INCORPORATION OF VARIABILITY INTO THE MODELING OF VIRAL DELAYS IN HIV INFECTION DYNAMICS

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ABSTRACT. We consider classes of functional differential equation models which arise in attempts to describe temporal delays in HIV pathogenesis. In particular, we develop methods for incorporating arbitrary variability (i.e., general probability distributions) for these delays into systems that cannot readily be reduced to a finite number of coupled ordinary differential equations (as is done in the method of stages). We discuss modeling from first principles, introduce several classes of nonlinear models (including discrete and distributed delays), and present a discussion of theoretical and computational approaches. We then use the resulting methodology to carry out simulations, perform parameter estimation calculations, and fit the models to experimental data. Results obtained suggest the statistical significance of the presence of delays and the importance of including delays in validating mathematical models with experimental data. We also show that the models are quite sensitive to the mean of the distribution which describes the delay in viral production, whereas the variance of this distribution has relatively little impact.

Keywords: HIV Pathogenesis; Eclipse Phase; Variability; Nonlinear Delay Equations

1. BACKGROUND

Viruses are obligate parasites with a multitude of pathways for infecting and reproducing within their target hosts. The Human Immunodeficiency Virus (HIV) is a lentivirus that is the causative agent for the slow, progressive, and fatal Acquired Immune Deficiency Syndrome (AIDS). According to a Joint United Nations Programme on HIV/AIDS June 2000 report, there were approximately 34.3 million

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individuals infected with HIV/AIDS worldwide at the end of 1999, including 24.5 million in sub-Saharan Africa [19]. HIV-related illness and death is and will continue to be an important clinical and public health issue as well as an international security, stability, and development issue. Clearly it is imperative that we attain a greater understanding of HIV/AIDS viral infection dynamics.

When an HIV virion comes into contact with an uninfected target cell, the viral envelope glycoproteins fuse to the cell's lipid bilayer at a CD4 receptor site. For HIV, the core of the virus is composed of single-stranded viral RNA and protein components. The moment a virion contacts the appropriate receptor site, the cell is described as being *acutely infected*. The viral core enters the cell where the protein components enable transcription and integration of the viral RNA into viral DNA and then incorporation into the cellular DNA (provirus). With its altered cellular DNA, the cell produces capsids and protein envelopes and transcribes multiple copies of viral RNA. The cell assembles a virion by encasing the viral RNA in a capsid and then a protein envelope. The new virion pushes out through the cell membrane budding off in chains of virions (though sometimes single virions do float away). If the acutely infected cell survives through this first viral release, it can subsequently become a *chronically infected* cell. Note that in the chronic stage, it is possible for the cells to continue to divide and to produce virions, albeit at a different rate than acutely infected or non-infected cells.

Clearly neither the time from viral infection to viral production (sometimes called the *eclipse phase* [10, 14, 18]) nor the transformation from acute infection to chronic infection are instantaneous. It is known that the first viral release occurs approximately 24 hours after the initial infection, while somewhat after this release (perhaps several hours), the cellular dynamics change to those characteristic of a chronic infection [8, 9, 10, 13, 16]. Development of mathematical models and the associated numerical techniques that incorporate these delays into models for HIV infection dynamics is the primary motivation for our efforts here.

One approach to modeling systems with delays is sometimes referred to as the *method of stages* and is described in [6, 7, 9, 12, 13]. Models of HIV infection dynamics with delays have been shown to produce dramatically different conclusions than those without delays. Mittler, et. al. [13] show that including a delay in the viral production of infected cells dramatically changes the estimates of viral clearance. Grossman, et. al., [9] show that including a delay model for death of infected cells results in different conclusions about residual transmission of infection in the presence of drugs that effectively

reduce viral load. Lloyd [12] shows that failing to include delays in models for HIV infection dynamics results in underestimates of the basic reproductive number R_0, which in turn results in overly optimistic conclusions about treatment efficacy. We remark that all of the previously cited papers represent the delays using a gamma distribution to describe the delay kernel, and reduce the resulting system of integro-differential equations to a system of (non-delayed) ordinary differential equations, which can easily be simulated using standard mathematical software. An alternative method (an implementation of which is a focus of this paper) that first converts a delay system into an abstract evolution equation (before numerical simulation) was described in [1, 2, 4]. This approach allows for simulation of systems with general kernels describing the delay distributions, and does not require that the model be reduced to a system of ODEs.

In this paper, we concentrate on the mathematical modeling of viral dynamics, focusing in particular on the mathematical aspects and biological nature of the delays. We also extend previous modeling work on HIV infection dynamics for *in vitro* laboratory experiments from the (continuous) delay differential equations developed in [5], which in turn were based on a discrete dynamical system from [11].

2. MODELS

We begin with a modification of the system of ordinary differential equations developed in [5] given by

$$\begin{aligned} \dot{V}(t) &= -cV(t) + n_A A(t) + n_C C(t) - pV(t)T(t) \\ \dot{A}(t) &= (r_v - \delta_A - \gamma - \delta X(t))A(t) + pV(t)T(t) \\ \dot{C}(t) &= (r_v - \delta_C - \delta X(t))C(t) + \gamma A(t) \\ \dot{T}(t) &= (r_u - \delta_u - \delta X(t) - pV(t))T(t) + S \ , \end{aligned} \tag{2.1}$$

for $0 \leq t \leq t_f$ with t_f finite, where the parameters and the compartments are described in Tables 1 and 2, respectively, and t is the continuous independent time variable. In the first equation, the $-pV(t)T(t)$ term is designed to account for the biological fact that upon infecting a cell, a virion is unable to infect additional target cells. Models possessing this term are inherently different from many *in vivo* models in which the (large) number of target cells is assumed to be constant. If over the time scale of interest,

Notation	*Description*
c	Infectious viral clearance rate
n_A	Infectious viral production rate for acutely infected cells
n_C	Infectious viral production rate for chronically infected cells
γ	Rate at which acutely infected cells become chronically infected
r_v	Birth-rate for virally infected cells
r_u	Birth-rate for uninfected cells
δ_A	Death-rate for acutely infected cells
δ_C	Death-rate for chronically infected cells
δ_u	Death-rate for uninfected cells
δ	Density dependent overall cell death-rate
p	Probability of infection
S	Constant rate of target cell replacement

TABLE 1. *in vitro* model parameters

Notation	*Description*
V	Infectious viral population
A	Acutely infected cells
C	Chronically infected cells
T	Uninfected or target cells
X	Total cell population (infected and uninfected) $(A + C + T)$

TABLE 2. *in vitro* model compartments

the T variable were a constant T_0 (such as in [13, 15, 17]), the equation would be

$$\dot{V}(t) = -(c + pT_0)V(t) + n_A A(t) + n_C C(t)$$

and we could define a new coefficient $c' = c + pT_0$ for the $V(t)$ term. For our *in vitro* model, we do not have this situation, as the target cell population is not replenished and thus not held constant in the experiment. In other computational results (not reported on here), we omitted the $-pV(t)T(t)$ term from the first equation of (2.1) and were also able to attain reasonable fits for our limited data set (albeit with different parameters in the models) along with statistically significant results analogous to those reported below.

This model, and all subsequent modifications, were designed with the goal of gaining a deeper understanding of *in vitro* experiments (such as those described in [20]). Thus, our model and discussions here deal exclusively with the simulation of and goodness of fit to *in vitro* data. However, we also

wish to develop approaches that may be used as an aid in understanding *in vivo* phenomena and thus our methods must be sufficiently flexible to accommodate information (from HIV infected subjects) concerning inter-individual and intra-individual delay time variability.

We call attention in (2.1) to the terms describing the rates of change in the population of virions, acutely infected cells, chronically infected cells, and uninfected cells ($\dot{V}(t)$, $\dot{A}(t)$, $\dot{C}(t)$, and $\dot{T}(t)$, respectively, in (2.1)). In particular, we should comment on the form of the nonlinear terms (e.g., $pV(t)T(t)$). Terms such as $pV(t)T(t)$ are obviously only first approximations to the density dependent (on V and T) component of the rate of new infections. A more realistic model requires that this term, dependent on both $V(t)$ and $T(t)$, be bounded in the limit, i.e., saturation should be modeled in the nonlinear term so that in the limit it is (at least) affine in V or T. While we use this term in our uncertainty analysis below, for well posedness considerations the term pVT is more appropriately replaced by a function $p(V,T)$ where $x \mapsto p(x)$, $x = (V,T)$ is globally Lipschitz (see [1] for the standard form of this assumption). However, for our initial purposes in modeling discussions, the simpler term will suffice.

The data from [20] is depicted on a log plot (note the exponential growth) in Figure 2.1. Clearly the number of data points is insufficient to carry out (with any degree of confidence) rigorous inverse problem (delay parameter estimation) investigations or to perform a legitimate statistical analysis with models such as (2.1) and its extensions discussed below. However, to illustrate our methodology, we can still perform the inverse problem calculations (fully aware of their inadequacies) to obtain an estimate of the delays and then compare these calculated values with the experimentally accepted ones.

3. Modeling of Delays and Variability

As mentioned in Section 1, it is known that there exist temporal delays between viral infection and viral production and between productive acute infection and chronic infection.

A central focus of our modeling effort has been on attempting to obtain reasonable mathematical representations of these delays. The problem of how to mathematically represent these phenomena is decidedly nontrivial and includes issues such as how to account for intra-individual variability (e.g., intercellular variability arising within a single infected individual or laboratory assay) and/or inter-individual variability arising between individual subjects or data from multiple assays. In the present paper, we do not specifically address these different sources of variability, although our model is sufficiently

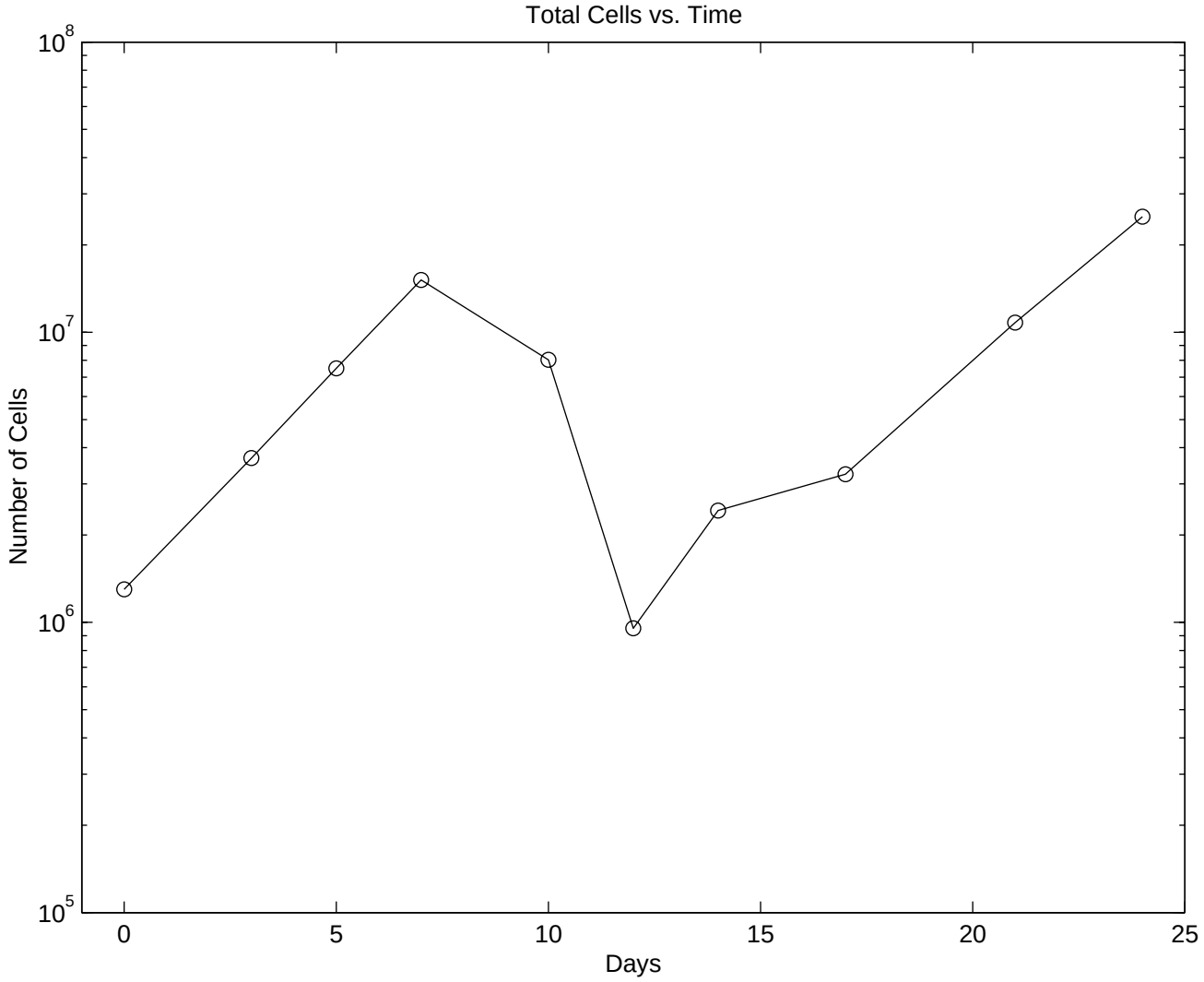

FIGURE 2.1. Log plot of experimental data (10 observations) from [20].

flexible to account for either type. These issues are highly significant and dealing with the levels of variability and the resulting mathematical ramifications is a primary focus of this paper.

