Effects of Benzodiazepines on the HPA Axis

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I. Introduction and Background:

The HPA axis plays a huge role in the nervous system and the endocrine system. It mediates the stress response and regulates the release of cortisol. Anatomical structures of the HPA axis are found in the central nervous system and peripheral nervous system. (Smith, 2006) The main effectors of the HPA axis are the paraventricular nucleus (PVN) in the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal gland. The PVN in the hypothalamus secretes cortical releasing hormone (CRH). In response to stress, CRH binds to the receptors on the anterior lobe of the pituitary gland and stimulates the release of adrenocorticotropic hormone (ACTH). ACTH is released from the pituitary gland into the bloodstream and binds to receptors on the adrenal gland, stimulating the release of cortisol. Cortisol plays two roles in this pathway: firstly, it acts as a negative regulator of CRH via a negative feedback loop. Second, cortisol binds to glucocorticoid receptors located on the pituitary gland forming a homodimer. This homodimer negatively regulates ACTH. Cortisol and the homodimer regulate the negative feedback of the production of cortisol. Gupta et. al has stated that glucocorticoid receptor expression has revealed bistability. In other words, glucocorticoid receptors and cortisol have two stable equilibrium states. During the time when the glucocorticoid receptor and cortisol levels are multivalued, the individual can be pushed from one value of the steady state to another by application of extreme stress. Moreover, due to negative feedback glucocorticoid receptors and cortisol have an inverse relationship. Therefore, when there is an increase in cortisol the levels of glucocorticoid receptors will decrease. Cortisol is the primary stress hormone and is secreted in response to stress. It has been shown that an increase in stress is linked with an increase in cortisol secretion. Increased cortisol levels have also been observed in people with anxiety disorders. (Faravelli, 2012)

As defined by the DSM-5, anxiety is a disorder that can cause severe fear and stress related behaviors. (Hinds, 2022) People experiencing anxiety symptoms can activate the stress response and can cause an increase in cortisol levels to be secreted. A popular treatment for anxiety is a benzodiazepine called alprazolam. The brand name of this drug is commonly known as Xanax. This treatment has a mechanism of action that is associated with activating the function of GABA in different areas of the CNS, including their potential role in regulation of the HPA axis. (Tafet, 2020) Alprazolam is a benzodiazepine agonist and it has been shown in vivo to exert inhibitory effects on the HPA axis. It binds to GABAergic receptors on the hypothalamus causing a hyperpolarization and an inhibition to the target neurons, thereby inhibiting the production of CRH. Decrease in the production of CRH results in the decrease of cortisol secretion. Decreasing the secretion of cortisol decreases the symptoms of stress and anxiety.

In this project, we will look at how the components of the HPA axis interact with one another during a normal stress response. The components that we will focus on are CRH, ACTH, cortisol, glucocorticoid receptor, and the homodimer of glucocorticoid receptors bound to cortisol. We will also discuss the regulation of the HPA axis when it is under the influence of

alprazolam, a benzodiazepine agonist. By modeling this process, we will show the dynamic effect of the components of the HPA axis as well as alprazolam. We will also perform a sensitivity analysis to determine which parameters are the most sensitive to the overall model.

I. Graphical Model:

This graphical model represents the dynamic effects of the components of the HPA axis. (Figure 1). Negative feedback of the HPA axis is shown in red arrows. The state variables in this diagram are cortical releasing hormone (CRH), adrenocorticotropic hormone (ACTH), cortisol (O), glucocorticoid receptor (R), and the homodimer of glucocorticoid receptors bound to cortisol (OR).

