et al., 2017). Using the activation and desensitization parameters presented above, we estimate that in the presence of 0.1 μM GABA, 0.05% of receptors are in the active state and 0.2% in the desensitized state. In the presence of 1 μM GABA, 1.2% of the receptors are in the active state and 5% in the desensitized state. Thus, up to 5% of synaptic GABA_A receptors are predicted to be unavailable for activation during a synaptic event simply as a consequence of being exposed to ambient GABA. In hippocampal neurons, exposure to 0.1 or 1 μM GABA reduced the subsequent response to saturating GABA by 1.2 or 7.3%, respectively (Orser et al., 1994). In the hippocampal autaptic preparation, pre-equilibration with 1 μM of externally-applied GABA reduced the amplitude of IPSCs by 23% (Overstreet et al., 2000).

Some portion of the synaptic response likely results from activation of δ -subunit containing receptors (Herd et al., 2013; Sun et al., 2018; Wei et al., 2003). The GABA_A receptor comprising $\alpha 4$, $\beta 2$ and δ subunits shows less desensitization to a given concentration of GABA, with an estimated value of $Q = 0.8$ (Pierce et al., 2019). As indicated in Fig. 5B this results in much less desensitization than what is observed for the $\alpha 1\beta 3\gamma 2$ receptor at comparable levels of steady-state levels of activation, suggesting that some synaptic transmission events would be more resistant to the desensitizing effects of increased tonic GABA or endogenous potentiator concentrations.

Other members of the pentameric ligand-gated ion channel family also show desensitization. In terms of rapidity and extent of the decay of the response to a high

concentration of agonist, desensitization ranges from minimal for the homomeric ρ GABA_A receptor (Amin and Weiss, 1994) to rapid and nearly complete for homomeric nicotinic $\alpha 7$ receptors (Corradi and Bouzat, 2016; Peng et al., 1994). The muscle nicotinic receptor has been most completely studied, and Auerbach (Auerbach, 2012) has estimated the values of L and c_{ACh} (using our nomenclature) of about 1.3×10^6 and 2×10^{-4} , while Elenes and Auerbach (Elenes and Auerbach, 2002) reported an energy difference between the open and the most deeply desensitized state of about 4.5 kcal/mol, corresponding to a Q value of 4×10^{-4} . These values correspond to values at saturating ACh of $P_{A,s.s.}$ of 4×10^{-4} and $P_{D,s.s.}$ close to 1. With estimated [ACh] in plasma < 5 nM, we can calculate that the fraction of desensitized muscle ACh receptors due to exposure to tonic ACh is 0.3%.

The data presented here and previously (Orser et al., 1994; Overstreet et al., 2000) indicate that an increase in cleft GABA concentration increases tonic activity but may reduce phasic activity. Comparison of total charge transfer involving synaptic receptors demonstrates a net gain of ~ 1 pC (assuming 100 receptors with 1 pA amplitude, a decay time constant of 30 ms and a frequency of 1 Hz and $P_{A,peak}$ of 1 for IPSCs) over 1 s when the cleft GABA concentration increases from 0.1 to 1 μ M, i.e., the increase in tonic current from the synaptic receptor overcompensates for the decrease in phasic response. However, the net change in charge transfer following an increase in cleft [GABA] from 0.1 to 1 μ M becomes 0 when the frequency of IPSCs is ~ 7 Hz, and is negative (i.e., a net decrease in cellular inhibition) at frequencies above that. The reversal point for net change in total charge transfer (~ 7 Hz) is independent of the magnitude of change in [GABA], because both the increase in tonic and decrease in phasic current are governed by the same activation and desensitization parameters.

Exposure to allosteric agonists, such as the endogenous steroid allopregnanolone, similarly to GABA increases tonic activity and desensitizes receptors. However, the resulting inhibitory effect on peak phasic response is to a degree compensated by slower decay of IPSCs. The anesthetic propofol increases the amplitude of tonic activity but is less efficient at stabilizing

desensitized receptors because it has higher affinity to the active than desensitized state ($d > 1$; Germann et al., 2019b). Accordingly, we predict minimal reduction in IPSC amplitude following exposure to propofol.

We emphasize that these calculations apply to a "closed system" involving solely synaptic GABA_A receptors. An increase in ambient [GABA] is expected to enhance tonic activity from extrasynaptic receptors (Bright et al., 2011; Nusser et al., 1998), and activate presynaptic GABA_B receptors to reduce presynaptic GABA release (Deisz and Prince, 1989; Khatri et al., 2019). It is not immediately clear what the overall sum effect is on the membrane potential of the postsynaptic cell.

This study adds to the growing body of evidence that synaptic GABA_A receptors undergo activation and desensitization between synaptic events in the presence of the background level of the transmitter. Tonic activation can be a significant contributor to total charge transfer, whereas desensitization can reduce the peak amplitude of a synaptic response. The findings also suggest a somewhat paradoxical phenomenon where an increase in ambient levels of GABAergic agents enhances tonic but reduces phasic activity from synaptic receptors with net change dependent on the frequency of phasic activity.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Evers, Steinbach, and Akk.