Let the delay in the first equation in (2.1) be modeled by treating the delay time τ between acute infection and viral production as a probabilistic quantity (i.e., a random variable) with distribution $P_1(\tau)$ so that the first equation in (2.1) is replaced by (see the appendix for a more detailed discussion of the foundations underlying such an equation)

$$\dot{V}(t) = -cV(t) + n_A \int_{-\infty}^{0} A(t+\tau) dP_1(\tau) + n_C C(t) - pV(t)T(t)\,. \tag{3.1}$$

Likewise, let the delay between acute infectivity and chronic infectivity (with distribution $P_2(\tau)$) be represented in altered forms of the second and third equations of (2.1) by

$$\dot{A}(t) = (r_v - \delta_A - \delta X(t))A(t) - \gamma \int_{-\infty}^{0} A(t+\tau)dP_2(\tau) + pV(t)T(t) \tag{3.2}$$

$$\dot{C}(t) = (r_v - \delta_C - \delta X(t))C(t) + \gamma \int_{-\infty}^{0} A(t+\tau)dP_2(\tau)\,. \tag{3.3}$$

Note that assuming Dirac distributions with atoms at $(-\tau_1)\,, (-\tau_1 - \tau_2) < 0$ respectively, for P_1, P_2 reduces the system to

$$\begin{aligned} \dot{V}(t) &= -cV(t) + n_A A(t-\tau_1) + n_C C(t) - pV(t)T(t) \\ \dot{A}(t) &= (r_v - \delta_A - \delta X(t))A(t) - \gamma A(t-\tau_1-\tau_2) + pV(t)T(t) \\ \dot{C}(t) &= (r_v - \delta_C - \delta X(t))C(t) + \gamma A(t-\tau_1-\tau_2) \\ \dot{T}(t) &= (r_u - \delta_u - \delta X(t) - pV(t))T(t) + S\ , \end{aligned} \tag{3.4}$$

for $0 \le t \le t_f$ where t_f finite. Moreover it becomes the special case

$$\begin{aligned} \dot{V}(t) &= -cV(t) + n_A \textstyle\int_{-\infty}^{0} A(t+\tau)k_1(\tau)d\tau + n_C C(t) - pV(t)T(t) \\ \dot{A}(t) &= (r_v - \delta_A - \delta X(t))A(t) - \gamma \textstyle\int_{-\infty}^{0} A(t+\tau)k_2(\tau)d\tau + pV(t)T(t) \\ \dot{C}(t) &= (r_v - \delta_C - \delta X(t))C(t) + \gamma \textstyle\int_{-\infty}^{0} A(t+\tau)k_2(\tau)d\tau \\ \dot{T}(t) &= (r_u - \delta_u - \delta X(t) - pV(t))T(t) + S\ , \end{aligned} \tag{3.5}$$

whenever P_1, P_2 possess probability densities k_1, k_2 respectively. In the subsequent discussions in this paper, all numerical simulations for each of the systems of functional differential equations (FDE) given above were performed using the methods described in Section 4. Figure 3.1 depicts simulations of the system (3.4) with and without simple discrete delays (i.e., $\tau_1 = 26$ and $\tau_2 = 3$ in (3.4) vs. the undelayed system in (2.1)). Both the undelayed system solutions and the delayed system solutions were computed using the method described in Section 4 for $N = 32$ with the parameters described in Table 3. Not surprisingly, the presence of nonzero delays has a dramatic effect upon the simulation. Issues relating to the exact nature of τ and whether or not it should be modeled as a fixed value for each cell or distributed across cells and how this distribution can be represented, are the focus of Section 3.1 and Appendix A.

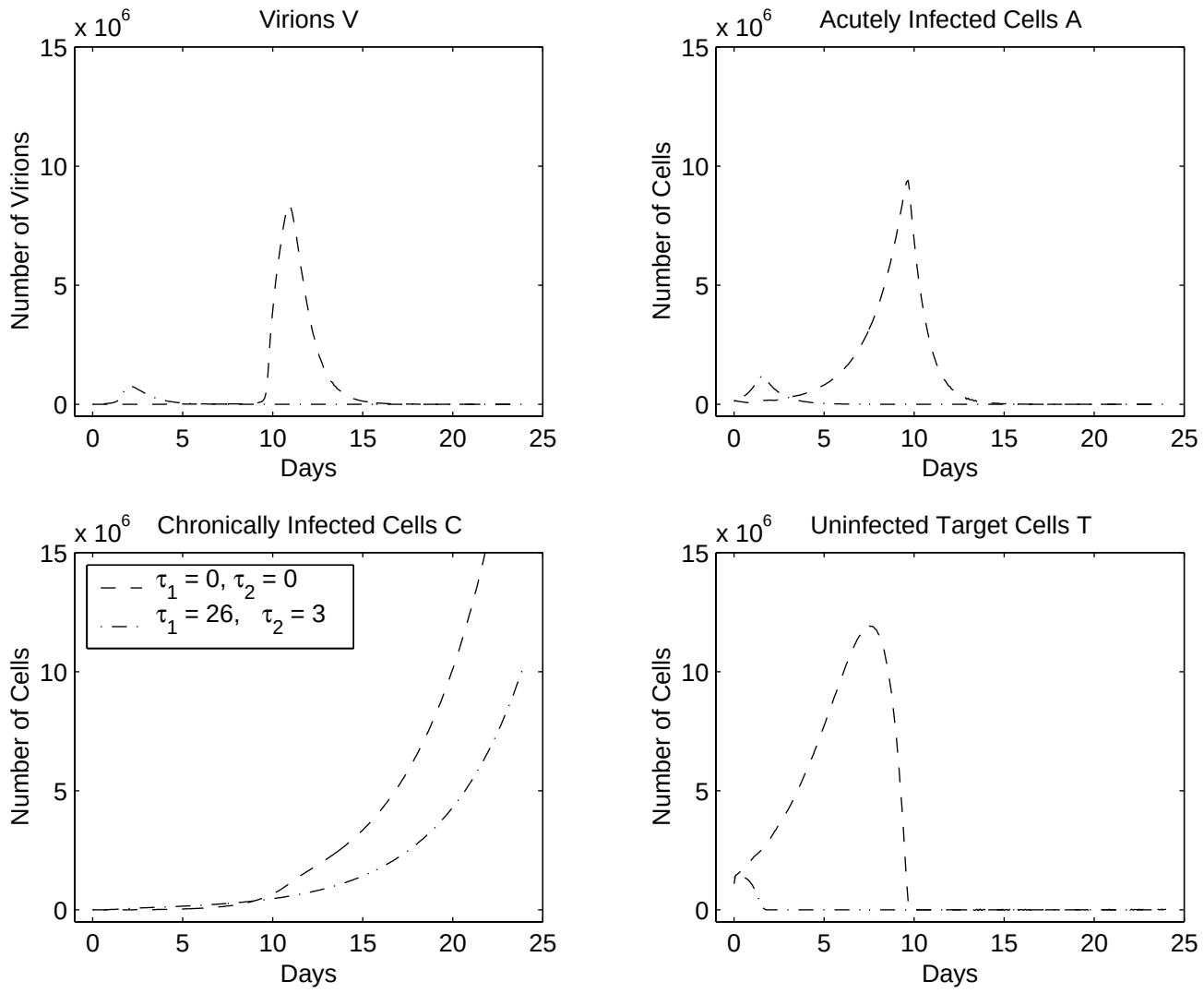

FIGURE 3.1. Simulations of (3.4) with ($\tau_1 = 26$, $\tau_2 = 3$) and without ($\tau_1 = 0$, $\tau_2 = 0$) discrete delays.

3.1. **Fixed Delays Versus Distributed Delays.** If we assume that the delays τ_1, τ_2 are fixed for each cell, then we can precisely describe the capacity of each member of the population (of infected cells) to produce virions as a function of time. In other words, exactly τ_1 units of time after a cell becomes infected, it begins producing virus. Exactly τ_2 units of time later, that same cell then becomes chronically infected (assuming it lives to this stage).

Such a system can be obtained from (3.1)-(3.3) along with the equation for $\dot{T}$ by assuming Heaviside distributions with unit jumps at $-\tau_1 < 0$ and $-\tau_1 - \tau_2 < 0$. This corresponds to Dirac delta "densities"

and results in the system

$$
\begin{aligned}
\dot{V}(t) &= -cV(t) + n_A \int_{-\infty}^{0} A(t+\tau)\,\delta_{-\tau_1}(\tau)\,d\tau + n_C C(t) - pV(t)T(t) \\
\dot{A}(t) &= (r_v - \delta_A - \delta X(t))\,A(t) - \gamma \int_{-\infty}^{0} A(t+\tau)\,\delta_{-\tau_1-\tau_2}(\tau)\,d\tau + pV(t)T(t) \\
\dot{C}(t) &= (r_v - \delta_C - \delta X(t))\,C(t) + \gamma \int_{-\infty}^{0} A(t+\tau)\,\delta_{-\tau_1-\tau_2}(\tau)\,d\tau \\
\dot{T}(t) &= (r_u - \delta_u - \delta X(t) - pV(t))\,T(t) + S\ ,
\end{aligned}
\tag{3.6}
$$

which is exactly the system (3.4). A simulation of this system is depicted in Figure 3.1.

In order to overcome the (biologically untenable) assumption that each cell begins producing virus at a fixed time after infection, a number of authors have used a Gamma function as the distribution for the time to viral production of infected cells (see Section 1). A Gamma distribution is just one example of a number of distributions which could be used to model this process. The primary advantage to using the Gamma distribution is that the distributed delay system can be rewritten as a system of ODE's and readily simulated using standard software packages. The derivation of this equivalent system of ODEs when the viral production delay is modeled with a Gamma distribution can be found in [13].

In this work, we considered a variety of normalized kernels for density functions (i.e., the model given by (3.5)) which we tacitly assumed exist for all distributions of interest (except of course, the Dirac distribution with masses at $-\tau_1$ and $-\tau_1 - \tau_2$). In particular, we considered simulations using density functions consisting of a triangular hat function and an inverted quadratic. Since these systems do not reduce to a system of ODE's, alternate numerical methods are required to simulate the dynamics of the modeled system. These are the subject of our discussions in Section 4.

