Structural Basis for the Allosteric Modulation of GABAA Receptors by Diazepam

Padmaja Senthil Kumar, *Lewis and Clark High School, Spokane, WA, USA*

Project Objectives

- The GABA type A receptor (GABAAR) is a pentameric ligand-gated ion channel, and is activated by GABA, a major inhibitory neurotransmitter in the human brain. GABAAR dysfunction is implicated in several CNS disorders, including anxiety, depression, and epilepsy.
- Allosteric modulators of GABAAR, such as benzodiazepines and neurosteroids, are used as antidepressant and antiseizure drugs.
- The objective of my project is to examine whether diazepam (DZP), a benzodiazepine, distinctly modulates the GABAA receptors as compared to ganaxolone (GNX), a neurosteroid.
- The results provide valuable insights into the fundamental mechanisms and may facilitate the design of new drugs with better efficacy and safety.

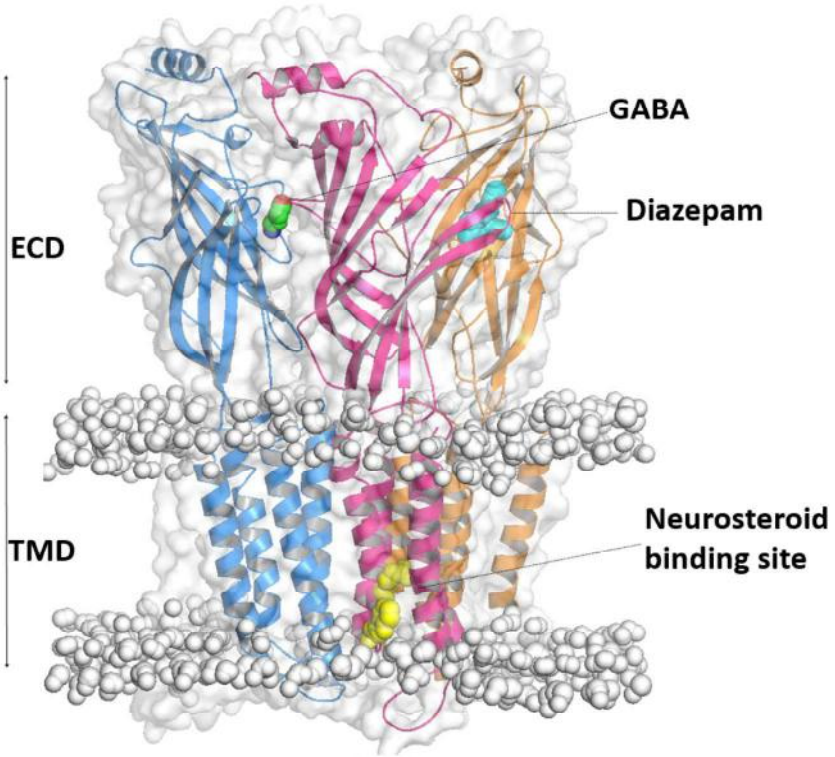


Fig. 1. GABAAR is a pentameric ligand-gated ion channel. Benzodiazepines and neurosteroids bind at the extracellular domain (ECD) and transmembrane domain (TMD), respectively, and act as allosteric modulators.