Conducted experiments: Pierce and Germann.

Contributed new reagents or analytical tools: N/A

Performed data analysis: Pierce, Germann, and Akk.

Wrote or contributed to the writing of the manuscript: Pierce, Germann, Evers, Steinbach, and Akk.

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Footnotes

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Reprint requests to: Gustav Akk, Department of Anesthesiology, Washington University, Campus Box 8054, 660 S. Euclid Ave, St. Louis, MO 63110. Email: akk@morpheus.wustl.edu

LEGENDS FOR FIGURES

Figure 1. Prolonged exposure to GABA reduces the number of activatable receptors. The current traces show responses to brief applications of 1 mM GABA before ("test application #1") and after ("test application #2") a 2-min desensitizing application of 1 μ M (Panel **A**) or 300 μ M GABA (Panel **B**). Panel **C** shows the GABA concentration dependence of the ratio of amplitudes of test responses (test application #2/test application #1). The data show mean \pm S.D. from 5-7 cells per concentration. Each cell was exposed to a single concentration of GABA. The curve was fitted with the Hill equation. The IC_{50} of the curve is 12.5 μ M, the Hill coefficient is -1.61, and the high concentration asymptote is 0.30.

Figure 2. A three-state Resting-Active-Desensitized model. The receptor can occupy a resting (R), active (A), or desensitized (D) state. The receptor has two binding sites for ligand X. The parameter L (=R/A) describes the equilibrium between the resting and active states. The parameter Q (=A/D) describes the equilibrium between the active and desensitized states. K_X , $K_X c_X$ and $K_X c_X d_X$ are the equilibrium dissociation constants for X in the resting, active, and desensitized receptor, respectively.

Figure 3. Calculated probability distributions. Panel **A** shows the probabilities of being in the resting ($P_{R,s.s.}$), active ($P_{A,peak}$ and $P_{A,s.s.}$), or desensitized states ($P_{D,s.s.}$) at various concentrations of agonist X. The modeling was done using the values of L of 8000 and Q of 0.2. The equilibrium dissociation constant of X in the resting state (K_X) was assigned a value of 10 μ M, c_X was 0.005, and d_X was 1. $P_{A,s.s.}$ and $P_{D,s.s.}$ were calculated using Eqs. 1 and 4, respectively. $P_{R,s.s.}$ was calculated as $1 - P_{A,s.s.} - P_{D,s.s.}$. $P_{A,peak}$ was calculated by assigning an arbitrary high value (10^7) to Q (ratio of active to desensitized receptors) in Eq. 1, effectively reducing the RAD model to a two-state Resting-Active model (Germann et al., 2019a). Panel **B**

shows the sum of the fractions of active and resting (i.e., activatable) receptors at different values of $P_{A,s.s.}$ (steady-state probability of being in the active state) with the value of Q constrained to 1000, 1, or 0.2. The maximal values for $P_{A,s.s.}$ in the presence of saturating agonist are calculated as $1/(1+1/(Qd^N)+Lc^N)$ and equal 0.83, 0.46, and 0.16 at these values of Q , while the corresponding values for the probability a receptor is either resting or active are 0.99, 0.54, and 0.19.

Figure 4. Activation and desensitization of the $\alpha 1\beta 3\gamma 2L$ GABA_A receptor. Panel **A** shows sample traces in the presence of 0.3, 3, 30, or 300 μ M GABA. The amplitudes of peak and steady-state currents were converted to units of open probability (P_A) by normalizing the current levels to the peak response to 1 mM GABA + 50 μ M propofol in the same cell. Panel **B** shows the peak and steady-state open probabilities as a function of GABA concentration. The data points show mean \pm S.D. from 5-6 cells per concentration. The curves were fitted with Eq. 5 (peak data) or Eq. 1 (steady-state data), yielding a K_{GABA} of 39 μ M, a c_{GABA} of 0.0027, and a Q of 0.24. The values of L and N_{GABA} were held at 8000 and 2, respectively.