The hat and inverted quadratic kernels (each of which have finite support) are described by

$$
\hat{k}(s;\mu,\sigma,s_1,s_2) = \frac{\hat{K}(s;\mu,\sigma)\chi_{[s_1,s_2]}(s)}{\int_{-\infty}^{0} \hat{K}(\xi;\mu,\sigma)\chi_{[s_1,s_2]}(\xi)d\xi}
\tag{3.7}
$$

where

$$
\begin{aligned}
\hat{K}(\xi;\mu,\sigma) &= \left(\frac{2\xi}{\sigma^2} + \frac{1}{\sigma}\left(1 - \frac{2\mu}{\sigma}\right)\right)\chi_{\left[\mu-\frac{\sigma}{2},\mu\right]}(\xi) \\
&\quad + \left(-\frac{2\xi}{\sigma^2} + \frac{1}{\sigma}\left(1 + \frac{2\mu}{\sigma}\right)\right)\chi_{\left[\mu,\mu+\frac{\sigma}{2}\right]}(\xi)
\end{aligned}
$$

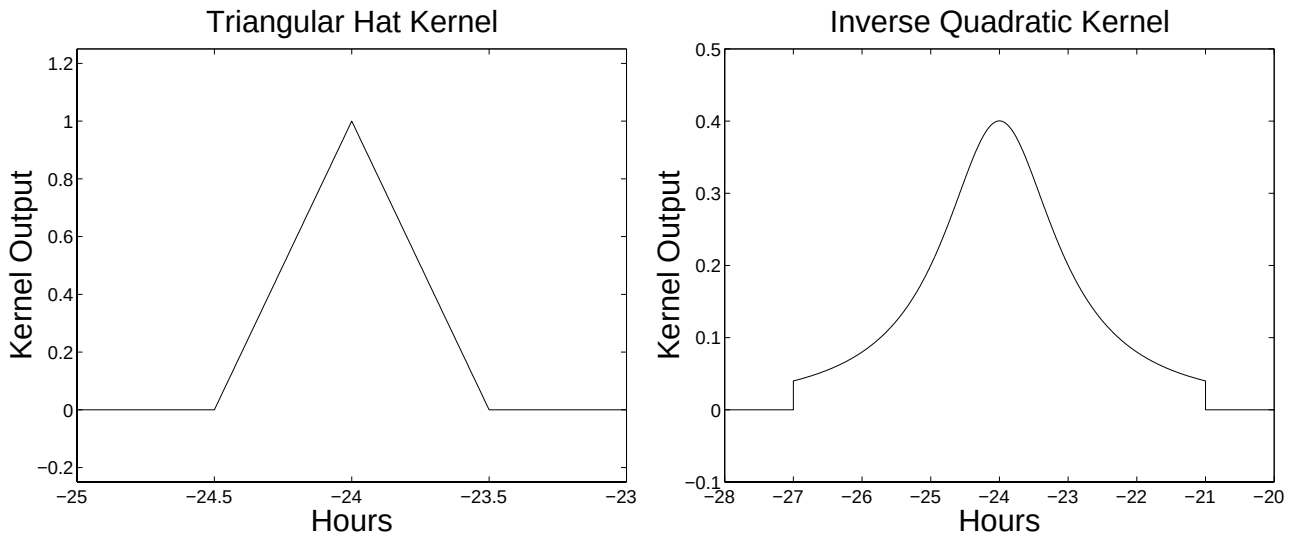

FIGURE 3.2. Sample graphs of the hat $\hat{k}$ and inverted quadratic $\tilde{k}$ kernels.

and

$$\tilde{k}(s;\mu,\sigma,s_1,s_2) \;=\; \frac{\tilde{K}(s;\mu,\sigma)\chi_{[s_1,s_2]}(s)}{\int_{-\infty}^{0}\tilde{K}(\xi;\mu,\sigma)\chi_{[s_1,s_2]}(\xi)d\xi} \tag{3.8}$$

where

$$\tilde{K}(\xi;\mu,\sigma) \;=\; \frac{1}{(\xi-\mu)^2+\sigma}$$

respectively and with mean μ, width σ, and support $[s_1, s_2] \subset \mathbb{R}$. Here $\chi_{[a,b]}$ is the indicator function of the interval $[a, b]$. Sample graphs of these functions are depicted in Figure 3.2. Note that in order to preserve the normalization, the height of these kernels automatically scales with changes in the width σ. Moreover, the support for the hat kernel $\hat{k}$ is always $\left[\mu - \frac{\sigma}{2}, \mu + \frac{\sigma}{2}\right]$ if s_1, s_2 are chosen so that $s_1 < \mu - \frac{\sigma}{2}$, $s_2 > \mu + \frac{\sigma}{2}$ (which we have done in all the calculations reported on in this paper). We use the $\chi_{[s_1,s_2]}$ notation in the $\hat{k}$ kernel only for consistency in notation in comparing kernels below.

4. NUMERICAL IMPLEMENTATION

For those interested in the mathematical aspects of simulating an FDE system, this section contains the necessary mathematical and numerical analysis foundations. In Section 4.1, we describe the conversion of the FDE system to an abstract evolution equation (AEE) system as well as provide existence and uniqueness results for a solution to the FDE (proofs are given in Appendix B). Section 4.2 contains details on the numerical implementation along with the convergence results for our numerical method.

In order to approximate solutions to the systems described in (3.4) and (3.5), we first converted them to an AEE and then approximated in a space spanned by piece-wise linear splines (i.e., in a Galerkin approach). We were able to numerically calculate the generalized Fourier coefficients of approximate solutions relative to the splines, and with these coefficients, recover an approximation to the solutions of (3.4) or (3.5).

4.1. **Abstract Evolution Equation Theory.** We briefly summarize the well-developed theory [1, 2, 3, 4] pertinent to our efforts. When considering the discrete delay system (3.4), we take $\tau_1,\ \tau_2 > 0$ and without loss of generality assume $r > \tau_1 + \tau_2 > 0$ is finite throughout.

Let

$$x(t) = (x_1(t), x_2(t), x_3(t), x_4(t))^T = (V(t), A(t), C(t), T(t))^T$$

and

$$x_t(\theta) = x(t+\theta)\ ,\ -r \leq \theta \leq 0,\ r \in \mathbb{R}^+ .$$

Our system, as described in (3.4) or (3.5), can then be written as

$$\begin{aligned} \dot{x}(t) &= L(x(t), x_t) + f_1(x(t)) + f_2(x(t-\tau_1)) + f_3(x(t-\tau_1-\tau_2)) + f_4(t) \\ &\qquad\qquad \text{for } 0 \leq t \leq t_f \end{aligned} \tag{4.1}$$

$$(x(0), x_0) = (\Phi(0), \Phi) \in Z\ ,\ \Phi \in L_2(-r, 0; \mathbb{R}^4)$$

where t_f is finite and for $(\eta, \phi) \in Z = \mathbb{R}^4 \times L_2(-r, 0; \mathbb{R}^4)$,

$$\begin{aligned} L(\eta, \phi) = &\begin{bmatrix} -c & 0 & n_C & 0 \\ 0 & r_v - \delta_A & 0 & 0 \\ 0 & 0 & r_v - \delta_C & 0 \\ 0 & 0 & 0 & r_u - \delta_u \end{bmatrix} \eta + n_A^1 \left[\delta_{(1,2)}\right]_{(4,4)} \int_{-r}^{0} \phi(\theta)\, dP_1(\theta) \\ &+\gamma^1 \left(\left[\delta_{(3,2)}\right]_{(4,4)} - \left[\delta_{(2,2)}\right]_{(4,4)} \right) \int_{-r}^{0} \phi(\theta)\, dP_2(\theta)\ , \end{aligned}$$

$$
\begin{aligned}
f_1(\eta) &= \begin{bmatrix} -p\eta_1\eta_4 \\ -\delta\left(\sum_{i=2}^{4}\eta_i\right)\eta_2 + p\eta_1\eta_4 \\ -\delta\left(\sum_{i=2}^{4}\eta_i\right)\eta_3 \\ -\delta\left(\sum_{i=2}^{4}\eta_i\right)\eta_4 - p\eta_1\eta_4 \end{bmatrix}, \\
f_2(\eta) &= n_A^2\left[\delta_{(1,2)}\right]_{(4,4)}\eta, \\
f_3(\eta) &= \gamma^2\left(\left[\delta_{(3,2)}\right]_{(4,4)} - \left[\delta_{(2,2)}\right]_{(4,4)}\right)\eta, \\
f_4(t) &= \left[0,0,0,S\right]^T,\ 0 \le t \le t_f\,.
\end{aligned}
$$

Moreover, Φ is the initial time history of the system on $[-r,0]$, $\{P_1, P_2\}$ are probability distributions, and $Z = \mathbb{R}^4 \times L_2(-r,0;\mathbb{R}^4)$ is the state space. Here $\left[\delta_{(i,j)}\right]_{(4,4)}$ denotes a 4 by 4 matrix with a one in the (i,j)th element and zeros elsewhere. The n_A^1 and the n_A^2 are model dependent parameters which are either zero or n_A depending on the delay distribution corresponding to the choice of either (3.4) or (3.5) as the model. Thus for a distributed delay (with distribution P_1), $n_A^1 = n_A$ and $n_A^2 = 0$. Conversely, for a discrete delay $n_A^2 = n_A$ and $n_A^1 = 0$. The γ^1 and γ^2 parameters are defined similarly.

Following the discussion in [1] regarding the existence and uniqueness of a solution to (4.1), we consider the following definitions and lemmas.

The norm on the space Z is defined as

$$
\|(\eta,\phi)\|_Z = \left(|\eta|^2 + \int_{-r}^{0}|\phi(\theta)|^2\,d\theta\right)^{1/2},\ (\eta,\phi) \in Z\,.
$$

Clearly, Z is a Hilbert space with inner product

$$
\langle(\eta,\phi),(\zeta,\psi)\rangle_Z = \eta^T\zeta + \int_{-r}^{0}\phi(\theta)^T\psi(\theta)\,d\theta\,,
$$

for $(\eta,\phi),\ (\zeta,\psi) \in Z$.

For the following discussion, denote $|\cdot|$ as the norm on $\mathbb{R}^n$ and the induced norm on $\mathbb{R}^{n\times n}$ and $\|\cdot\|$ as the norm on $L_2(-r,0;\mathbb{R}^4)$.

As discussed in Section 2, the nonlinearities exemplified by terms such as px_1x_4 are biologically unrealistic. However, these nonlinear terms in f_1 can be replaced by standard saturation limited nonlinearities such as

$$\begin{aligned} px_1x_4 \quad &\text{by} \quad p_1(x_1)\,x_4 \\ \text{and } \delta x_i x_j \quad &\text{by} \quad \delta_i(x_i)\,x_j \quad (\text{for } i,j=2,3,4)\ , \end{aligned}$$

where

$$p_1(x_1) \;=\; \begin{cases} 0 & x_1 < 0 \\ px_1 & 0 \le x_1 \le \bar{x}_1 \\ p\bar{x}_1 & \bar{x}_1 < x_1\ , \end{cases} \tag{4.2}$$

and

$$\delta_i(x_i) = \begin{cases} 0 & x_i < 0 \\ \delta x_i & 0 \le x_i \le \bar{x}_i \\ \delta\bar{x}_i & \bar{x}_i < x_i\ , \end{cases} \tag{4.3}$$

(for finite upper bounds $\bar{x}_i \in \mathbb{R}^+, i = 1,2,3,4$), and where f_1 can be replaced by

$$\tilde{f}_1(\eta) \;=\; \begin{bmatrix} -p_1(\eta_1)\,\eta_4 \\ -\left(\sum_{i=2}^4 \eta_i\right)\delta_2(\eta_2) + p_1(\eta_1)\,\eta_4 \\ -\left(\sum_{i=2}^4 \eta_i\right)\delta_3(\eta_3) \\ -\left(\sum_{i=2}^4 \eta_i\right)\delta_4(\eta_4) - p_1(\eta_1)\,\eta_4 \end{bmatrix},\ \eta \in \mathbb{R}^4. \tag{4.4}$$

Note that p_i and δ_i are globally bounded functions satisfying $p_1(x_1) \le p\bar{x}_1$ and $\delta_i(x_i) \le \delta\bar{x}_i$. Indeed they are differentiable, satisfying $p_1'(x_1) \le p$ and $\delta_i'(x_i) \le \delta$.