The image in Fig. 1 was created by Padmaja Senthil

Current Knowledge

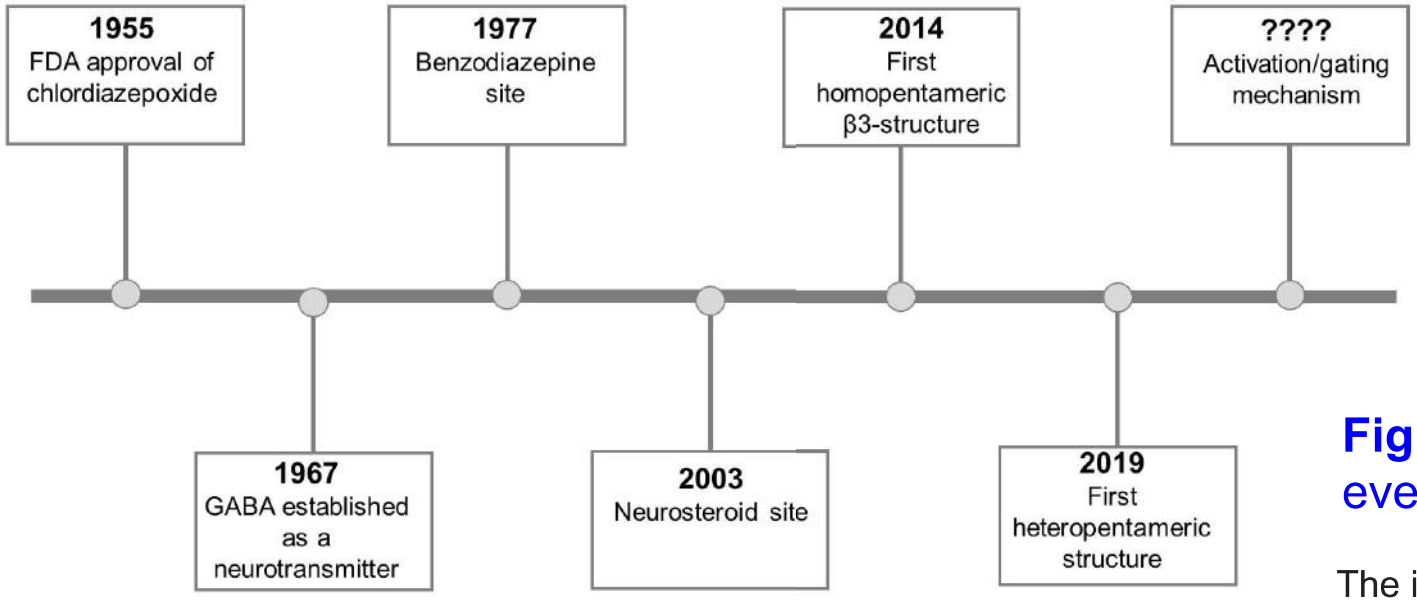


Fig. 2. Historical timeline of the events related to GABAAR

The image was adopted from *Front.* Neurosci. **2021** Vol. 14, Article. 616289

Benzodiazepines vs. Neurosteroids

- Benzodiazepines, such as diazepam, have been widely used for many decades as antidepressant and antiepileptic drugs, despite many side effects, including tolerance and potential for abuse.
- Recently, two neurosteroids (ganaxolone and allopregnanolone) have been approved by the US-FDA and these drugs demonstrate better efficacy and safety profiles. However, the structural basis for the allosteric modulation of the receptor by benzodiazepines and neurosteroids remain poorly understood.
- A recent study showed that ligands binding at the neurosteroid site induce GABA dissociation and promote the recovery of the receptor from the desensitized state (Gc et al. *Biophysical Journal*, **2023**, 122, 849-867).
- **My project aims to elucidate whether diazepam acts on the receptor in a similar or distinct manner to that of neurosteroids.**

Methodology

- I used classical molecular dynamics (MD) simulation to investigate the allosteric mechanisms of diazepam.
- GABAAR in its desensitized state (PDB ID 6HUP) bound to diazepam was subjected to 1 μs simulations.
- The trajectory obtained from the simulations were analyzed for various structural characteristics.
- **DISCLAIMER**: I only studied the system with DZP. The results/trajectory discussing ganaxolone systems were from previous research in the lab and are used for comparison.

Steps	Unbiased Simulations
Structure Preparation	Protein-ligand complex preparation PDB ID: 6HUP
Membrane Preparation	POPC, 10% cholesterol (CHARMM-GUI)
MD simulation	Unbiased MD (1 μs each)
Trajectory Analysis	VMD, PyMOL, Wordom, HOLE, and Matplotlib