Figure 5. Desensitization of the $\alpha 1\beta 3\gamma 2L$ GABA_A receptor in the presence of GABA or GABA + 3 α 5 α P. Panel **A** shows the fraction of desensitized receptors ($P_{D,s.s.}$) following different levels of desensitizing activity ($P_{A,s.s.}$) elicited by GABA or the combination of GABA + 3 α 5 α P. The data points give mean \pm S.D. from 5 to 7 cells per experimental condition. For GABA, the symbols show data obtained in the presence of 1, 2, 5, 10, 30, 100, and 300 μ M GABA (order from low to high $P_{A,s.s.}$). For GABA + 3 α 5 α P, the symbols show data obtained in the presence of 1 μ M GABA + 0.1 μ M 3 α 5 α P, 2 μ M GABA + 0.5 μ M 3 α 5 α P, 3 μ M GABA + 1 μ M 3 α 5 α P, 5 μ M GABA + 1 μ M 3 α 5 α P, 20 μ M GABA + 1 μ M 3 α 5 α P, and 100 μ M GABA + 1 μ M 3 α 5 α P (order from low to high $P_{A,s.s.}$). The solid line is not fit to the data; it was calculated using Eq. 4 and the activation and desensitization parameters established for the $\alpha 1\beta 3\gamma 2L$ receptor ($K_{GABA} = 39$ μ M,

$c_{\text{GABA}} = 0.0027$, $N_{\text{GABA}} = 2$, $L = 8000$, $Q = 0.24$) with no free parameters. In the absence of agonist, $P_{\text{A,s.s.}}$ is calculated as $1/(1+1/Q+L)$. In the presence of saturating agonist, $P_{\text{A,s.s.}}$ is calculated as $1/(1+1/(Qd^N)+Lc^N)$. Panel **B** compares the fraction of desensitized receptors following different levels of desensitizing activity in the $\alpha 1\beta 3\gamma 2\text{L}$ (black lines) and $\alpha 4\beta 2\delta$ receptor (blue lines). The activation and desensitization parameters for the $\alpha 4\beta 2\delta$ receptor are: $K_{\text{GABA}} = 15.7$ nM, $c_{\text{GABA}} = 0.45$, $N_{\text{GABA}} = 2$, $Q = 0.78$, $L = 6.7$ (Pierce et al., 2019). The regions in the plot indicated with thick solid lines give the levels of $P_{\text{A,s.s.}}$ and the associated $P_{\text{D,s.s.}}$ in the presence of putative ambient GABA (0.1-1 μM).