Now we can prove the global existence and uniqueness of a solution to

$$\begin{aligned} \dot{x}(t) \;&=\; L(x(t), x_t) + \tilde{f}_1(x(t)) + f_2(x(t-\tau_1)) \\ &\qquad + f_3(x(t-\tau_1-\tau_2)) + f_4(t) \qquad \text{for } 0 \le t \le t_f\,, \\ (x(0), x_0) \;&=\; (\Phi(0), \Phi) \in Z \end{aligned} \tag{4.5}$$

with t_f finite, through the following series of steps. We first define $\mathcal{F} = L + \tilde{f}_1 + f_2 + f_3$ on $Z \times \mathbb{R}^4 \times \mathbb{R}^4$ by

$$\mathcal{F}\left(\left(\eta, \phi\right), \nu, \xi\right) = L\left(\eta, \phi\right) + \tilde{f}_1\left(\eta\right) + f_2\left(\nu\right) + f_3\left(\xi\right) .$$

Lemma 4.1. *The function $\mathcal{F} = L + \tilde{f}_1 + f_2 + f_3 : Z \times \mathbb{R}^4 \times \mathbb{R}^4 \to \mathbb{R}^4$ is differentiable.*

Proof. Given that all pertinent components of the equation (4.5) are differentiable, we can conclude that the function $\mathcal{F}$ is differentiable.

Lemma 4.2. *For all $\left(\left(\eta, \phi\right), \nu, \omega\right), \left(\left(\zeta, \psi\right), \xi, \lambda\right) \in Z \times \mathbb{R}^4 \times \mathbb{R}^4$ the function $\mathcal{F} = L + \tilde{f}_1 + f_2 + f_3$ satisfies a global Lipschitz condition*

$$\left|\mathcal{F}\left(\left(\eta, \phi\right), \nu, \omega\right) - \mathcal{F}\left(\left(\zeta, \psi\right), \xi, \lambda\right)\right| \leq K_L \left\{\left|\eta - \zeta\right| + \left\|\phi - \psi\right\| + \left|\nu - \xi\right| + \left|\omega - \lambda\right|\right\} \tag{4.6}$$

for some fixed constant $K_L > 0$.

Proof: See B.1 for proof.

Remark 4.3. Note that in the above Lemma, K_L may not necessarily be the minimal Lipschitz constant; we merely wish to emphasize its existence for use in a subsequent theorem.

Following the standard arguments for the existence and uniqueness of the solution to an ODE on a finite interval $I = [0, t_f]$, we begin by noting that as a consequence of the Second Fundamental Theorem of Calculus, we can rewrite (4.5) as

$$\begin{aligned} x\left(t\right) &= \Phi\left(0\right) + \textstyle\int_0^t \{L\left(x\left(\sigma\right), x_\sigma\right) + \tilde{f}_1\left(x\left(\sigma\right)\right) + f_2\left(x\left(\sigma - \tau_1\right)\right) \\ &\qquad + f_3\left(x\left(\sigma - \tau_1 - \tau_2\right)\right) + f_4\left(\sigma\right)\} d\sigma \qquad t \in I\,, \\ &= \Phi\left(t\right) \qquad - r \leq t < 0\,. \end{aligned} \tag{4.7}$$

We now make the following definition.

Definition 4.4. Let *successive approximations* to the solution of (4.7) on $[-r, t_f]$ be defined for $j = 0, 1, 2, \ldots$, as

$$
\begin{aligned}
y_0(t) &= \begin{cases} \Phi(0) & t \in I \\ \Phi(t) & -r \le t < 0 \end{cases} \\
y_{j+1}(t) &= \Phi(0) + \int_0^t \{L\left(y_j(\sigma), (y_j)_\sigma\right) + \tilde{f}_1(y_j(\sigma)) + f_2(y_j(\sigma - \tau_1)) \\
&\qquad + f_3(y_j(\sigma - \tau_1 - \tau_2)) + f_4(\sigma)\} d\sigma \qquad t \in I\,, \\
&= \Phi(t) \qquad -r \le t < 0\,.
\end{aligned} \tag{4.8}
$$

Theorem 4.5. *Given a finite interval I, suppose $L + \tilde{f}_1 + f_2 + f_3$ satisfies both Lemma 4.1 and Lemma 4.2. Then there exists a unique solution to (4.5) on I.*

Proof: See Appendix B.2 for proof.

In our simulations, where the states do not exceed the predefined upper bounds in (4.2), (4.3), we know that these solutions solve (4.1) as well as (4.5). In any case (4.5) is the biologically meaningful system.

4.2. **The Abstract Evolution Equation Implementation.** The system described by (4.5) can be written in a form that facilitates a discussion regarding its approximation which is developed fully in [4] and will only be summarized here.

Define the nonlinear operator $\mathcal{A} : \mathcal{D}(\mathcal{A}) \subset Z \to Z$ by

$$
\begin{aligned}
\mathcal{D}(\mathcal{A}) &= \{(\psi(0), \psi) \in Z : \psi \in H^1(-r, 0; \mathbb{R}^4)\} \\
\mathcal{A}(\psi(0), \psi) &= \left(L(\psi(0), \psi) + \tilde{f}_1(\psi(0)) + f_2(\psi(-\tau_1)) + f_3(\psi(-\tau_1 - \tau_2)), \tfrac{d}{d\theta}\psi\right)\,.
\end{aligned}
$$

With this definition, we can then write (4.5) in the form

$$
\begin{aligned}
\dot{z}(t) &= \mathcal{A}z(t) + f_5(t) \\
z(0) &= z_0\,,
\end{aligned} \tag{4.9}
$$

where $f_5(t) = (f_4(t), 0) \in Z$ and $z_0 \in Z$.

Let $\left\{Z^N, P^N, \mathcal{A}^N\right\}$ be our approximation scheme for (4.9) satisfying the conditions of Theorem 3.1 in [4], where Z^N is a spline subspace of Z, P^N is the orthogonal projection of Z onto Z^N, and $\mathcal{A}^N$ is the approximating operator $\mathcal{A}^N = P^N \mathcal{A} P^N$. Thus, using $\left\{Z^N, P^N, \mathcal{A}^N\right\}$ we can generate an

approximation to the formulation described by (4.9), which we denote by

$$\begin{aligned} \dot{z}^N(t) &= \mathcal{A}^N z^N(t) + P^N\left(f_4(t), 0\right) \\ z^N(0) &= P^N(\eta, \phi) \ . \end{aligned} \tag{4.10}$$

As before, the second Fundamental Theorem of Calculus implies that an alternative description of (4.10) is

$$z^N(t) = P^N(\eta, \phi) + \int_0^t \left\{\mathcal{A}^N z^N(\sigma) + P^N\left(f_4(\sigma), 0\right)\right\} d\sigma \ .$$

Theorem 4.6. *Given the systems described in (4.5) and (4.10) with* $(\eta, \phi) = (\psi(0), \psi)$, $\psi \in H^1(-r, 0; \mathbb{R}^4)$, *under the conditions of Lemmas 4.1 and 4.2, we have* $z^N(t) \to y(t) = \left(x(t; \psi, f_4), x_t(\psi, f_4)\right)$, *as* $N \to \infty$, *uniformly in* t *on the finite interval* I.

Proof. The function f_4 is clearly in $L_2(I)$ and thus Theorem 2.2 in [1] directly implies our desired conclusion.

As in [4], we choose hat functions (piece-wise linear splines) as our basis for Z_1^N (the subspace of Z^N spanned by the hat functions). Thus, if we partition $[-r, 0]$ by $t_j^N = -j(r/N)$, $j = 0, \ldots, N$, we can then define the basis $\hat{\beta}^N = \left(\beta^N(0), \beta^N\right)$ by

$$\beta^N = \left(e_0^N, e_1^N, \ldots, e_N^N\right) \otimes I_n$$

where I_n is the $n \times n$ identity matrix and the e_j^N's are characterized by

$$e_j^N\left(t_i^N\right) = \delta_{ij}; \ i, j = 0, \ldots, N \ .$$

Therefore, an element in Z_1^N can be written as

$$z^N = \hat{\beta}^N \alpha^N = \sum_{j=0}^{N} \left(e_j^N(0), e_j^N\right) a_j^N \ , \text{ with } a_j^N \in \mathbb{R}^N \ .$$

Denote A^N as the matrix representation of $\mathcal{A}^N$ restricted to Z_1^N and let $w^N(t)$ and $F^N(t)$ be defined such that $z^N(t) = \hat{\beta}^N w^N(t)$ and $P^N\left(f_4(t), 0\right) = \hat{\beta}^N F^N(t)$ respectively. By construction we have

that $\mathcal{A}^N \hat{\beta}^N = \hat{\beta}^N A^N$, which implies that solving (4.5) for $z^N(t)$ is equivalent to solving

$$\begin{aligned} \dot{w}^N(t) &= A^N w^N(t) + F^N(t) \quad t \in I\,, \\ w^N(0) &= w_0^N \end{aligned} \tag{4.11}$$

for $w^N(t)$, where $\hat{\beta}^N w_0^N = P^N(\eta, \phi)$.

We remark that if we are able to obtain w^N, the product $\hat{\beta}^N w^N$ converges uniformly in t on I to the solution of (4.5)

$$\lim_{N\to\infty} \hat{\beta}^N w^N\left(t, w_0^N, F^N\right) = \left(x\left(t;\eta,\psi,f_4\right), x_t\left(\eta,\psi,f_4\right)\right)\,.$$

For the numerical simulation of (4.11), it is necessary to compute $P^N(\gamma,\psi)$ for any $(\gamma,\psi) \in Z$ and $A^N\alpha^N$ for $\alpha^N \in \mathbb{R}^n$. Since $P^N(\gamma,\psi)$ is the orthogonal projection of $(\gamma,\psi) \in Z$ onto Z^N, $P^N(\gamma,\psi)$ is uniquely determined by the $\mu^N \in \mathbb{R}^N$ such that

$$\left\langle \hat{\beta}^N \mu^N - (\gamma,\psi), \hat{\beta}^N \right\rangle_Z = 0$$

or equivalently,

$$\left\langle \hat{\beta}^N, \hat{\beta}^N \right\rangle_Z \mu^N = \left\langle \hat{\beta}^N, (\gamma,\psi) \right\rangle_Z\,. \tag{4.12}$$

Thus, solving (4.12) for μ^N yields $P^N(\gamma,\psi) = \hat{\beta}^N\mu^N$ for any $(\gamma,\psi) \in Z$ and implies that $F^N(t)$ is uniquely defined by

$$F^N(t) = \left(\left\langle \hat{\beta}^N, \hat{\beta}^N \right\rangle_Z\right)^{-1} \left\langle \hat{\beta}^N, (f_4(t), 0) \right\rangle_Z\,.$$

To calculate $A^N\alpha^N$, first consider the action of $\mathcal{A}^N$ applied to $\hat{\beta}^N\alpha^N$, an element of Z^N. We know that for any $\alpha^N \in \mathbb{R}^n$

$$\begin{aligned} \mathcal{A}^N\hat{\beta}^N\alpha^N = P^N\left(\mathcal{A}\hat{\beta}^N\alpha^N\right) &= P^N\Big(L\left(\left(\beta^N(0)\,\alpha^N\right), \beta^N\alpha^N\right) + \tilde{f}_1\left(\beta^N(0)\,\alpha^N\right) \\ &\quad + f_2\left(\beta^N(-\tau_1)\,\alpha^N\right) + f_3\left(\beta^N(-\tau_1-\tau_2)\,\alpha^N\right), D\left(\beta^N\alpha^N\right)\Big) \end{aligned}$$

and that $\mathcal{A}^N\hat{\beta}^N\alpha^N = \hat{\beta}^N A^N\alpha^N$. Thus

$$\begin{aligned} 0 = \hat{\beta}^N A^N\alpha^N - P^N\{&L\left(\left(\beta^N(0)\,\alpha^N\right), \beta^N\alpha^N\right) + \tilde{f}_1\left(\beta^N(0)\,\alpha^N\right) + f_2\left(\beta^N(-\tau_1)\,\alpha^N\right) \\ &+ f_3\left(\beta^N(-\tau_1-\tau_2)\,\alpha^N\right)), \tfrac{d}{d\theta}\left(\beta^N\alpha^N\right)\} \end{aligned}$$

and

$$0 = \langle\, \hat{\beta}^N, \hat{\beta}^N A^N \alpha^N - \{L\left(\left(\beta^N(0)\,\alpha^N\right), \beta^N \alpha^N\right) + \tilde{f}_1\left(\beta^N(0)\,\alpha^N\right) + f_2\left(\beta^N(-\tau_1)\,\alpha^N\right) \\ + f_3\left(\beta^N(-\tau_1-\tau_2)\,\alpha^N\right), \tfrac{d}{d\theta}\left(\beta^N \alpha^N\right)\}\,\rangle_Z$$

which implies that

$$\left\langle \hat{\beta}^N, \hat{\beta}^N \right\rangle_Z \left(A^N \alpha^N\right) = \langle\, \hat{\beta}^N, \{L\left(\left(\beta^N(0)\,\alpha^N\right), \beta^N \alpha^N\right) + \tilde{f}_1\left(\beta^N(0)\,\alpha^N\right) + f_2\left(\beta^N(-\tau_1)\,\alpha^N\right) \\ + f_3\left(\beta^N(-\tau_1-\tau_2)\,\alpha^N\right), \frac{d}{d\theta}\left(\beta^N \alpha^N\right)\}\,\rangle_Z\ .$$

Therefore, for any $\alpha \in \mathbb{R}^n$, the action of A^N on α^N is defined by

$$A^N \alpha^N = \left(\left\langle \hat{\beta}^N, \hat{\beta}^N \right\rangle_Z\right)^{-1} \langle\, \hat{\beta}^N, L\left(\left(\beta^N(0)\,\alpha^N\right), \beta^N \alpha^N\right) + \tilde{f}_1\left(\beta^N(0)\,\alpha^N\right) + f_2\left(\beta^N(-\tau_1)\,\alpha^N\right) \\ + f_3\left(\beta^N(-\tau_1-\tau_2)\,\alpha^N\right), \frac{d}{d\theta}\left(\beta^N \alpha^N\right)\,\rangle_Z\ .$$

With these characterizations, we can now calculate $w^N(t)$ and thus $z^N(t)$ (on I), and thus a numerical approximation to the solution of (4.5). Note that the characterization of the FDE system allows us to include both discrete and distributed delays in any modeling and simulation investigations.