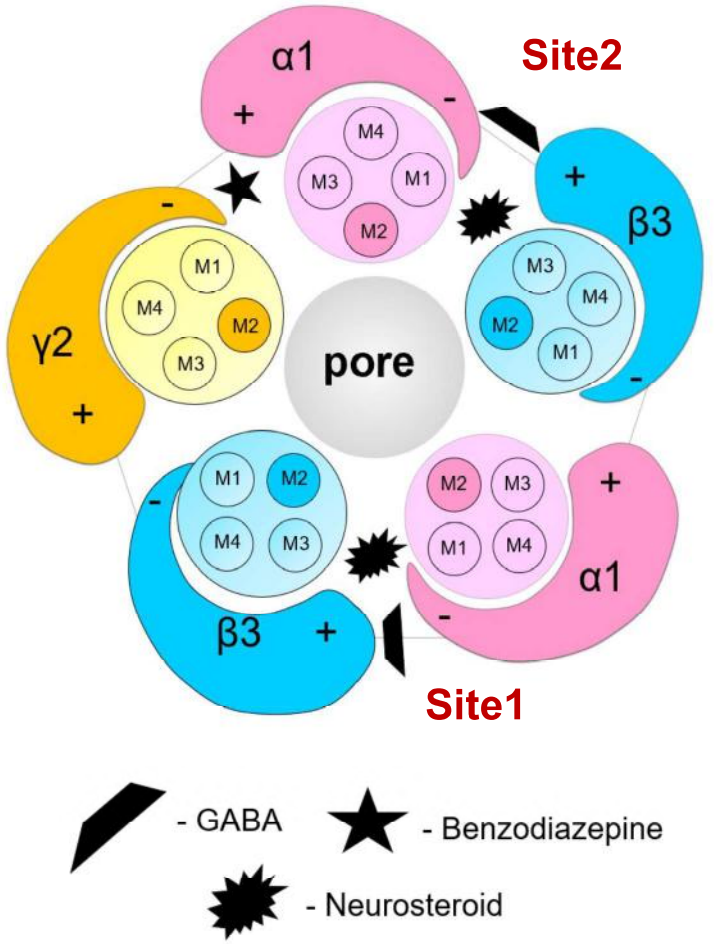


Fig. 3. Simulated systems

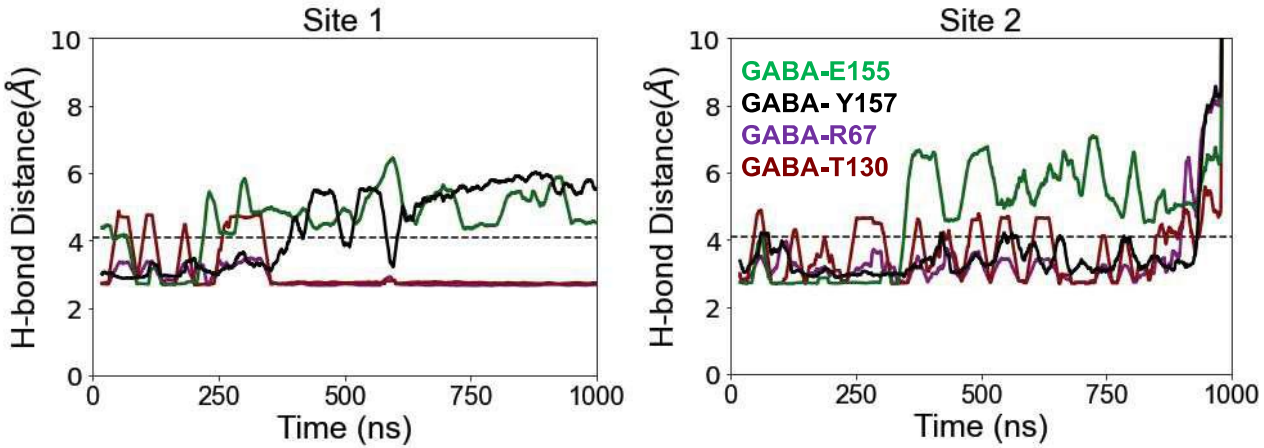
System 1	**Only GABA**
System 2	GABA + ganaxolone
System 3	GABA + diazepam

The images in Fig. 3 were created by Padmaja Senthil

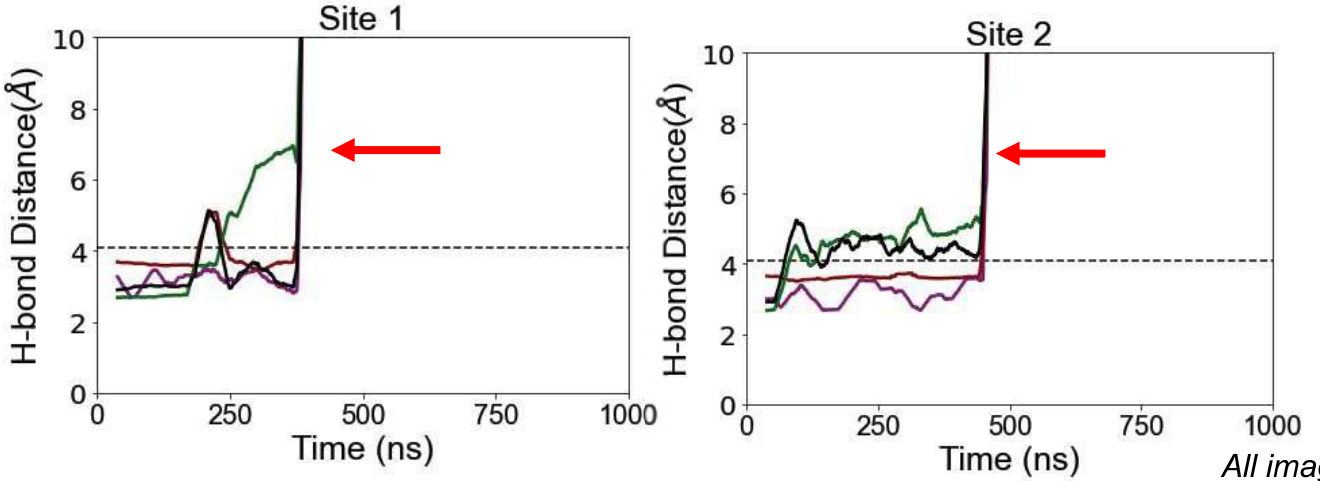
GABA Stability and affinity

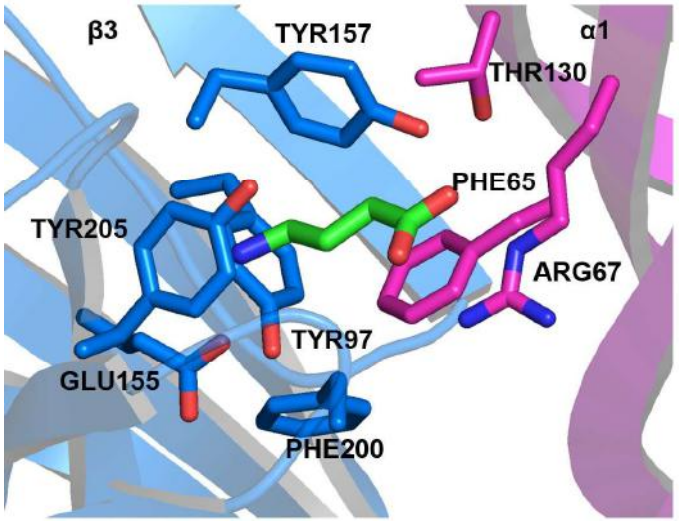
- I examined the GABA stability by quantifying the polar interactions between GABA and the binding-site residues.
- In the presence of GNX, GABA dissociated from both site 1 and site 2 (indicated by red arrows); however, diazepam did not induce such dissociation of GABA.

GABA + Diazepam



GABA + Ganaxolone





System	Binding site	
	1	2
GABA		
GABA + DZP		
GABA + GNX		

Stable Dissociation

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Diazepam distinctly affect GABA interactions at the orthosteric sites

- In addition to polar interactions, contacts between GABA and several binding site residues were analyzed over a period of 1μs.
- In the GABA only and GABA+DZP systems, GABA made higher contacts with multiple residues, indicating better stability.
- In the GABA + GNX system, there were less frequent contacts in both binding sites, indicating less stability at both binding sites. GABA dissociated ~400-500 ns.