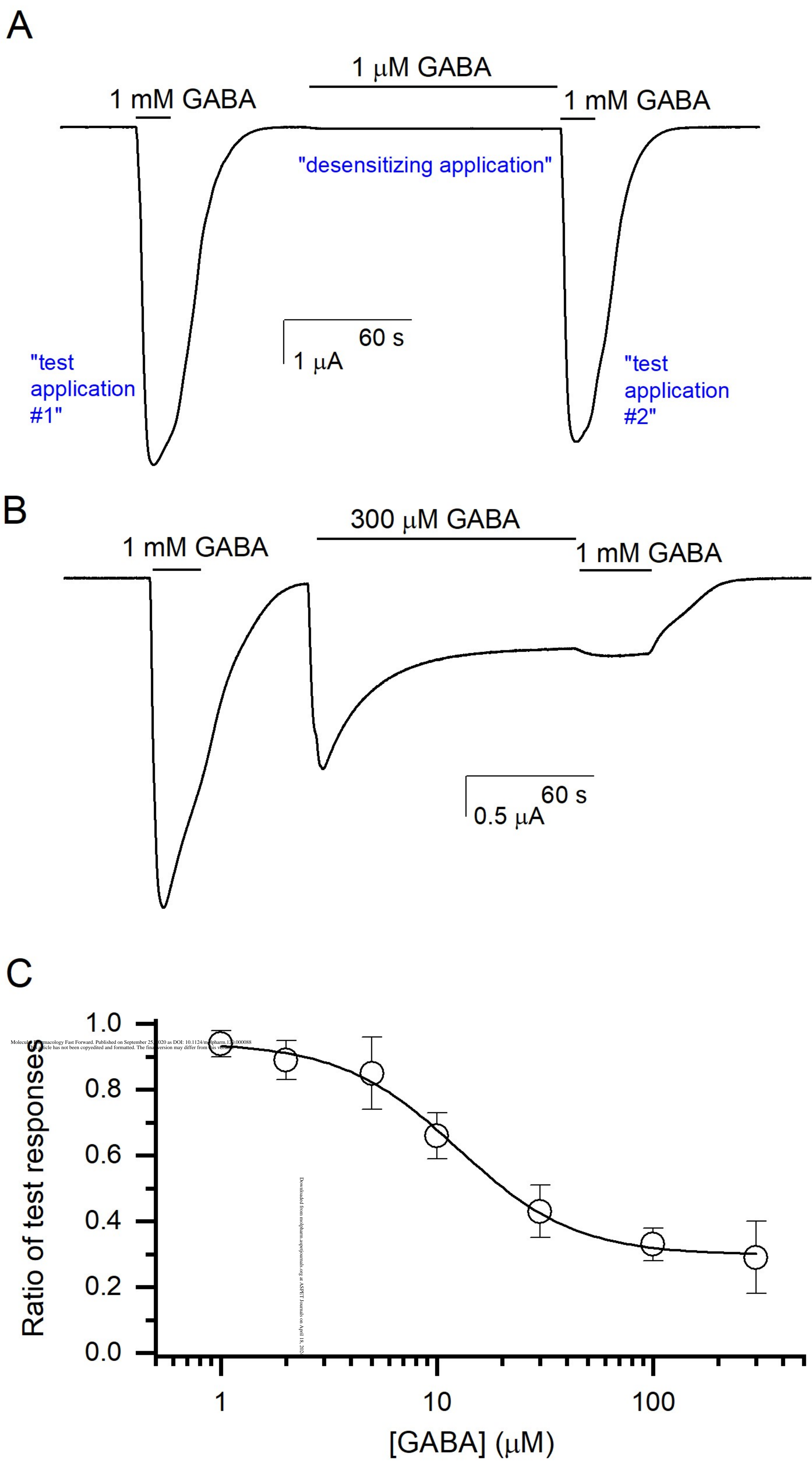
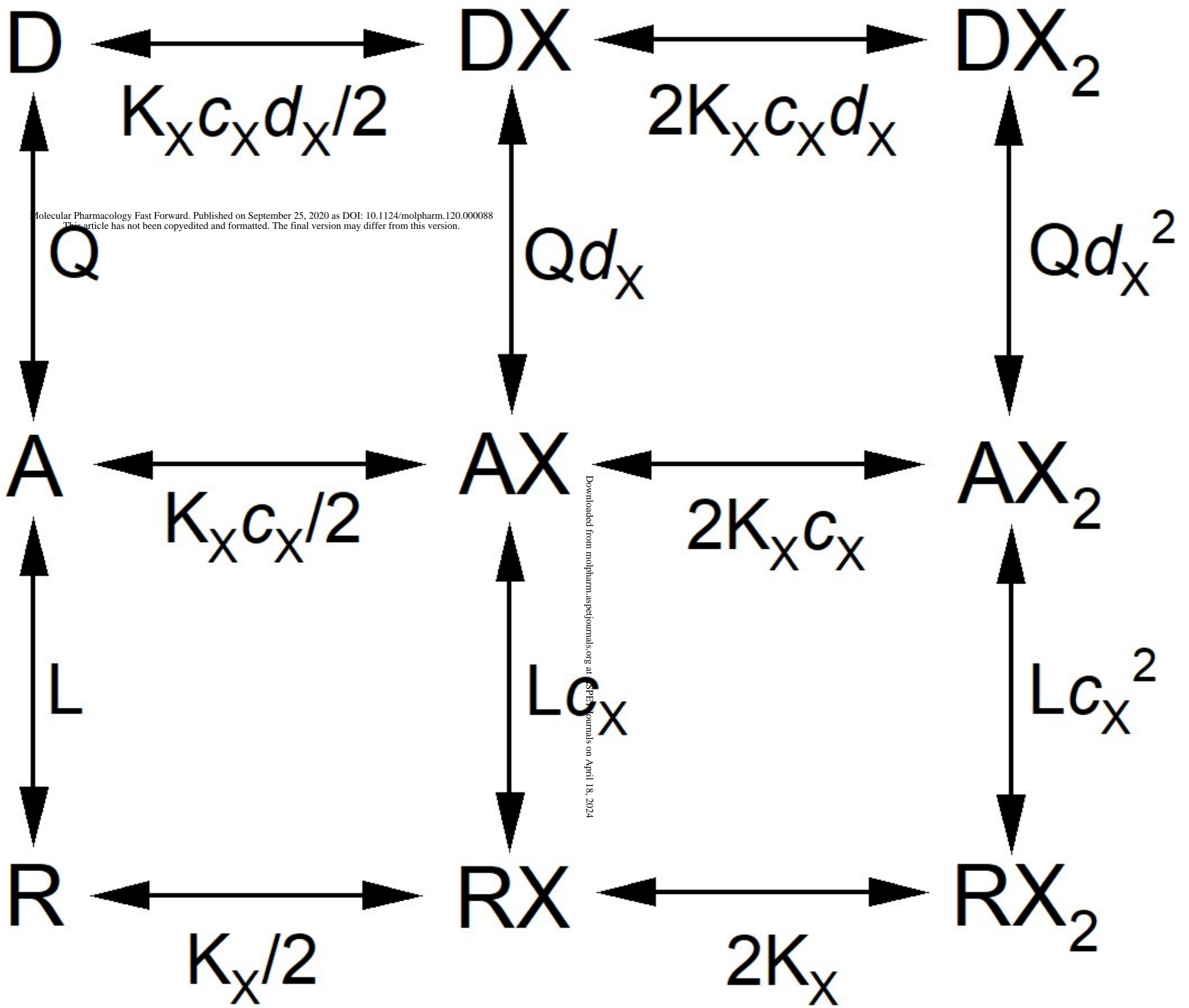