5. Numerical Results

5.1. **Results for the FDE Approach.** We carried out numerous simulations and inverse problem calculations using the methodology outlined above for problems with discrete and distributed delay systems.

The initial conditions for all our simulations were

$$(V_0, A_0, C_0, T_0) = (0, 1.5 \times 10^5, 0, 1.35 \times 10^6)\,,$$

the parameters are those defined in Tables 1 and 3, and all of the presented plots are from simulations run with $N = 32$ basis elements.

5.1.1. *Statistical Significance of the Delays.* Employing ideas given in the discussions regarding a statistical testing methodology for model comparisons in inverse problems in [3], we examined the statistical significance of the presence of both types of delays in fitting the models (3.4) to experimental data provided by Dr. Michael Emerman. We used the data consisting of the total cells X from [20] (sampled at time-points t_i; $i = 1, 2, \ldots, 10$, denoted by the vector $\hat{y}$, and depicted in Figure 2.1). We

$Parameter$	$Value$
c	0.12
n_A	0.1194
n_C	1.6644×10^{-6}
γ	8.7625×10^{-4}
r_v	0.035
r_u	0.035
δ_A	0.0775
δ_C	0.0257
δ_u	0.0160
δ	5.4495×10^{-13}
p	1.3359×10^{-6}
S	0.0

TABLE 3. *in vitro* model parameter values

Optimization Variables	p^*	τ_1^*	τ_2^*	J^*
$q = (p, 0, 0)$	4.0115×10^{-7}	-	-	8.6329×10^5
$q = (p, \tau_1, 0)$	1.3696×10^{-6}	24.278	-	6.1638×10^4
$q = (p, \tau_1, \tau_2)$	1.3321×10^{-6}	24.234	2.8806	4.2386×10^4

TABLE 4. Results from the inverse problem.

then carried out inverse problems for estimating the parameters p, p and τ_1, and p, τ_1, and τ_2, using a least squares criterion. That is, we first estimate p holding $\tau_1 = \tau_2 = 0$, then estimate p and τ_1 with $\tau_2 = 0$, and finally estimated p, τ_1, and τ_2 simultaneously. Note that we used delta distributions for both delays (in the appropriate simulations) in solving the inverse problem, although the methods apply readily to more general distributions.

We employed a cost function using a residual sum of squares formula

$$J(q) = J(p, \tau_1, \tau_2) = \frac{1}{10}\sqrt{\sum_{i=1}^{10} \left|\hat{y}_i - \left(A(t_i, q) + C(t_i, q) + T(t_i, q)\right)\right|^2} \tag{5.1}$$

and optimized J using the Nelder-Mead nonlinear iterative routine in Matlab (*fminsearch*). The results of the inverse problems are summarized in Table 4, with the optimal parameter values denoted by p^*, τ_1^*, τ_2^* and the corresponding fit with the value $J(q^*)$ by J^*.

We then investigated the statistical significance by using the test described on page 523 of [3]. The reader should be aware that the statistics we used here are only asymptotically χ^2's as the sample size becomes infinite. With only the ten data points we have to use here, one can rightfully question the legitimacy of our use of the tests given in [3]. None the less, we use these tests here to give some

indications of the relevance of improved fits to data. We first considered a null hypothesis of *no dela*y in the acutely infected to viral production step. This generated a test statistic of

$$U_{10}^{N}\left((p^*,0,0),(p^*,\tau_1^*,0)\right) = 10\frac{J\left(4.0115\times10^{-7},0,0\right) - J(1.3696\times10^{-6},24.278,0)}{J(1.3696\times10^{-6},24.278,0)} \cong 130.06\,.$$

With this test statistics, we can use a $\chi^2(1)$ test to reject the hypothesis at all (useful) confidence levels. This suggests that the presence of a delay in the model is statistically significant. That is, the improved fit to data obtained by including the delay is not simply due to the increased degrees of freedom in the model. We also calculated the statistic to determine the significance of both delays versus no delay and found

$$U_{10}^{N}\left((p^*,0,0),(p^*,\tau_1^*,\tau_2^*)\right) = 193.67$$

and the significance of two delays versus one delay, obtaining

$$U_{10}^{N}\left((p^*,\tau_1^*,0),(p^*,\tau_1^*,\tau_2^*)\right) = 4.54\,.$$

As expected, the presence of two delays also appears to be statistically significant. However, it is interesting to note that for a null hypothesis of only *one delay* (i.e., $\tau_2 = 0$), the improvement in the fit to data due to the addition of a second delay to the inverse problem is not significant (i.e., we can only reject the hypothesis $\tau_2 = 0$ at 95% or lower confidence levels). This suggests that the modeling of the delay between infection and production is somewhat more critical than modeling a delay between acute productivity and chronic infection in developing an accurate mathematical representation.

5.1.2. *Comparison to data.* In Figure 5.1 we compare experimental data and an AEE based simulation (generated using parameters from Tables 3 and 4) with two optimal discrete delays of $\tau_1^* = 24.23$ and $\tau_2^* = 2.88$. Clearly, our simulation (with the above parameters) is a very good fit to the experimental measurements. However, as mentioned before, we should be wary of drawing decisive conclusions given the sparsity of experimental observations.

5.1.3. *Kernel Analysis.* Given our limited data and the results from Section 5.1.1, we further examined the nature of the delay by numerically simulating our system using the method described in Section 4 and the different kernels described in Section 3.1. Specifically, we studied the effect of different μ's,

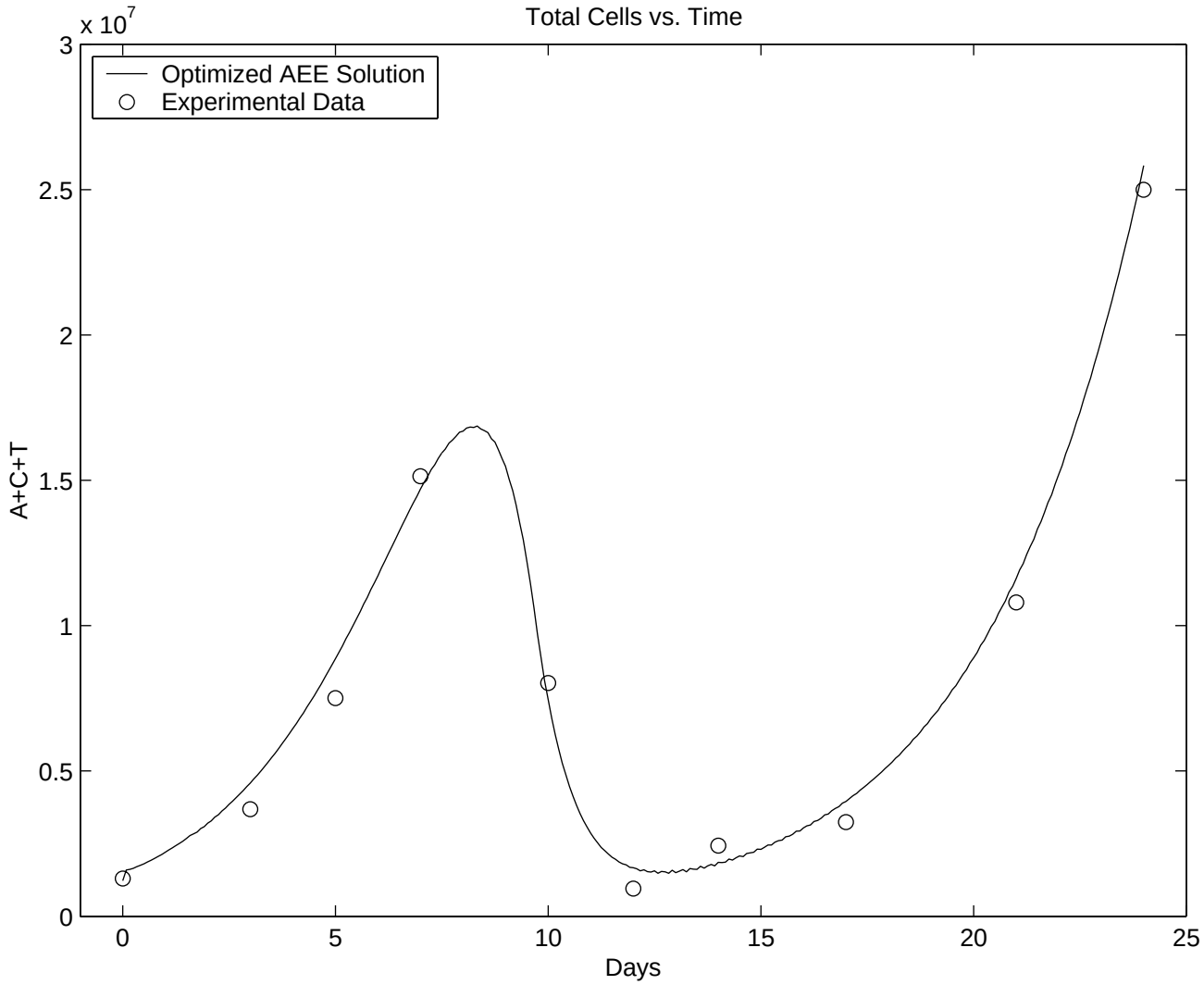

FIGURE 5.1. Data from [20] and simulation using $\tau_1^* = 24.23$, $\tau_2^* = 2.88$, and parameters from Tables 3 and 4.

different σ's, and kernel smoothness upon the system, and only present here some of our findings from these investigations.

To assess the effect of the mean on the kernel k_1 (the kernel from the $\dot{V}$ equation), we let $k_1 = \hat{k}$ and performed simulations for a variety of means, the results of which are depicted in Figure 5.2. For this simulation, we let the second kernel $k_2 = \hat{k}$, but kept its mean fixed at $\mu_2 = \mu_1 - 3$ (where μ_1 is the mean of k_1). Note that as the mean varies, we observe a dramatic temporal shift in the peaks of various compartments. The simulations run using the hat kernel $\tilde{k}$ exhibited virtually identical sensitivity to the perturbation of μ.