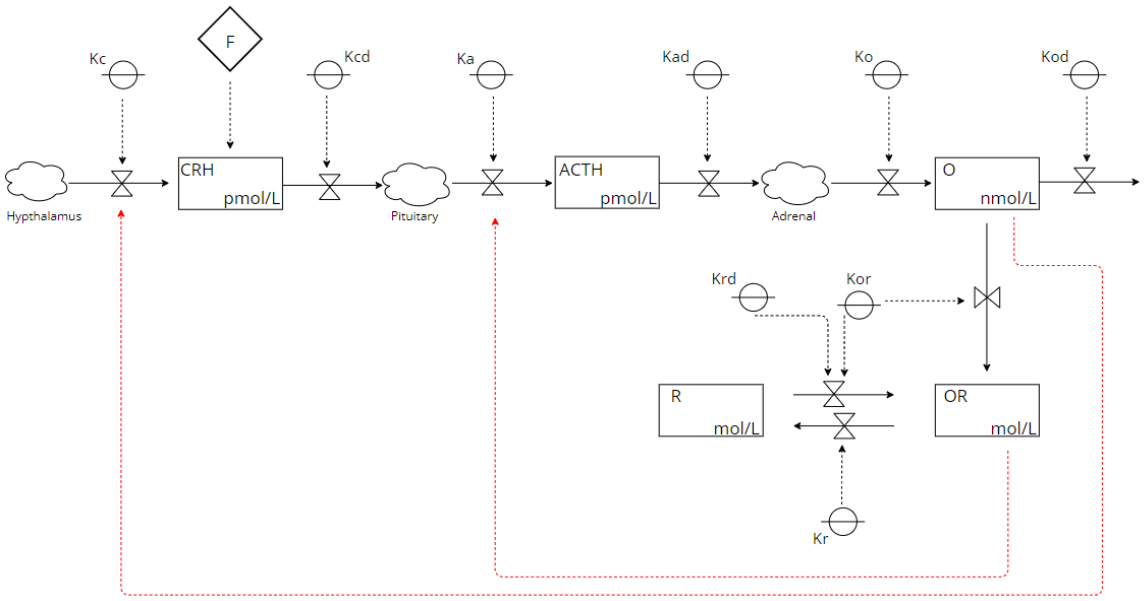

Figure 1. Forrester diagram of the HPA axis

II. Mathematical Equations

The model equations used in the regulation of the HPA axis are Michaelis-Menten ordinary differential equations (ODE) and they were derived by Sriram et al. and Gupta et al. According to Gupta et al. these models were successful at capturing negative feedback features. They were derived with the goal to understand the dynamic effects of CRH, ACTH, and cortisol and gain insight into the HPA axis. The parameter values for normal state were referenced from Gupta et al. and are listed in Table 1. The initial conditions for this model are listed in Table 2. To note, the homodimer (OR) doesn't have an equation included because it is in constant quasi-equilibrium and the model is assuming that O and R are binding with very fast kinetics

compared to the rest of the four state variables. Equations have variables scaled in order for the simulation to operate with fewer parameters provided by Gupta et al.

- **CRH**

$$\frac{dC}{dt} = \frac{K_c + F}{1 + \frac{O}{K_{i1}}} - K_{cd}C$$

= Production - Degradation

- **ACTH**

$$\frac{dA}{dt} = \frac{K_a C}{1 + \frac{OR}{K_{i2}}} - K_{ad}A$$

= Production - Degradation

- **Cortisol (O)**

$$\frac{dO}{dt} = K_o A - K_{od}O$$

= Production - Degradation

- **Glucocorticoid Receptor (R)**

$$\frac{dR}{dt} = \frac{K_r(OR)^2}{K + (OR)^2} + K_{or} - K_{rd}R$$

= Represents homodimer dimerizing with fast kinetics + Production - Degradation

Table 1. Model Parameter values

Parameter Description	Symbol	Unit Value
Production of CRH	K_c	1 M
Constant Degradation of CRH	K_{cd}	1 M
Production of ACTH	K_a	1 M
Constant Degradation of ACTH	K_{ad}	10 M
Production of cortisol	K_o	1 M
Constant Degradation of cortisol	K_{od}	1 M
Production of glucocorticoid receptor	K_r	1 M
Constant Degradation of glucocorticoid receptor	K_{rd}	0.9 M