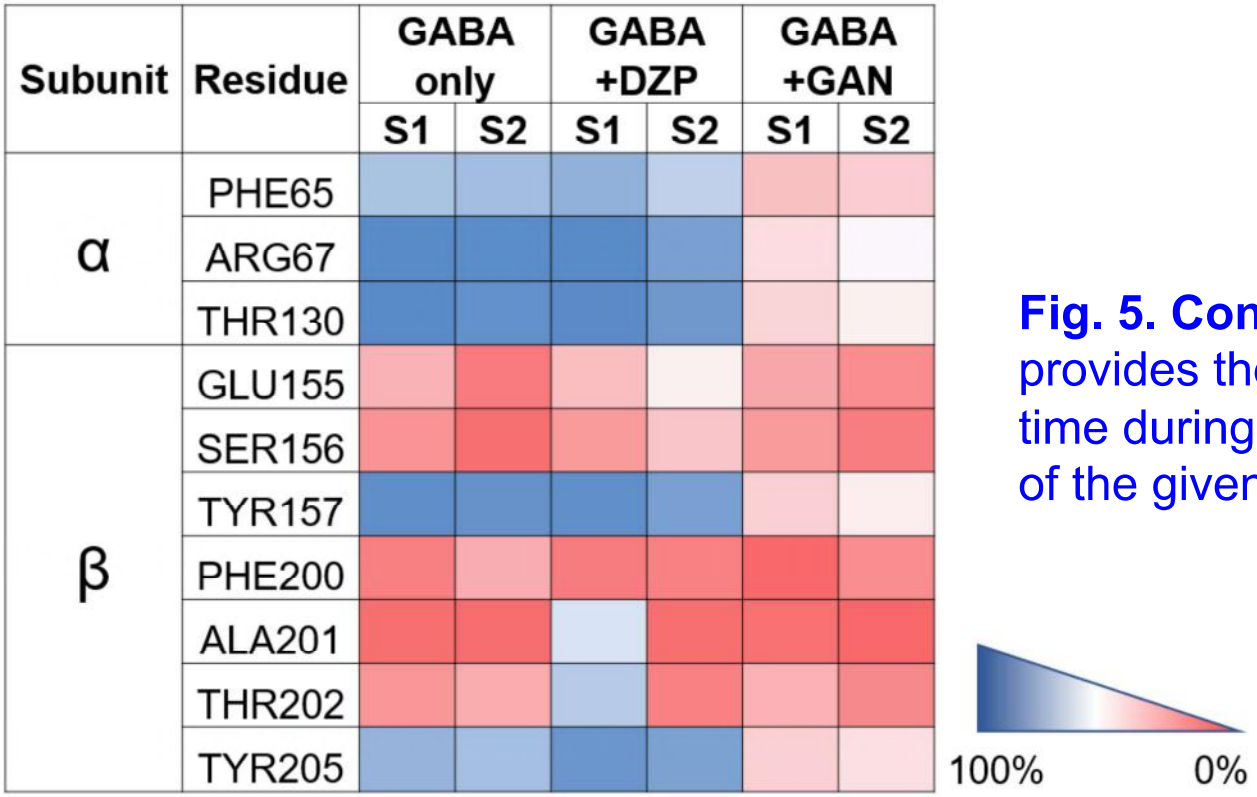
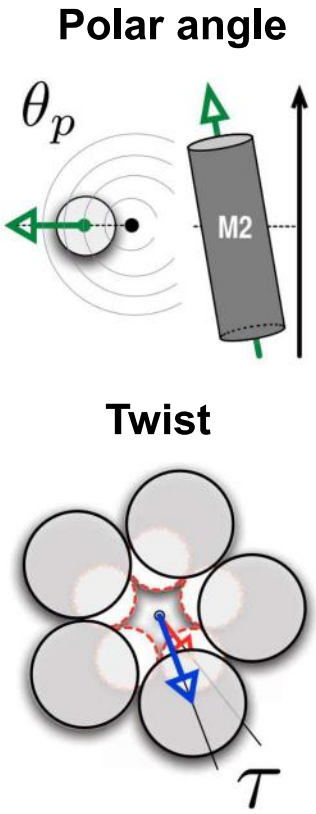


Fig. 5. Contact occupancy % - provides the fraction of the simulation time during which GABA is within 4 Å of the given residue.

The heatmap in this page was created by Padmaja Senthil

Diazepam induces distinct conformational changes

- The conformational changes in GABAAR were characterized by the twist angle (τ) of the extracellular domain over the transmembrane domain, and polar tilt angle (θ_p) of the M2 helices.
- The twist angles for the GABA only and GABA + DZP systems were very similar, staying relatively high. However, in the GABA + GNX system, the twist angle decreased significantly, indicating the active open state of the pore channel.