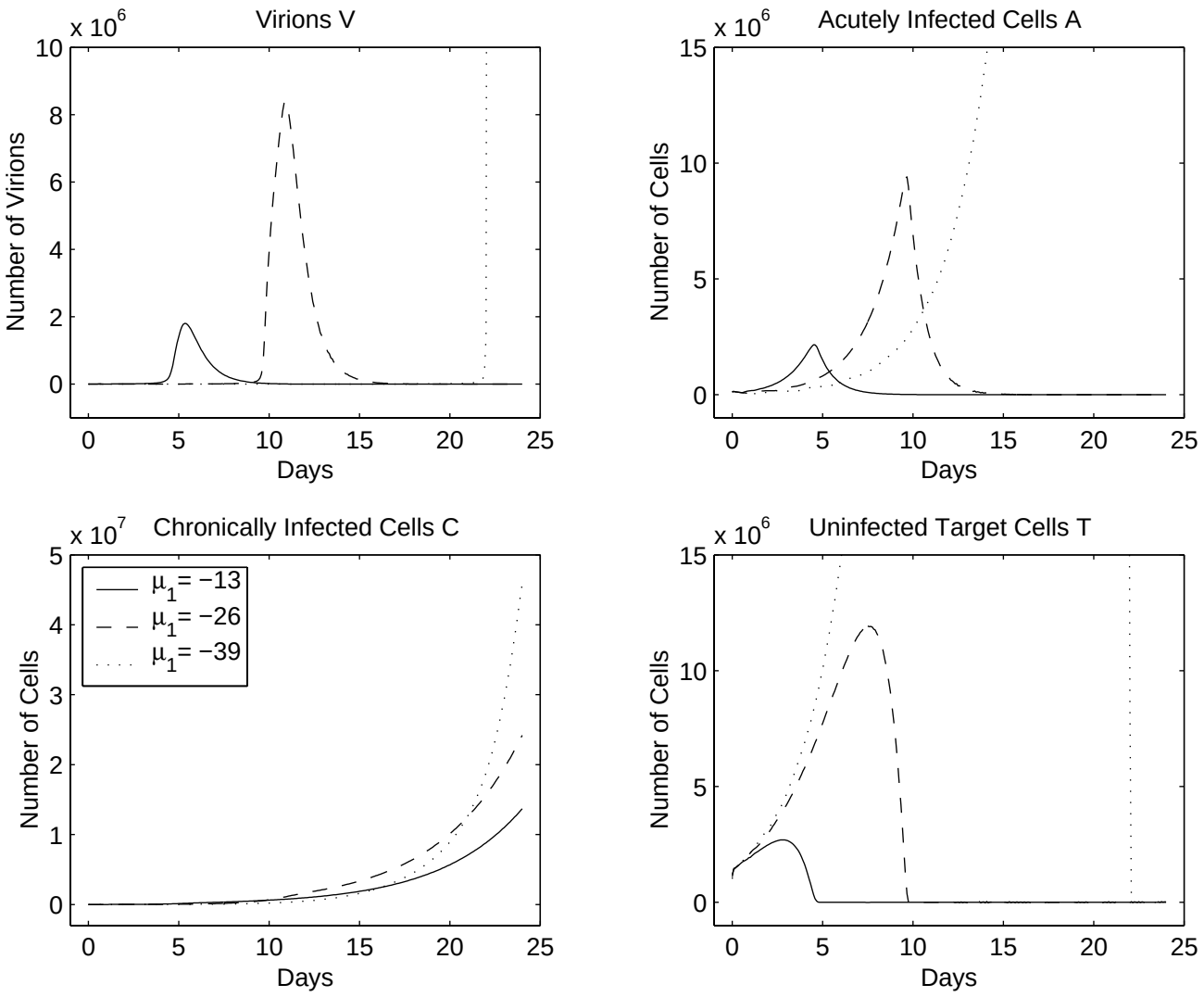

FIGURE 5.2. Simulations of (3.5) using $k_1 = \hat{k}(t, \mu_1, 1, -48, 0)$ and $k_2(t) = \hat{k}(t, \mu_1 - 3, 1, -48, 0)$ for several values of μ_1.

Figure 5.3 depicts simulations in which we vary the width σ of a kernel. We let

$$k_1(t) = \hat{k}(t, -26, \sigma, -48, 0)$$

$$k_2(t) = \hat{k}(t, -29, \sigma, -48, 0)$$

and simulated (3.5). As the σ decreases, the numerical solution calculated using the hat kernel rapidly approaches the one calculated using the Heaviside distribution. This coincides with the intuitive notion that as $\sigma \to 0$, the hat kernel approaches a Dirac delta function. Indeed, from the graphs, it appears that changing the σ has little to no effect upon the simulation. We remark that the simulations computed

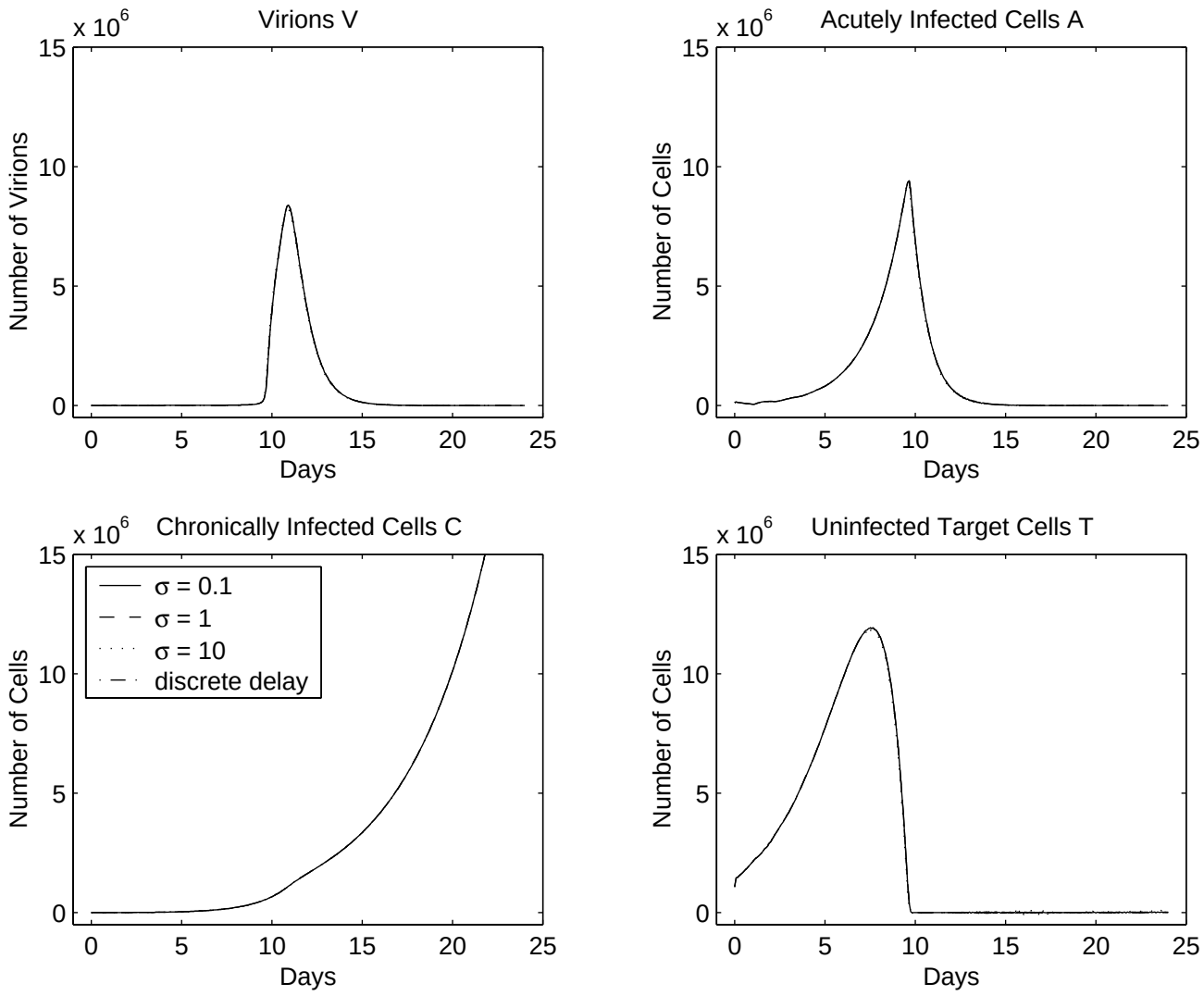

FIGURE 5.3. Simulations of (3.5) using $k_1 = \hat{k}(t, -26, \sigma, -48, 0)$ and $k_2(t) = \hat{k}(t, -29, \sigma, -48, 0)$ for several values of σ.

using the inverted quadratic kernel with varying σ exhibit behavior virtually identical to those using the hat kernel and are thus not depicted here.

For $(\mu_1, \mu_2, \sigma, s_1, s_2) = (-26, -29, 1, -48, 0)$, the numerical simulations of (3.5), using the normalized hat kernel and the normalized inverted quadratic kernel are compared in Figure 5.4. If we compare these behaviors with that of the discrete delay system depicted in Figure 3.1, we observe that kernel shape does not appear to have a significant effect upon the simulation. Other simulations also confirmed that the qualitative behavior of solutions does not vary greatly between the discrete delay systems and systems with the continuous kernels $\tilde{k}$, $\hat{k}$ with mean equal to the discrete delay.

Figure 5.2 depicts simulations of (3.5) with $k_1(s) = \hat{k}(s; \mu_1, 1, -48, 0)$ for a variety of means μ_1, while Figure 5.3 depicts simulations of the same system with $k_1(s) = \hat{k}(s, -26, \sigma, -48, 0)$ and a variety

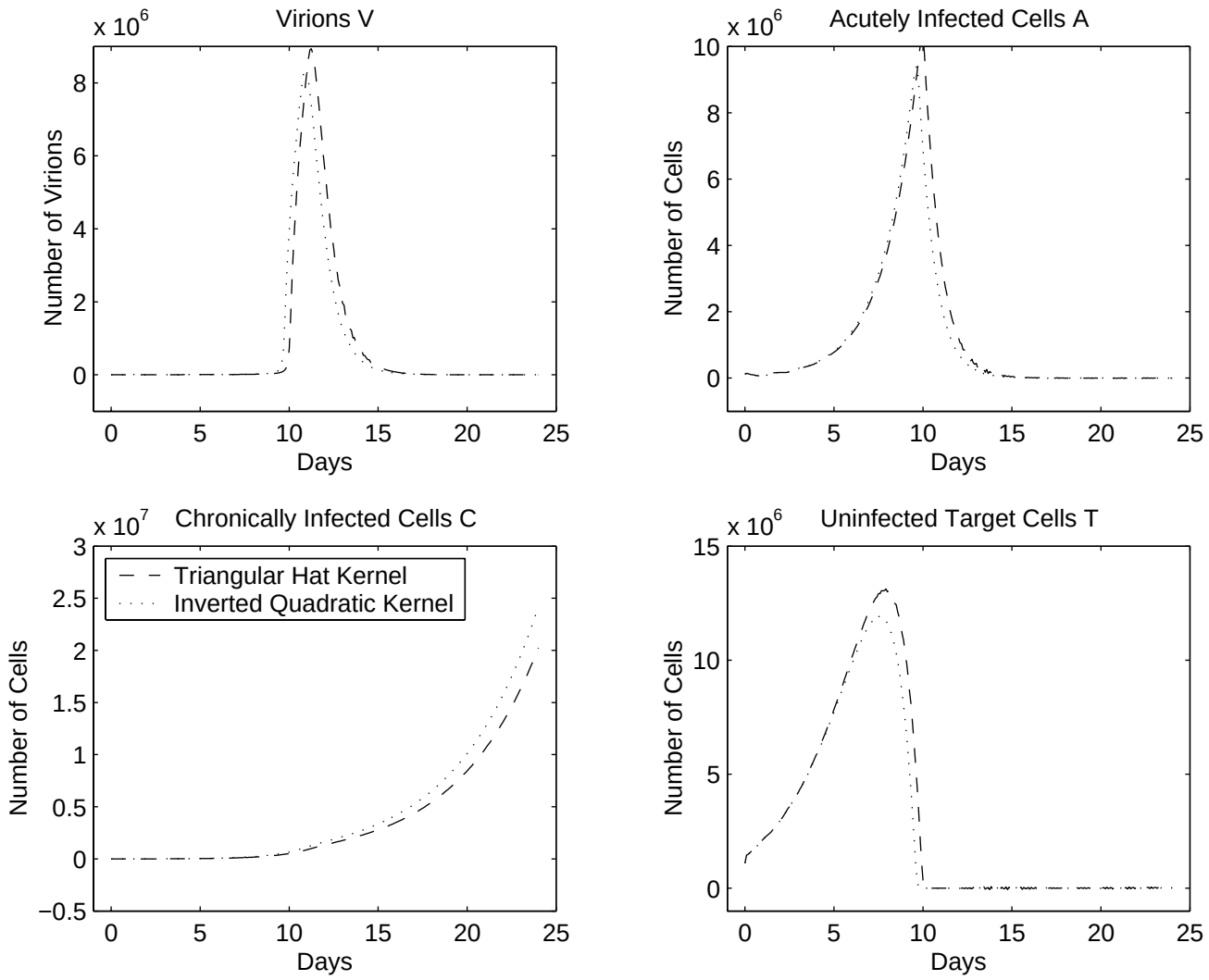

FIGURE 5.4. Simulations of (3.5) with the $\hat{k}$ and $\tilde{k}$ kernels.

of widths (σ). Both of these cases suggest that the dominant parameter is μ_1. However, it is still possible that s_1 or s_2 has a significant influence upon the system, since they determine the kernel support (at least in the case of $\tilde{k}$). It is not useful to study the support independent of σ when using $k_1(s) = \hat{k}(s, \mu, \sigma, s_1, s_2)$, and thus we only examine the ramifications of varying the support of $k_1 = \tilde{k}$. Our interests were focused upon parameter ranges in which $k_1(\mu+\frac{\sigma}{2}) \gg k_1(s_2)$ and $k_1(\mu-\frac{\sigma}{2}) \gg k_1(s_1)$ to prevent interference between σ and the domain of the kernel support. Over a wide range of values for s_1 and s_2 (that satisfied our criteria) there were negligible differences between the simulations, with the results being almost identical to those in Figure 5.4. Clearly the dominant parameter in $\tilde{k}$, from among μ_1, σ, s_1, and s_2, is the mean μ_1.