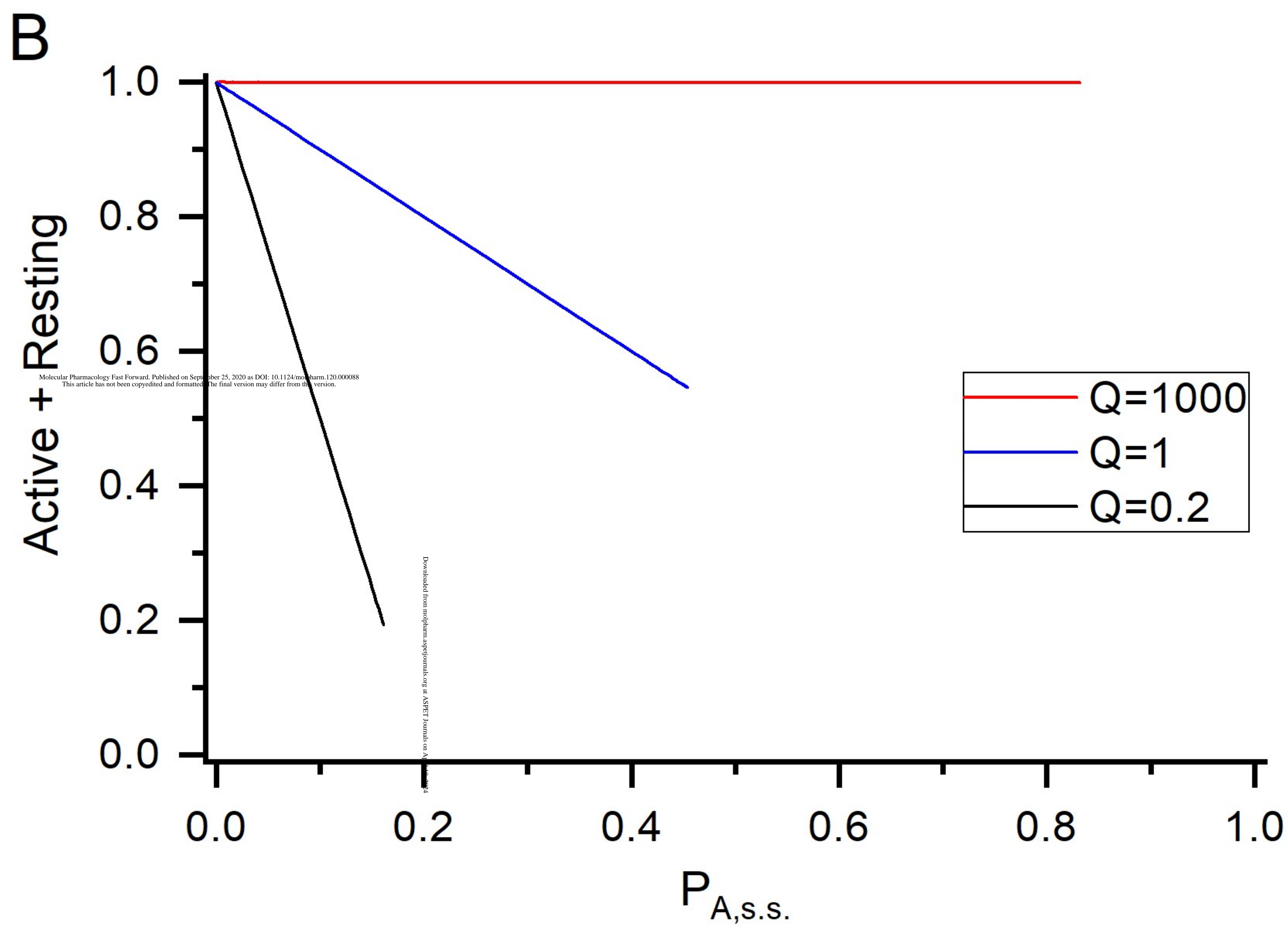
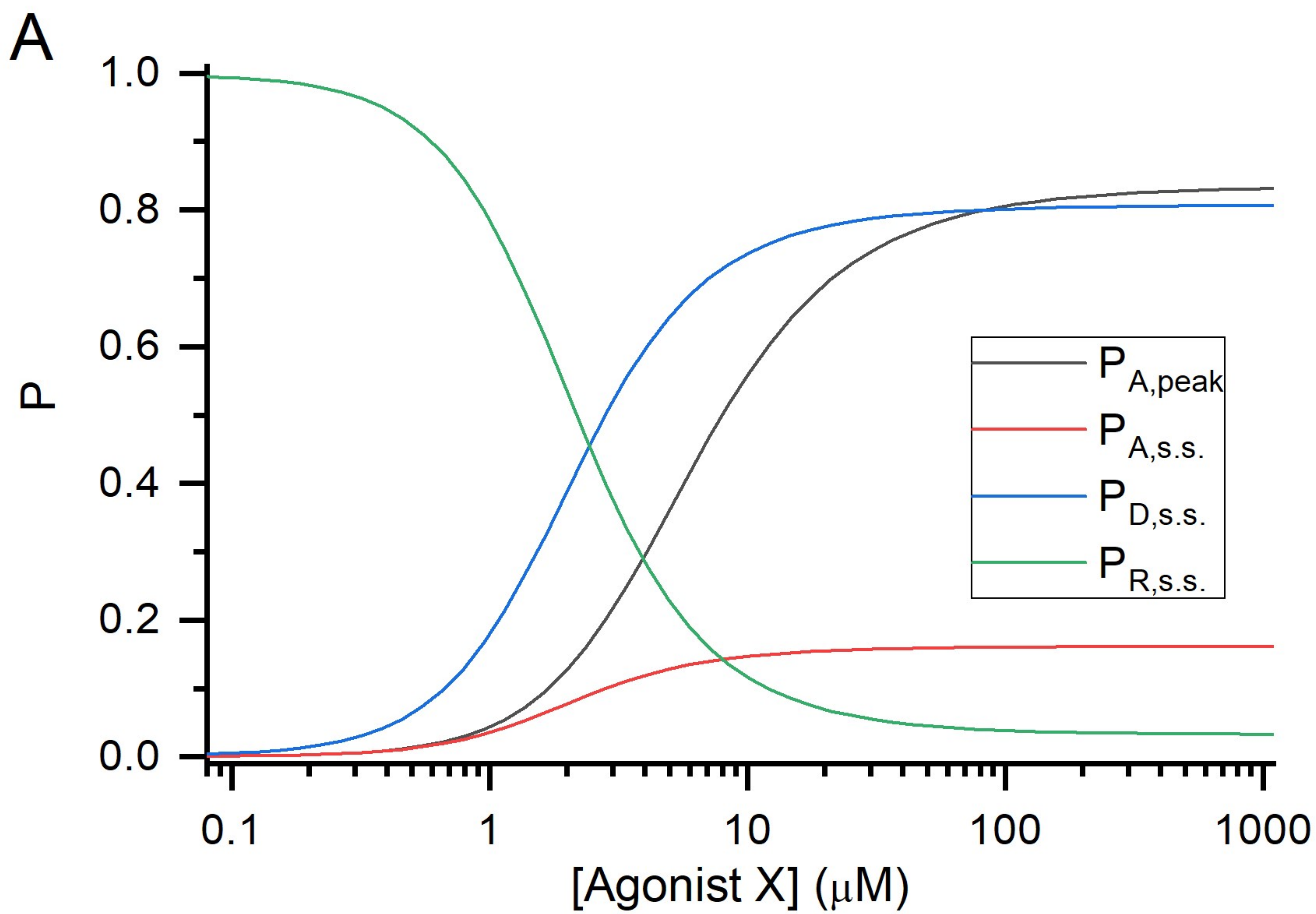