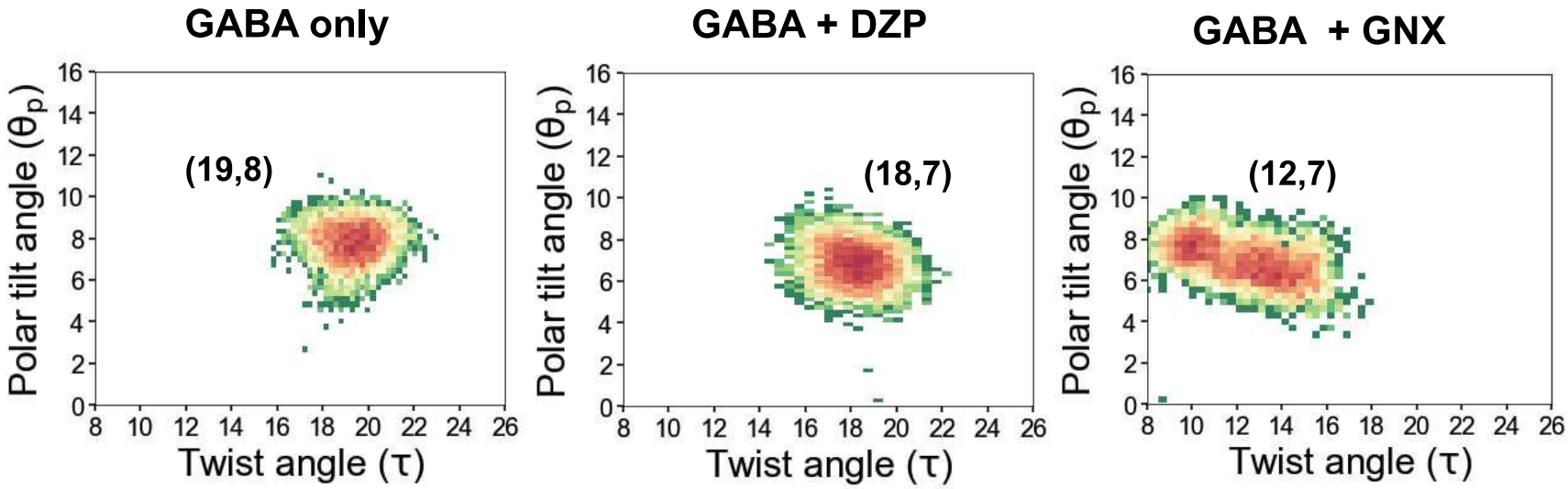


Fig. 6. Global conformational changes measured as polar and twist angles in $GABA_A$ receptor in the presence of diazepam and ganaxolone. The twist angle is significantly reduced in the presence of GNX, but not DZP.

Image courtesy: PNAS USA ***2013,*** *110(42), E3987-E3996.*

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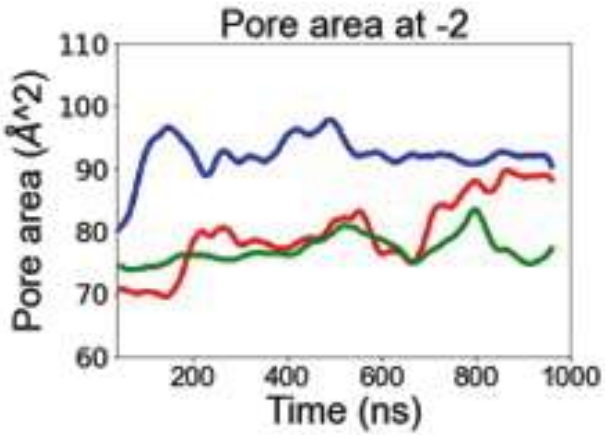
Receptor pore dynamics differ between systems

- Below are representations of the pore across all three systems. Areas colored in blue indicated pore radius > 2Å, the necessary dimensions for ion diffusion, whereas areas in green represent < 2Å. Results indicated that GNX increased the pore radius at the desensitized gate (2') more significantly than DZP, potentially retrieving the receptor from its desensitized state.