Production of homodimer	K_{or}	0.05 M
Inhibition constant 1	K_{i1}	0.1
Inhibition constant 2	K_{i2}	0.1
Equilibrium binding affinity	K	0.001

Table 2. Model Initial Values

State Variable	**Initial Value**
CRH	0 pmol/L
ACTH	0 pmol/L
Cortisol	0 nmol/L
Glucocorticoid Receptor	0 mol/L

III. Simulation Results

Following a short stress that was applied for one hour, the response of cortisol and ACTH showed an initial increase, followed by a decrease to a plateau due to the synthesis of CRH. Once the stress ended, CRH levels quickly decreased due to its degradation. The response of the glucocorticoid receptor was shown to slightly elevate and then return to baseline. These responses were also seen when stress was applied repeatedly. Glucocorticoid receptors increased when cortisol and ACTH decreased due to negative feedback. During the application of chronic stress for ten hours, the increase in glucocorticoid receptor concentration resulted in lower than basal levels of cortisol and ACTH production.

The HPA axis under the influence of benzodiazepines would decrease the parameters for the production and degradation of CRH, ACTH, O, and R, resulting in a smaller amplitude compared to the normal state due to the drug's inhibitory effects. (Arvat, 1999) For this simulation, the parameters for the production and degradation of CRH, ACTH, O and R were decreased by 70% to model the inhibitory effects of Alprazolam. All parameters show a decrease in concentration due to the effects of the drug. The value for applied stress (F), according to Sriram et. al, was 7.6 μM. We scaled that up, and used 7600 as our value for stress.