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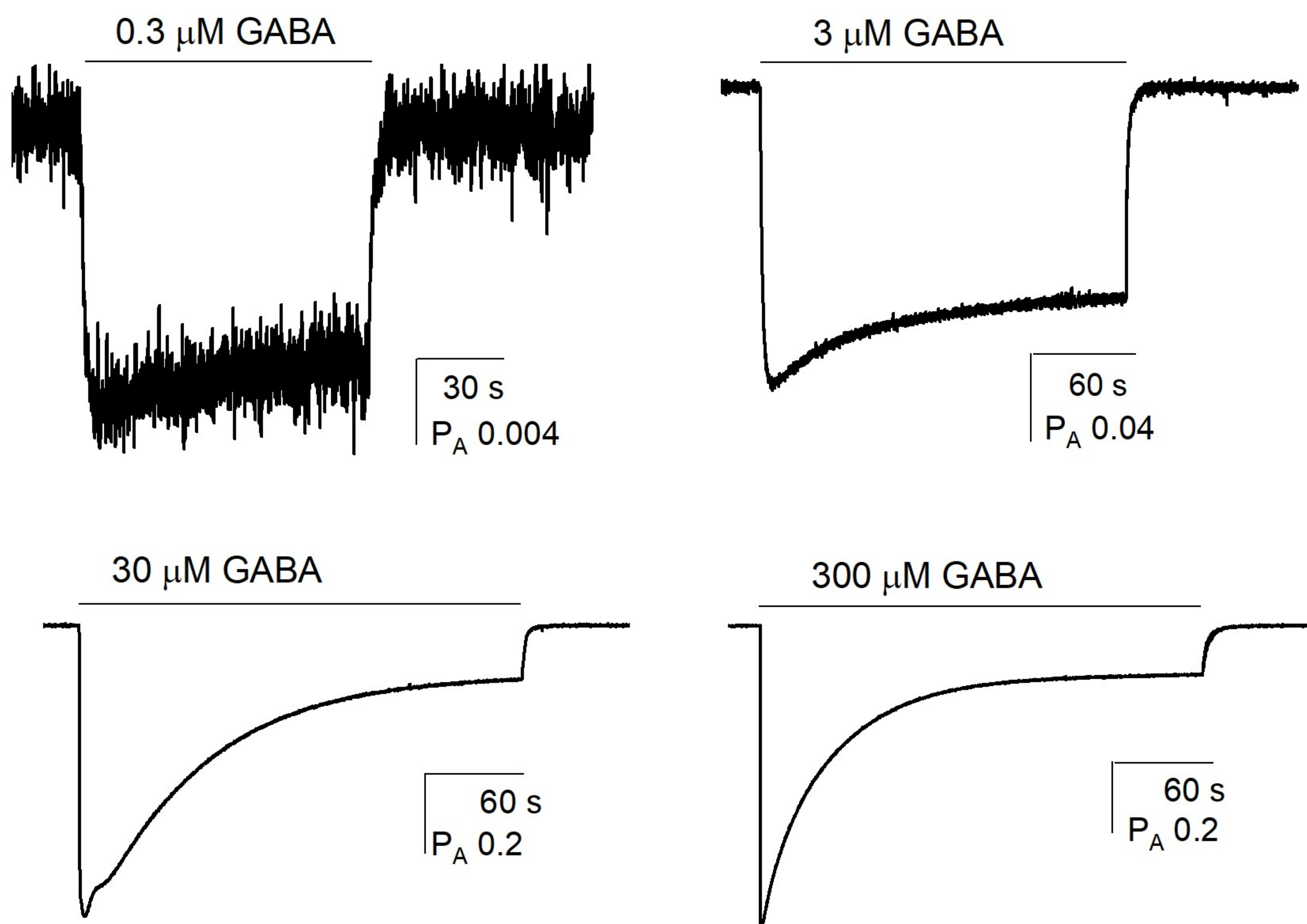
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