GABA only
GABA + Diazepam
GABA + Ganaxolone

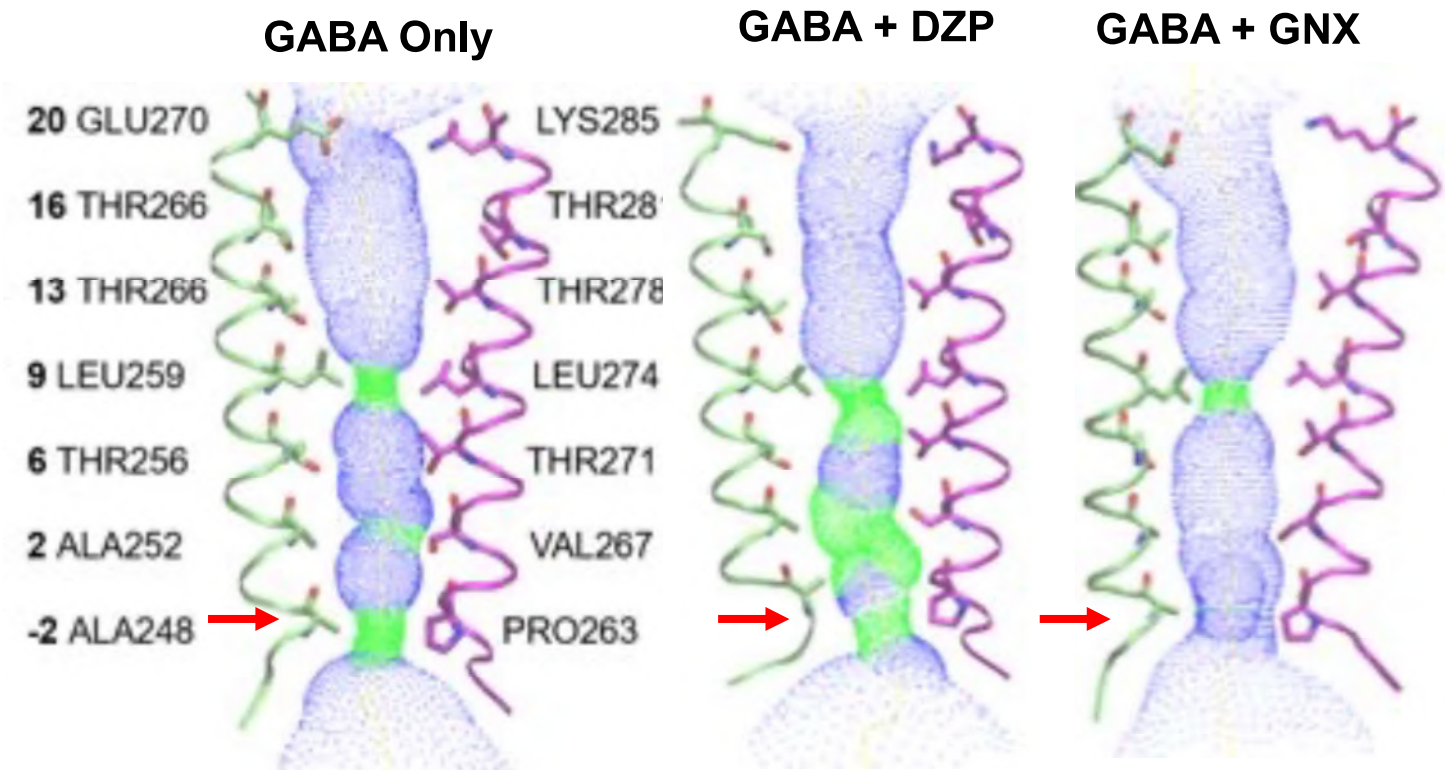


Fig. 7. Pore geometry is distinctly affected in the presence of diazepam and ganaxolone. Ganaxolone induced opening at -2' position of the channel (shown in red arrows).

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Water Flux: the hydration status of the channel

- To investigate the effect of diazepam on the functional state of the channel the water flow (hydration status) was quantified. The number of water molecules crossing the channel was counted in each frame (5000 frames) of the trajectory.

- The GABA only and GABA + DZP systems had a maximum of only one water molecule, and rarely two. However, in the presence of ganaxolone, the channel was hydrated with up to three water molecules, indicating the opening of the pore.

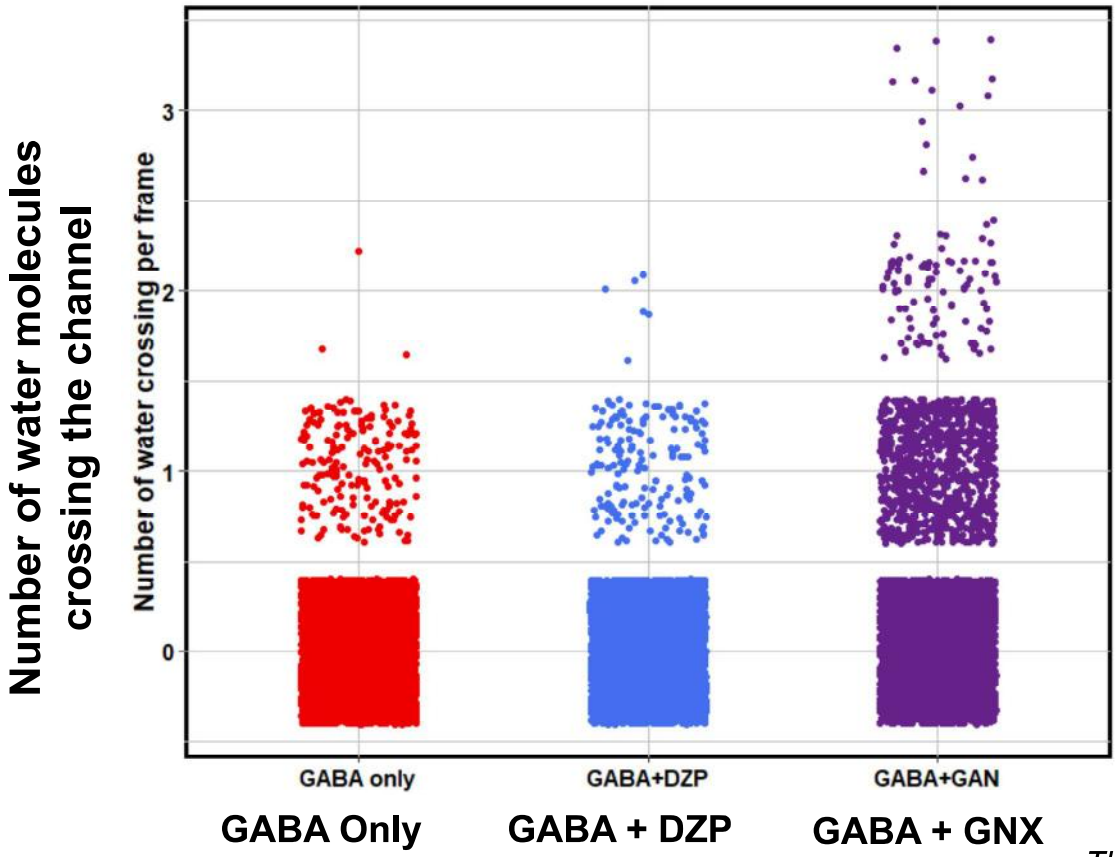

Fig. 8. Water flux: The number of water molecules crossing the channel during the simulation time.

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Limitations of this study

1. This computational study represents a simplistic representation of the human physiological system. The study did not include the complex extracellular matrix and other cell environments. However, the lack of this complexity is common among all the studied systems.