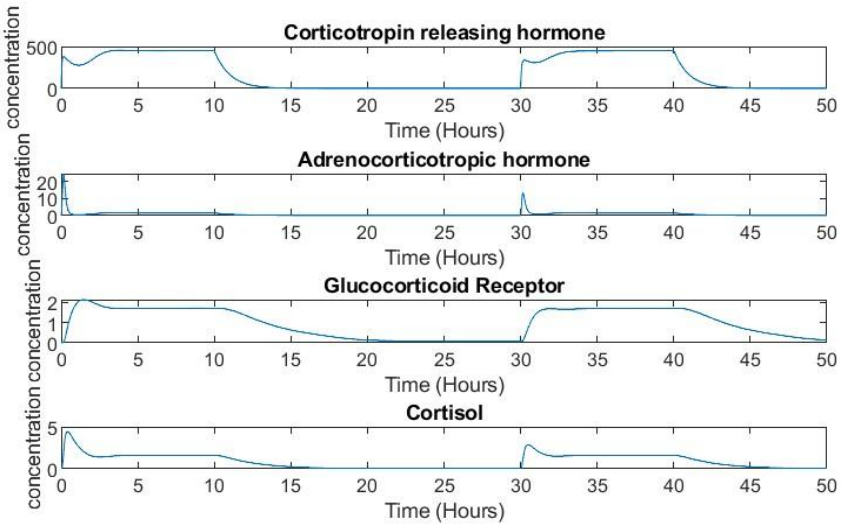

Figure 2: Transient responses of HPA axis to recursive stress

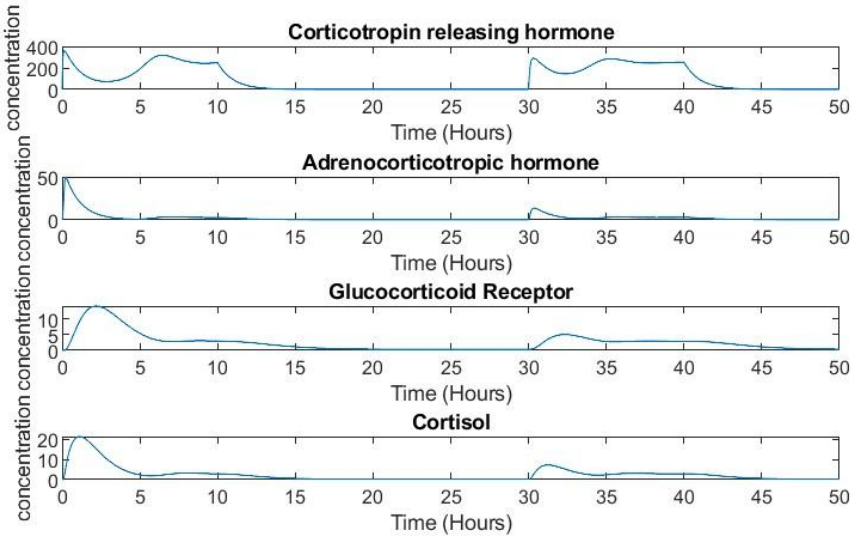

Figure 3: Transient responses of HPA axis under the influence of benzodiazepines to recursive stress

IV. Sensitivity Analysis

The CRH concentration was found to be most sensitive to changes in the degradation of CRH, K_{cd}, and cortisol, K_{od} . This is suggestive of the roles that the concentrations of CRH and cortisol play in the control of and regulation of the HPA axis' reaction to stress. The analysis

shows CRH was more sensitive to increases in K_{cd} ,with increases in this value resulting in a decrease in the concentration of CRH. This indicates that the roles of CRH and cortisol concentration is in a negative feedback loop and cortisol plays a prominent role in the concentration of CRH, most likely due to negative feedback. Similarly, cortisol and ACTH concentrations were found to be most sensitive to perturbations to the CRH degradation constant, K_{cd}, and the cortisol degradation constant , K_{od}. Cortisol concentrations were affected equally by the same increases and decreases in K_{od} and K_{cd}. This points to the importance of cortisol concentrations as perturbations to these parameters cause an increase in cortisol which increases the amount of negative feedback control that cortisol is able to exert, i.e. the more cortisol present the stronger the negative feedback. Lastly, the glucocorticoid receptor concentration was found to be most sensitive to changes in the rate of cortisol degradation, K_{od}, and the rate of glucocorticoid receptor degradation K_{rd}. Similar to cortisol, receptor concentrations were more sensitive to increases in these parameters, resulting in higher receptor concentrations. These increases result in down regulation of the production of ACTH which in turn causes a reduction in cortisol synthesis.

The cortisol and ACTH concentration was found to be most sensitive to changes in the degradation in cortisol, K_{od} , and CRH, K_{cd}. Cortisol and ACTH are more sensitive to the degradation of cortisol compared to CRH. The glucocorticoid receptor concentration was found to be most sensitive to the changes in the degradation of cortisol K_{od} and changes in the degradation in glucocorticoid receptor, K_{rd} . This is likely because the glucocorticoid receptor plays an important role when interacting with cortisol and changes in cortisol levels can affect the concentration of activated glucocorticoid receptors.

Sensitivity Analysis for CRH

	5% Decrease	5% Increase	10% Decrease	10% Increase
Kcd	0.771	0.713	0.808	1.369
Kod	0.269	0.253	0.273	0.493
ki1	0.016	0.016	0.019	0.032
kad	0.016	0.013	0.014	0.026
krd	0.003	0.003	0.003	0.006
kor	0.288	0.269	0.294	0.525
ki2	0.010	0.013	0.011	0.022
k	0.000	0.000	0.000	0.000
Kcd	0.000	0.000	0.000	0.000
kad	0.000	0.000	0.000	0.000
Kr	0.509	0.489	0.518	0.976
Ko	0.000	0.000	0.000	0.000

Sensitivity Analysis for ACTH

	5% Decrease	5% Increase	10% Decrease	10% Increase
Kod	0.735	0.668	0.751	1.269
Kcd	0.735	0.668	0.751	1.269
kad	0.033	0.067	0.050	0.100
ki1	0.033	0.033	0.033	0.067
krd	0.000	0.033	0.000	0.033
ki2	0.267	0.267	0.267	0.501
kor	0.033	0.033	0.033	0.067
k	0.000	0.000	0.000	0.000
Kcd	0.000	0.000	0.000	0.000
kad	0.000	0.000	0.000	0.000
Kr	1.336	1.336	1.352	2.604
Ko	0.000	0.000	0.000	0.000