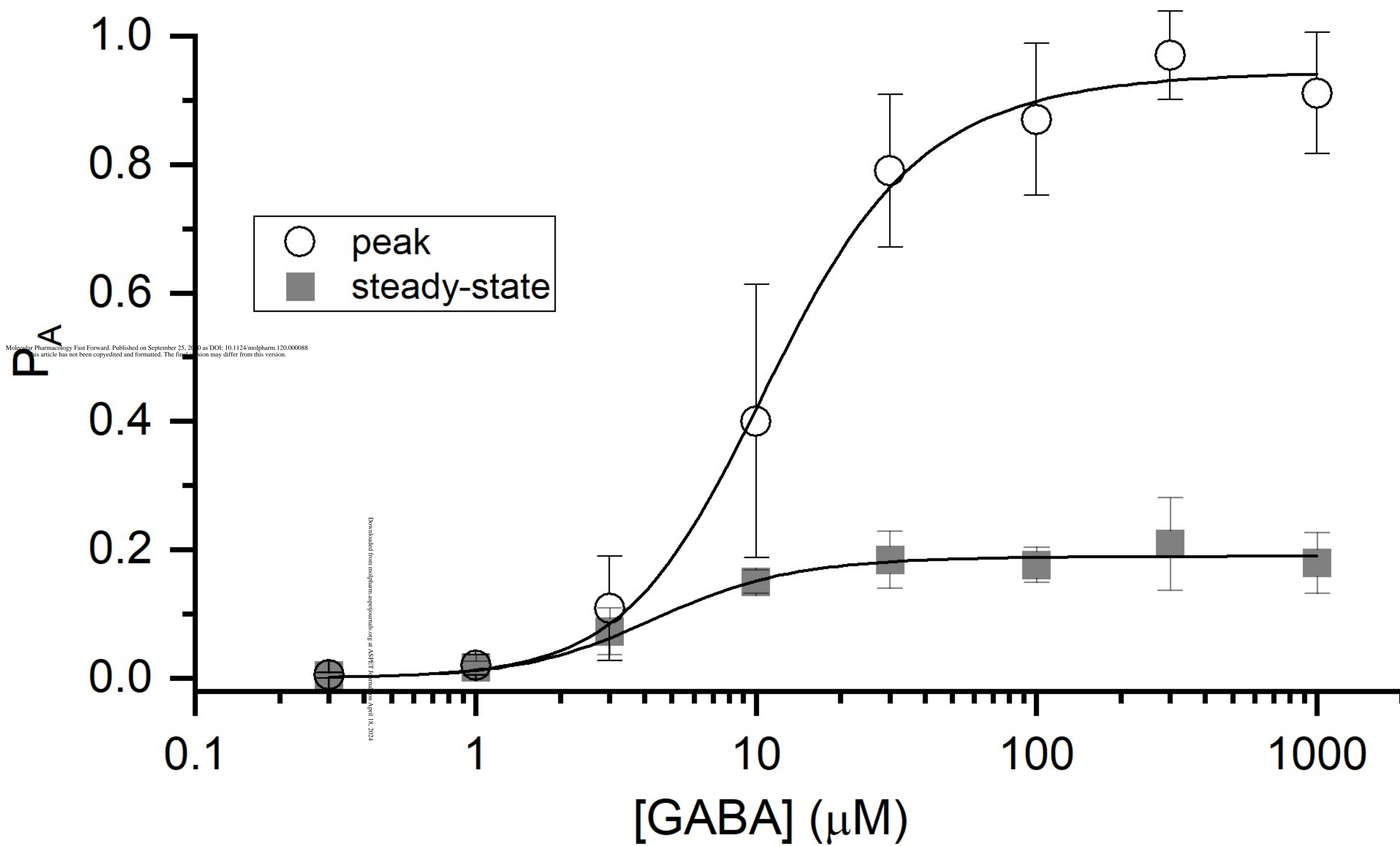
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