2. The used experimental structure may have errors. These *in silico* findings need further validation by experimental methods.

3. Currently, there is no experimental structure of GABAAR available in the open state. Results may be different if the simulations began with a receptor in the closed or open state rather than in the desensitized state.

Summary and conclusions

1. This computational study provides structural and dynamic analyses of the allosteric modulation of the GABAA receptor by diazepam (benzodiazepine) and ganaxolone (neurosteroid).

2. The structural observables such as twist and tilt angles, polar interactions, and pore size, clearly elucidate that ganaxolone exerts a greater allosteric effect on GABA at the orthosteric site than that of diazepam, illustrating distinct mechanisms on the desensitized state of the receptor.

3. These results enhances our fundamental understanding of the channel and may facilitate in identifying allosteric modulators with better efficacy and safety profiles.

References

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RESEARCH OBJECTIVE

- The GABA type A receptor (GABAAR) is a pentameric ligand-gated ion channel activated by GABA.
- GABAAR dysfunction is implicated in several CNS disorders, including anxiety, depression, and epilepsy. Allosteric modulators of GABAAR, such as benzodiazepines and neurosteroids, are used as antidepressants and antiseizure drugs.
- **The main objective of this project is to examine whether diazepam (DZP), a benzodiazepine, distinctly modulates the GABAA receptors as compared to ganaxolone (GNX), a neurosteroid.**

HYPOTHESIS

Benzodiazepines induce the allosteric modulation of the $GABA_A$ receptors by distinct mechanisms as compared to neurosteroids.

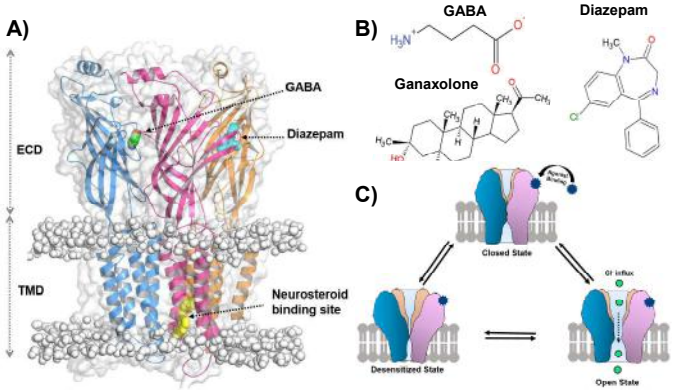


Fig. 1 Structure and topology of the heteropentameric (α1β3γ2) $GABA_A$ receptor (GABAAR). (A) Side view of the receptor along the membrane (in gray). (B) 2D-structures of GABA, diazepam, and ganaxolone. C) The three functional states of GABA: closed, open, and desensitized states. *All images were created by Padmaja Senthil.*

METHODOLOGY

- Classical molecular dynamics (MD) simulations were used to investigate the allosteric mechanisms of diazepam.
- $GABA_A$R, in its desensitized state (PDB ID 6HUP) bound to diazepam, was subjected to 1 μs simulations.
- The trajectories obtained from the simulations (three replicates) were analyzed for various structural characteristics.

Experimental Design: Schematic workflow

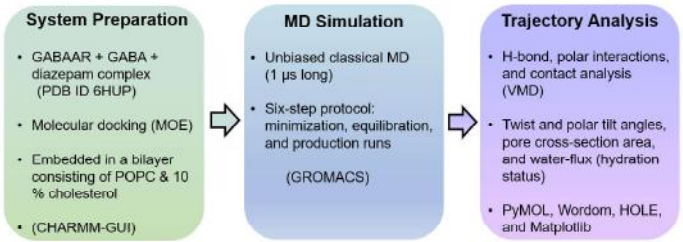
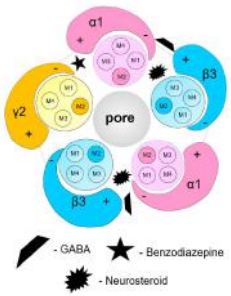


Fig. 2. The schematic workflow summarizing the input data, simulation steps, software programs, and trajectory analysis. *The workflow diagram was created by Padmaja Senthil.*

RESULTS

GABA stability is distinctly affected by DZP and GNX



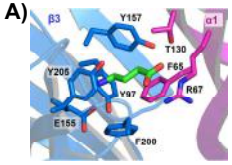
- In three replicates, the GABA-bound receptor was simulated alone, and in the presence of diazepam or ganaxolone.
- In the presence of GNX, GABA dissociated from both site 1 and site 2; however, diazepam did not induce such dissociation of GABA.

System	Ligands	Binding site	
		1	2
1	GABA		
2	GABA + DZP		
3	GABA + GNX		

Stable / Dissociation

Fig. 3. The schematic diagram of heteropentameric $GABA_A$R showing five subunits and the binding site locations for GABA, diazepam, and neurosteroids. The table summarizes GABA stability in the various studied systems. The *diagram and table were created by Padmaja Senthil.*

DZP distinctly affects GABA interactions at the orthosteric sites



- GABA stability was examined by quantifying the polar interactions between GABA and the binding-site residues. In the presence of GNX, GABA dissociated from both site 1 and site 2 (indicated by red arrows); however, diazepam did not induce such dissociation of GABA.