Sensitivity Analysis for Receptor

	5% Decrease	5% Increase	10% Decrease	10% Increase
Kod	0.499	0.443	0.540	0.803
krd	0.499	0.443	0.540	0.803
kor	1.690	1.440	1.856	2.687
Kcd	1.219	1.191	1.205	2.410
kad	0.305	0.277	0.319	0.526
k	0.194	0.166	0.180	0.332
ki1	0.028	0.000	0.028	0.028
ki2	0.000	0.000	0.000	0.000
Kcd	0.000	0.000	0.000	0.000
kad	0.000	0.000	0.000	0.000
Kr	2.216	2.825	1.981	6.537
Ko	0.000	0.000	0.000	0.000

Sensitivity Analysis for Cortisol

	5% Decrease	5% Increase	10% Decrease	10% Increase
Kod	0.735	0.668	0.751	1.269
Kcd	0.735	0.668	0.751	1.269
kad	0.033	0.067	0.050	0.100
ki1	0.033	0.033	0.033	0.067
krd	0.000	0.033	0.000	0.033
ki2	0.267	0.267	0.267	0.501
kor	0.033	0.033	0.033	0.067
k	0.000	0.000	0.000	0.000
Kcd	0.000	0.000	0.000	0.000
kad	0.000	0.000	0.000	0.000
Kr	1.336	1.336	1.352	2.604
Ko	0.000	0.000	0.000	0.000

V. Conclusion

To conclude, the value for K_{rd} affects the concentration of glucocorticoid receptors. Changes in this can also affect the concentration of cortisol as cortisol and glucocorticoid receptors are sensitive to the degradation of cortisol. Moreover, K_{rd} also plays a role in the bistability of glucocorticoid receptors and cortisol. Genetic differences in individuals contribute to the variations in this parameter. When the value for K_{rd} is high, glucocorticoid receptor concentration is at a low steady state and cortisol concentration is at a high steady state. When the value for K_{rd} is low, glucocorticoid receptor concentration is at a high steady state and cortisol concentration is at a low steady state. This inverse relationship is due to negative feedback. Transient increases in cortisol levels are important for the maintenance of the body's homeostasis. That being the case, this altered state of low cortisol concentration can be observed in hypocortisolism. An altered state where there is high cortisol concentration can be observed in other stress-related illnesses such as chronic anxiety. For that reason, it is important to also look

into how the effects of CRH, ACTH, cortisol, and glucocorticoid receptors can lead to targeted treatments. Alprazolam, a popular treatment for anxiety, is shown to inhibit the HPA axis. Inhibiting the HPA axis causes a downstream effect and ultimately decreases the levels of cortisol production. This model could assist in creating models for different drug treatments, which could be the focus for future studies.

VI. Statement of Contribution

In terms of the contributions, the members did the following: Krupa suggested the topic and article, as well as completed the Introduction, Diagram, Mathematical Equations, and Conclusion. Alex and Daniel both contributed to MATLAB, more specifically Alex did the Simulations and Daniel did the Sensitivity Analysis. The paper was edited and reviewed by everyone.