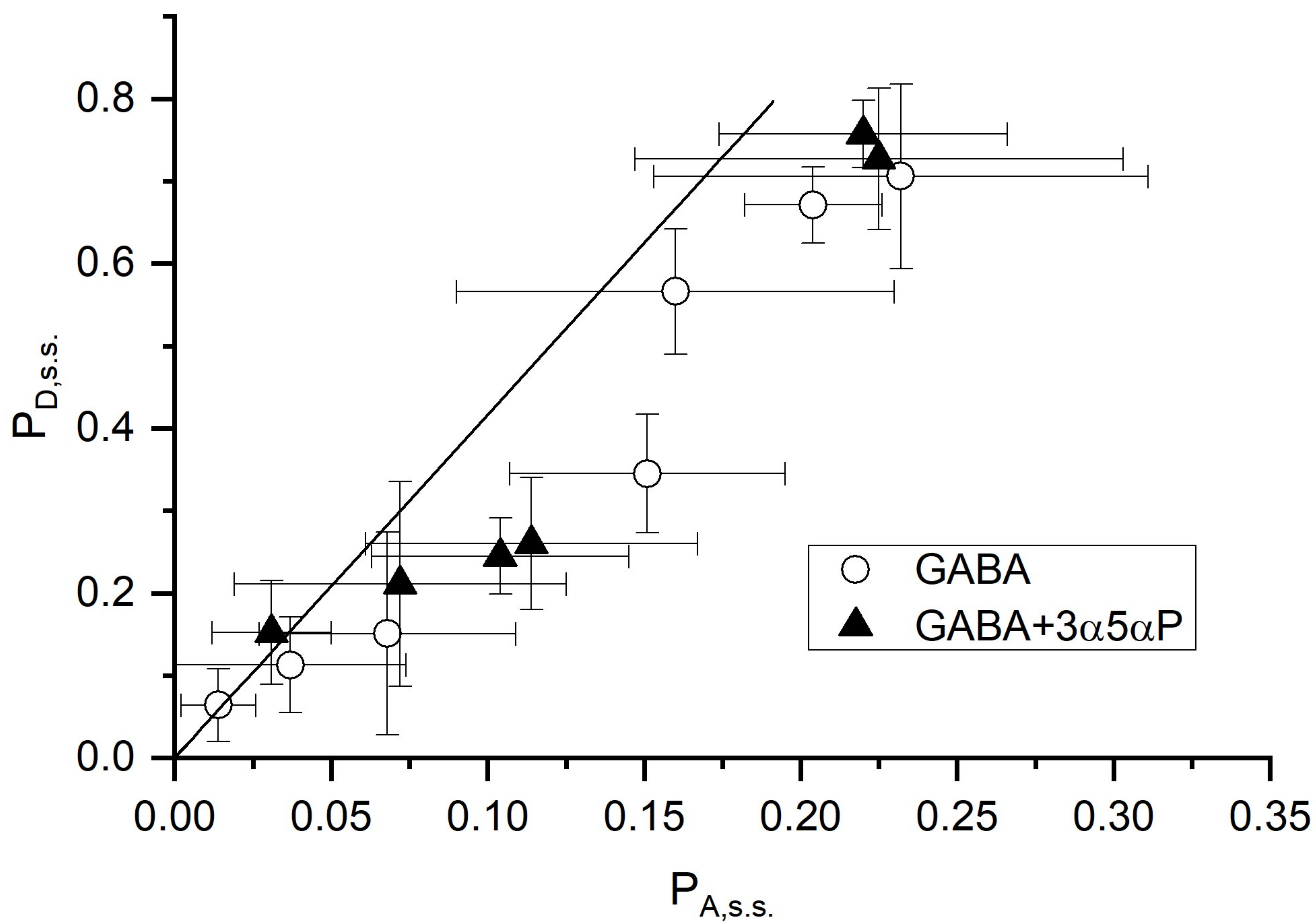
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