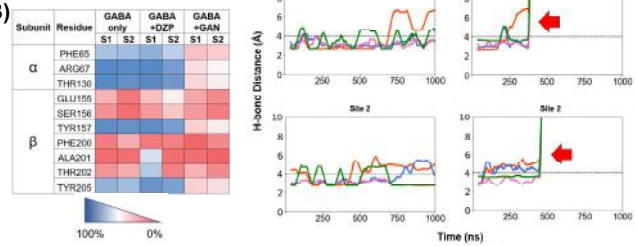
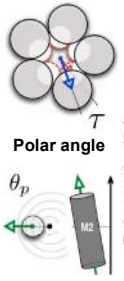


Fig. 4. The stability of GABA at two binding sites was altered by ganaxolone and diazepam in distinct ways. A) The binding mode and residue interactions of GABA at the orthosteric sites. **B)** Contact occupancy % - provides the fraction of the simulation time during which GABA is within 4 Å of the given residue. **C)** Hydrogen bond distances (in angstrom) plotted against simulation time (in nanoseconds) at both GABA sites in all three systems. GABA dissociates from both sites in the presence of GNX but not DZP. *All Images were created by Padmaja Senthil.*

DZP induces distinct conformational changes



- The conformational changes in GABAAR were characterized by the extracellular domain's twist angle (τ) over the transmembrane domain and polar tilt angle (θ_p) of the M2 helices.
- The twist angles for the GABA-only and GABA + DZP systems were similar, staying relatively high. However, in the GABA + GNX system, the twist angle decreased significantly, indicating the active open state of the channel pore.

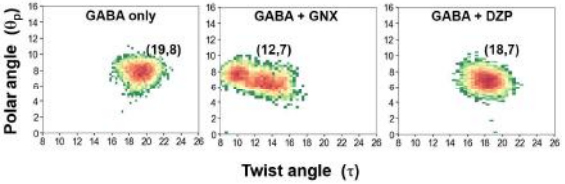


Fig. 5. Global conformational changes in $GABA_A$ receptor in the presence of diazepam and ganaxolone. The conformational changes were characterized by the extracellular domain's twist angle (τ) over the transmembrane domain and polar tilt angle (θ_p) of the M2 helices. **A)** Schematic representation of polar tilt and twist angles are shown. **B)** Polar tilt angles plotted against the twist angle in systems consisting of only GABA alone, GABA and ganaxolone, and GABA and diazepam are shown. *All images were created by Padmaja Senthil.*

Receptor pore dimensions significantly differ among the systems

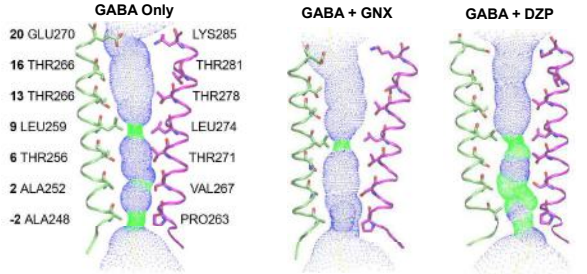


Fig. 6. The pore radius along the channel across all three studied systems. Results indicate that GNX increased the pore radius at the desensitized gate (2') more significantly than DZP, potentially retrieving the receptor from its desensitized state. The pore radius along the channel after 1 μs simulation is indicated by openings (radius > 2 Å in blue) and constrictions (< 2 Å in green). *All Images were created by Padmaja Senthil.*

The pore area and hydration significantly differ among the systems

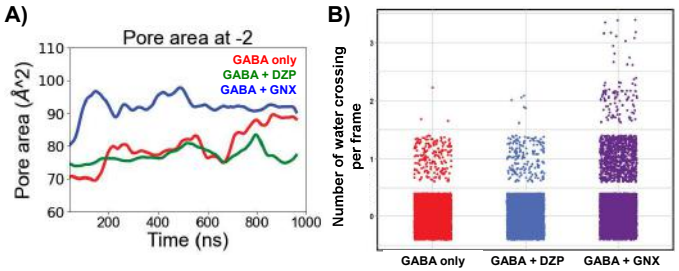


Fig. 7. Dynamics of the $GABA_A$ receptor pore in all studied systems. A) The time evolution of the pore size area at the desensitization gate (position '-2') in the three studied systems (GABA only, GABA + DZP, and GABA + GNX)**. B)** Water flux: The number of water molecules crossing the channel during the simulation time. The number of water molecules crossing the channel was counted in each frame (5000 frames) of the trajectory. The GABA-only and GABA + DZP systems had a maximum of only one water molecule and rarely two. However, in the presence of ganaxolone, the channel was hydrated with up to three water molecules, indicating the open state of the pore. *All Images were created by Padmaja Senthil.*

SUMMARY AND FUTURE DIRECTIONS

Limitations of the study

1) This computational study represents a simplistic representation of the human physiological system. The study did not include the complex extracellular matrix and other cell environments. However, the lack of this complexity is common for all the studied systems.
2) The used experimental structure may have errors. These *in silico* findings need further validation by experimental methods.

Conclusions

1) This computational study provides structural and dynamic analyses of the allosteric modulation of the $GABA_A$ receptor by diazepam (benzodiazepine) and ganaxolone (neurosteroid).
2) The structural observables, such as twist and tilt angles, polar interactions, and pore size, clearly elucidate that ganaxolone exerts a greater allosteric effect on GABA at the orthosteric site than that of diazepam, illustrating distinct mechanisms on the desensitized state of the receptor.
3) These results may be useful in identifying allosteric modulators with better efficacy and safety profiles.

References

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Acknowledgements

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