6. CONCLUSIONS

We have mathematically modeled a biological system (using coupled functional differential equations) that arises in the study of HIV infection dynamics and offered a derivation supporting a non-deterministic mathematical treatment of the biological delays. We converted these equations to an abstract evolution equation to facilitate analysis and numerical approximation of the system. We used a χ^2 statistical test to support our claim as to the significance of the presence of the delays in fitting experimental data. Additionally, our numerical sensitivity analysis for a distributed delay (i.e., FDE) approach yielded the information that the approximate system appears to be highly sensitive to the mean of the delay kernel but not to the width or the smoothness of the kernel.

7. ACKNOWLEDGMENTS

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APPENDIX A. DERIVATION OF MODEL

Here we present a brief derivation from first principles (with assumptions based on the biology) that supports the mathematical form in treating the delays as stochastic or random variables.

Let us first consider the delay between initial acute infection and initial chronic infection of a cell. It is biologically unrealistic to expect an entire population of cells to simultaneously change infection characteristics $\bar{\mu}_2$ ($\bar{\mu}_2 > 0$) hours after initial viral infection. Therefore, suppose that the delay between initial acute infection and chronic infection varies across the cell population (thus mathematically characterizing the intercellular variability) according to a probabilistic distribution $\bar{P}_2$ with density $\bar{k}_2$. We denote by $C(t;\tau)$ the subpopulation consisting of chronically infected cells that either maintained their acute infection characteristics for τ time units or are the progeny of those same cells. In other words, for some $\tau > 0$, there exists a subpopulation $C(t;\tau)$ of the chronically infected cells which either spent τ hours as acutely infected cells (before converting to chronically infected cells) or are descendants of

cells that spent exactly τ hours as acutely infected cells. Thus, the rate of change in this subpopulation of cells is governed by

$$\dot{C}(t;\tau) = (r_v - \delta_C - \delta X(t)) C(t;\tau) + \gamma A(t-\tau) ,$$

where

$$X(t) = A(t) + C(t) + T(t)$$

and the expected value of the population of chronic cells is given by integrating over the distribution $\bar{P}_2$, over all possible delays, obtaining

$$C(t) = \mathcal{E}_2[C(t;\tau)] = \int_0^\infty C(t;\tau)\bar{k}_2(\tau)d\tau . \tag{A.1}$$

Therefore, the rate of change in the total population of chronic cells is governed by

$$\begin{aligned} \dot{C}(t) &= \mathcal{E}_2[\dot{C}(t;\tau)] \\ &= (r_v - \delta_C - \delta X(t)) C(t) + \gamma \int_0^\infty A(t-\tau)\bar{k}_2(\tau)\, d\tau \\ C(0) &= C_0 , \end{aligned} \tag{A.2}$$

where C_0 is the initial condition for the total chronically infected cell population.

Next, we consider the delay between viral infection and viral production for the acutely infected cells $A(t)$. Again, it is unreasonable to expect the entire population of acutely infected cells to simultaneously commence viral production $\bar{\mu}_1$ ($\bar{\mu}_1 > 0$) hours after infection. We suppose that the delay between infection and production (for acutely infected cells $A(t)$) varies across the population with probability distribution $\bar{P}_1$ and corresponding density $\bar{k}_1$. We also partition the expected total viral population V into those virions V_A produced by acutely infected cells and those virions V_C produced by chronically infected cells so that

$$V = V_A + V_C .$$

We then denote by $V_A(t;\tau)$ the subpopulation of virus which are produced by an acutely infected cell τ hours after being infected. Thus, the rate of change in this subgroup of virions is governed by

$$\dot{V}_A(t;\tau) = -cV_A(t;\tau) + n_A A(t-\tau) - pV_A(t;\tau)T(t) .$$

To obtain the (expected) number of virus at time t that have been produced by acutely infected cells, we must integrate over the distribution $\bar{P}_1$, over all possible delays

$$V_A(t) = \mathcal{E}_1[V_A(t;\tau)] = \int_0^\infty V_A(t;\tau)\bar{k}_1(\tau)d\tau\,,$$

which yields the governing equation for this larger subpopulation of virions

$$\begin{aligned}\dot{V}_A(t) &= \mathcal{E}_1[\dot{V}_A(t;\tau)] \\ &= -cV_A(t) + n_A\int_0^\infty A(t-\tau)\bar{k}_1(\tau)d\tau - pV_A(t)T(t)\,.\end{aligned}$$

To account for the chronically infected cells as a source of virions, we denote V_C as the subpopulation of virions produced by chronically infected cells. Thus the equation describing the rate of change in the size of this subpopulation is

$$\dot{V}_C(t) = -cV_C(t) + n_C C(t) - pV_C(t)T(t)\,,$$

where the expected value C of the total population of chronically infected cells is defined in (A.1). Therefore, the governing equations for the total population of virus are described by

$$\begin{aligned}\dot{V}(t) &= \mathcal{E}_1[\dot{V}_A(t;\tau) + \dot{V}_C(t)] \\ &= -c(V_A(t)+V_C(t)) + n_A\int_0^\infty A(t-\tau)\bar{k}_1(\tau)d\tau + n_C C(t) - p(V_A(t)+V_C(t))T(t) \\ &= -cV(t) + n_A\int_0^\infty A(t-\tau)\bar{k}_1(\tau)d\tau + n_C C(t) - pV(t)T(t) \\ V(0) &= V_0\,,\end{aligned}$$

where V_0 is the initial condition for the total virions population.

Moreover, we assume that the A and T subclasses have no subpopulation structures, and are therefore governed by

$$\dot{A}(t) = (r_v - \delta_A - \delta X(t)) A(t) - \gamma \int_0^\infty A(t-\tau) \bar{k}_2(\tau) d\tau + pV(t) T(t) \quad \text{(A.3)}$$

$$A(0) = A_0 \quad \text{(A.4)}$$

$$\dot{T}(t) = (r_u - \delta_u - \delta X(t) - pV(t)) T(t) + S \quad \text{(A.5)}$$

$$T(0) = T_0\,, \quad \text{(A.6)}$$

with initial conditions A_0 and T_0. Note that in (A.3), the rate term with the delay (representing the delayed conversion of A to C) is simply the negative of the delay rate term in (A.2).

Finally, we make the change of variables $k_i(\xi) = \bar{k}_i(-\xi)$ so that the densities are now defined on $(-\infty, 0)$ instead of $(0, \infty)$ (we do this to be consistent with the notation of Section 4 which is standard in the FDE literature), and obtain the system

$$\begin{aligned}
\dot{V}(t) &= -cV(t) + n_A \int_{-\infty}^0 A(t+\tau) k_1(\tau) d\tau + n_C C(t) - pV(t) T(t) \\
\dot{A}(t) &= (r_v - \delta_A - \delta X(t)) A(t) - \gamma \int_{-\infty}^0 A(t+\tau) k_2(\tau) d\tau + pV(t) T(t) \\
\dot{C}(t) &= (r_v - \delta_C - \delta X(t)) C(t) + \gamma \int_{-\infty}^0 A(t+\tau) k_2(\tau) d\tau \\
\dot{T}(t) &= (r_u - \delta_u - \delta X(t) - pV(t)) T(t) + S\,,
\end{aligned}$$

which is identical to (3.5).

APPENDIX B. DETAILS OF PROOFS

B.1. ***Proof of Lemma* 4.2:** Let

$$M = \begin{bmatrix} -c & 0 & n_C & 0 \\ 0 & r_v - \delta_A & 0 & 0 \\ 0 & 0 & r_v - \delta_C & 0 \\ 0 & 0 & 0 & r_u - \delta_u \end{bmatrix}$$

and observe that we have

$$\begin{aligned}(\text{B.1}) \qquad |\mathcal{F}((\eta,\phi),\nu,\omega)-\mathcal{F}((\zeta,\psi),\xi,\lambda)| &\leq |L(\eta,\phi)-L(\zeta,\psi)|+\left|\tilde{f}_1(\eta)-\tilde{f}_1(\zeta)\right| \\ &\quad +|f_2(\nu)-f_2(\xi)|+|f_3(\omega)-f_3(\lambda)|\ .\end{aligned}$$

The first, third, and forth terms in the sum on the right hand side of (B.1) are easily bounded by

$$\begin{aligned}|L(\eta,\phi)-L(\zeta,\psi)| &= \left|M(\eta-\zeta)+n_A^1\left[\delta_{(1,2)}\right]_{(4,4)}\int_{-r}^{0}(\phi(\theta)-\psi(\theta))\,dP_1(\theta)\right. \\ &\quad \left.+\gamma^1\left(\left[\delta_{(3,2)}\right]_{(4,4)}-\left[\delta_{(2,2)}\right]_{(4,4)}\right)\int_{-r}^{0}(\phi(\theta)-\psi(\theta))\,dP_2(\theta)\right| \\ &\leq \max\{|M|,|n_A|,2|\gamma|\}\left(|\eta-\zeta|+\|\phi-\psi\|\right)\ ,\end{aligned}$$

$$\begin{aligned}|f_2(\nu)-f_2(\xi)| &= \left|n_A^2\left[\delta_{(1,2)}\right]_{(4,4)}(\nu-\xi)\right| \\ &\leq |n_A|\,|\nu-\xi|\ ,\end{aligned}$$

and

$$\begin{aligned}|f_3(\omega)-f_3(\lambda)| &= \left|\gamma^2\left(\left[\delta_{(3,2)}\right]_{(4,4)}-\left[\delta_{(2,2)}\right]_{(4,4)}\right)(\omega-\lambda)\right| \\ &\leq 2|\gamma|\,|\omega-\lambda|\ .\end{aligned}$$

To bound the second term, note that the multidimensional Mean Value Theorem implies that for $\eta,\zeta\in\mathbb{R}^4$

$$\tilde{f}_1(\eta)-\tilde{f}_1(\zeta) = \int_0^1\left\langle \mathbf{D}\tilde{f}_1(\eta+\theta(\zeta-\eta)),\eta-\zeta\right\rangle d\theta\,,$$

where the 4×4 matrix valued function is given by

$$\mathbf{D}\tilde{f}_1 = \left[\begin{array}{cccc}\frac{\partial\tilde{f}_1}{\partial\lambda_1} & \frac{\partial\tilde{f}_1}{\partial\lambda_2} & \frac{\partial\tilde{f}_1}{\partial\lambda_3} & \frac{\partial\tilde{f}_1}{\partial\lambda_4}\end{array}\right].$$

Define $\bar{\Omega}=\{x\in\mathbb{R}^4 : x_i\leq\bar{x}_i, i=1,2,3,4\}$ and recall the definition of $\tilde{f}_1$ in (4.4). For $x\in\bar{\Omega}$, $\mathbf{D}\tilde{f}_1$ is linear and $\left|\mathbf{D}\tilde{f}_1\right|\leq K_L^1\left(\bar{\Omega}\right)$ for some constant $K_L^1>0$ that depends upon $\bar{\Omega}$. On $\mathbb{R}^4\setminus\bar{\Omega}$, $\mathbf{D}\tilde{f}_1$ is constant and hence $\left|\mathbf{D}\tilde{f}_1\right|\leq K_L^2\left(\bar{\Omega}\right)$ for some constant $K_L^2>0$ that depends upon $\bar{\Omega}$.