VII. Reference

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VIII. Appendix

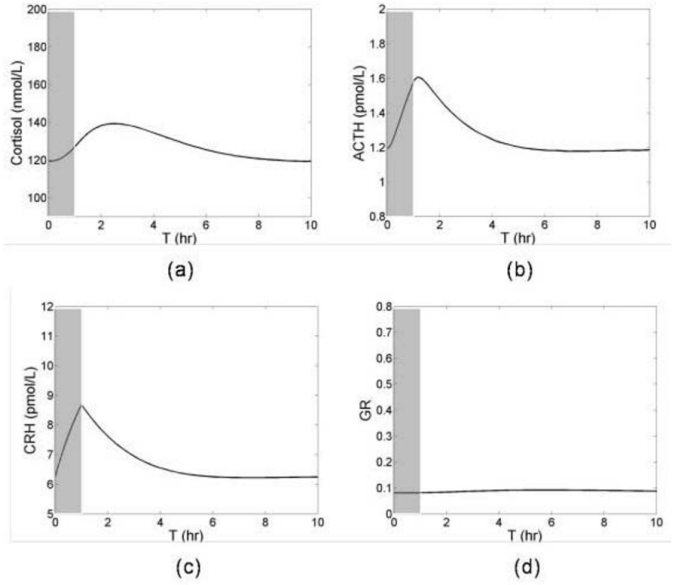

Figure 4
The response of the HPA axis following a short stress. Short time stress as indicated by the shaded larea was given for 0<T<1 hr.

(Gupta, 2007)

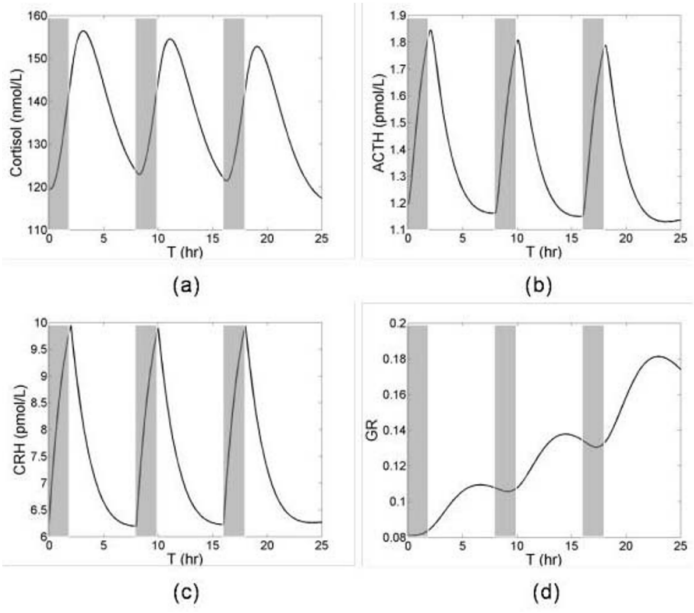

Figure 5
Transient responses of HPA axis to recursive stresses. Initially HPA axis was at a lower GR steady state and stress was given at T = 0, 8 and 16 for 2 hours. Repeated stresses are shown by shaded areas.

(Gupta, 2007)

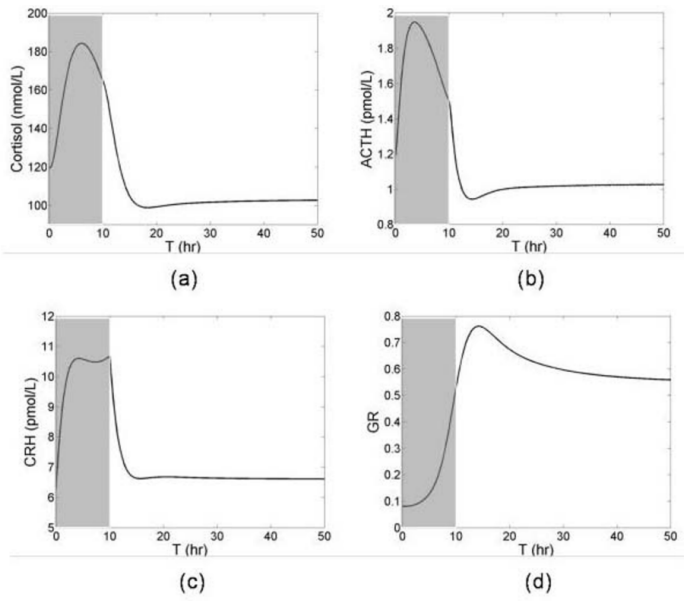

Figure 6
Transient responses of HPA axis to chronic stress. Extended length stress was given for 0<T<10. Stress is indicated with shading.

(Gupta, 2007)