By the properties of integrals and Cauchy-Schwarz we then know that

$$\begin{aligned}\left|\tilde{f}_1(\eta)-\tilde{f}_1(\zeta)\right| &\leq \int_0^1 \left|\left\langle \mathbf{D}\tilde{f}_1(\eta+\theta(\zeta-\eta)),\zeta-\eta\right\rangle\right| d\theta \\ &\leq \int_0^1 \left|\mathbf{D}\tilde{f}_1(\eta+\theta(\zeta-\eta))\right| |\zeta-\eta|\, d\theta \\ &\leq \max\left\{K_L^1,K_L^2\right\}|\eta-\zeta|\ .\end{aligned}$$

Combining these results we obtain the global Lipschitz condition (4.6) for

$$K_L=\max\left\{|M|,|n_A|,2|\gamma|,K_L^1,K_L^2\right\}\ .$$

B.2. ***Proof of Theorem* 4.5:** The general idea of our proof is to show that the successive approximations defined in (4.8) converge to a unique solution of (4.7).

Let the residual function of two functions z, w be defined as

$$\begin{aligned}e(t;z,w) &= |z(t)-w(t)|+\|z_t-w_t\|+|z(t-\tau_1)-w(t-\tau_1)| \\ &\quad +|z(t-\tau_1-\tau_2)-w(t-\tau_1-\tau_2)|\ ,\end{aligned} \tag{B.2}$$

for $z,w\in L_2(-r,t_f:\mathbb{R}^4)$, $t\in[-r,t_f]$ and denote

$$E(t;z)=L(z(t),z_t)+\tilde{f}_1(z(t))+f_2(z(t-\tau_1))+f_3(z(t-\tau_1-\tau_2))$$

for $z\in L_2(-r,t_f;\mathbb{R}^4)$, $t\in[-r,t_f]$. Note that both e and E are L_2 functions and as such may not be defined in a pointwise sense, but will be used in the L_2 sense below.

If we consider the residual for the functions y_{j+1} and y_j, we find that for $t\in I$ and $j>0$

$$\begin{aligned}e(t;y_{j+1},y_j) &= \left|\int_0^t (E(\sigma;y_j)-E(\sigma;y_{j-1}))\,d\sigma\right| + \left\|\int_0^{t+\cdot} (E(\sigma;y_j)-E(\sigma;y_{j-1}))\,d\sigma\right\| \\ &\quad +\left|\int_0^{t-\tau_1} (E(\sigma;y_j)-E(\sigma;y_{j-1}))\,d\sigma\right| + \left|\int_0^{t-\tau_1-\tau_2} (E(\sigma;y_j)-E(\sigma;y_{j-1}))\,d\sigma\right| \\ &\leq K_L\int_0^t e(\sigma;y_j,y_{j-1})\,d\sigma + \left\|K_L\int_0^{t+\cdot} e(\sigma;y_j,y_{j-1})\,d\sigma\right\| \\ &\quad +K_L\int_0^{t-\tau_1} e(\sigma;y_j,y_{j-1})\,d\sigma + K_L\int_0^{t-\tau_1-\tau_2} e(\sigma;y_j,y_{j-1})\,d\sigma\end{aligned}$$

$$
\begin{aligned}
&\leq K_L\left(3\int_0^t e\left(\sigma;y_j,y_{j-1}\right)d\sigma+\left\|\int_0^t e\left(\sigma;y_j,y_{j-1}\right)d\sigma\right\|\right)\\
&\leq K_L\left(3+\max\left\{1,\sqrt{r}\right\}\right)\int_0^t e\left(\sigma;y_j,y_{j-1}\right)d\sigma
\end{aligned}
$$

and thus

$$
e\left(t;y_{j+1},y_j\right)\leq K_R\int_0^t e\left(\sigma;y_j,y_{j-1}\right)d\sigma\,, \tag{B.3}
$$

where $K_R=4K_L\max\left\{1,\sqrt{r}\right\}$.

Note that the case for $j=0$ (with $t\in I$) is special

$$
\begin{aligned}
e\left(t;y_1,y_0\right) &= \left|y_1\left(t\right)-y_0\left(t\right)\right|+\left\|\left(y_1\right)_t-\left(y_0\right)_t\right\|+\left|y_1\left(t-\tau_1\right)-y_0\left(t-\tau_1\right)\right|\\
&\quad+\left|y_1\left(t-\tau_1-\tau_2\right)-y_0\left(t-\tau_1-\tau_2\right)\right|\\
&= \left|\int_0^t\left(E\left(\sigma;y_0\right)+f_4\left(\sigma\right)\right)d\sigma\right|+\left\|\int_0^{t+\cdot}\left(E\left(\sigma;y_0\right)+f_4\left(\sigma\right)\right)d\sigma\right\|\\
&\quad+\left|\int_0^{t-\tau_1}\left(E\left(\sigma;y_0\right)+f_4\left(\sigma\right)\right)d\sigma\right|+\left|\int_0^{t-\tau_1-\tau_2}\left(E\left(\sigma;y_0\right)+f_4\left(\sigma\right)\right)d\sigma\right|\\
&\leq \int_0^t\left(K_L\left|e\left(\sigma,y_0,0\right)\right|+\left|S\right|\right)d\sigma+\left\|\int_0^{t+\cdot}\left(K_L\left|e\left(\sigma,y_0,0\right)\right|+\left|S\right|\right)d\sigma\right\|\\
&\quad+\int_0^{t-\tau_1}\left(K_L\left|e\left(\sigma,y_0,0\right)\right|+\left|S\right|\right)d\sigma+\int_0^{t-\tau_1-\tau_2}\left(K_L\left|e\left(\sigma,y_0,0\right)\right|+\left|S\right|\right)d\sigma\\
&\leq 3\left|S\right|\left|t\right|+3K_L\int_0^t\left|e\left(\sigma,y_0,0\right)\right|d\sigma+\left\|\int_0^{t+\cdot}\left(K_L\left|e\left(\sigma,y_0,0\right)\right|+\left|S\right|\right)d\sigma\right\|\\
&\leq 3\left|S\right|\left|t\right|+3K_L\left(\left|\Phi\left(0\right)\right|\left|t\right|+\left|t\right|^{\frac{1}{2}}\left(\int_{-\tau_1}^{t-\tau_1}\left|y_0\left(\theta\right)\right|^2d\theta\right)^{\frac{1}{2}}+\left|t\right|^{\frac{1}{2}}\left(\int_{-\tau_1-\tau_2}^{t-\tau_1-\tau_2}\left|y_0\left(\theta\right)\right|^2d\theta\right)^{\frac{1}{2}}\right.\\
&\quad\left.+\int_0^t\left(\int_{\sigma-r}^{\sigma}\left|y_0\left(\theta\right)\right|^2d\theta\right)^{\frac{1}{2}}d\sigma\right)+\left(\int_{-r}^0\left(\int_0^{t+\theta}\left(K_L\left|e\left(\sigma,y_0,0\right)\right|+\left|S\right|\right)d\sigma\right)^2d\theta\right)^{\frac{1}{2}}\\
&\leq 3\left|S\right|\left|t\right|+3K_L\left(\left|\Phi\left(0\right)\right|\left|t\right|+2\left|t\right|^{\frac{1}{2}}\left(\int_{-r}^t\left|y_0\left(\theta\right)\right|^2d\theta\right)^{\frac{1}{2}}+\int_0^t\left(\int_{-r}^t\left|y_0\left(\theta\right)\right|^2d\theta\right)^{\frac{1}{2}}d\sigma\right)\\
&\quad+\int_0^t\left(K_L\left|e\left(\sigma,y_0,0\right)\right|+\left|S\right|\right)d\sigma\left(\int_{-r}^0d\theta\right)^{\frac{1}{2}}\\
&\leq 3G\left(t;\Phi\right)+\sqrt{r}G\left(t;\Phi\right)
\end{aligned}
$$

where

$$G(t;\Phi) = \{|S| + K_L(3|\Phi(0)| + \|\Phi\|)\}|t| + K_L|\Phi(0)||t|^{\frac{3}{2}} + 2K_L\|\Phi\||t|^{\frac{1}{2}} .$$

Thus

$$e(t;y_1,y_0) \leq \frac{K_R K_G}{K_L}\max\left\{|t|^{\frac{1}{2}}, |t|^{\frac{3}{2}}\right\}$$

for

$$K_G = |S| + K_L(4|\Phi(0)| + 3\|\Phi\|) .$$

We claim that from (B.3) and the $j = 0$ case, we have

$$e(t;y_{n+1},y_n) \leq \frac{K_R K_G}{K_L}\frac{(K_R|t|)^n}{(n)!}\max\left\{\sqrt{t}, |t|^{\frac{3}{2}}\right\}, t \in I . \tag{B.4}$$

Clearly, this is true for $n = 0$, and the general case follows easily from induction using (B.3). Using the estimate (B.4), we can then infer that

$$\begin{aligned}\sum_{j=0}^{\infty} e(t;y_j,y_{j-1}) &\leq \frac{K_R K_G}{K_L}\sum_{j=0}^{\infty}\frac{(K_R|t|)^{(j)}}{(j)!}\max\left\{\sqrt{t}, |t|^{\frac{3}{2}}\right\} \\ &\leq \frac{K_R K_G}{K_L}\max\left\{\sqrt{t}, |t|^{\frac{3}{2}}\right\}e^{K_R|t|} .\end{aligned}$$

Thus, by the comparison test, $\sum_{j=0}^{\infty} e(t;y_j,y_{j-1})$ converges uniformly for $t \in I$, which proves that $\{y_j(t)\}$ converges uniformly for all $t \in I$. Denote $\lim_{j\to\infty} y_j(t)$ as $y(t)$. Since the y_j's are continuous and converge uniformly to y, we see that y is both continuous on I and satisfies (4.5) by taking limits in (4.8). Note that this also yields y absolutely continuous on $[0, t_f]$

To prove the uniqueness of our solution, suppose we have two distinct solutions $\{y, \tilde{y}\} \in L_2(-r, t_f; \mathbb{R}^4)$ to (4.7). Using the same arguments as in establishing (B.3), we have

$$\begin{aligned}e(t;y,\tilde{y}) &\leq \left|\int_0^t (E(\sigma;y) - E(\sigma;\tilde{y}))\,d\sigma\right| + \left\|\int_0^{t+\cdot} (E(\sigma;y) - E(\sigma;\tilde{y}))\,d\sigma\right\| \\ &\quad + \left|\int_0^{t-\tau_1} (E(\sigma;y) - E(\sigma;\tilde{y}))\,d\sigma\right| + \left|\int_0^{t-\tau_1-\tau_2} (E(\sigma;y) - E(\sigma;\tilde{y}))\,d\sigma\right| \\ &\leq K_R\int_0^t e(\sigma;y,\tilde{y})\,d\sigma .\end{aligned}$$

Thus by Gronwall's inequality we have that

$$\begin{aligned} 0 \quad \leq \quad & |y(t) - \tilde{y}(t)| + \|y_t - \tilde{y}_t\| + |y(t-\tau_1) - \tilde{y}(t-\tau_1)| \\ & + |y(t-\tau_1-\tau_2) - \tilde{y}(t-\tau_1-\tau_2)| \leq 0 \end{aligned} \quad \text{for } t \in I,$$

and thus $y(t) = \tilde{y}(t)$ for $t \in I$ and also for $t \in [-r, 0]$ since they satisfy the same initial condition.

We have therefore now proven that there exists a unique solution (for $[-r, t_f]$) to (4.7) and thus to (4.5), which is in fact absolutely continuous on $[0, t_f]$.

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