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Synaptic transmission and the susceptibility of HIV infection to anti-viral drugs

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Cell-to-cell viral transmission via virological synapses has been argued to reduce susceptibility of the virus population to anti-viral drugs through multiple infection of cells, contributing to low-level viral persistence during therapy. Using a mathematical framework, we examine the role of synaptic transmission in treatment susceptibility. A key factor is the relative probability of individual virions to infect a cell during free-virus and synaptic transmission, a currently unknown quantity. If this infection probability is higher for free-virus transmission, then treatment susceptibility is lowest if one virus is transferred per synapse, and multiple infection of cells increases susceptibility. In the opposite case, treatment susceptibility is minimized for an intermediate number of virions transferred per synapse. Hence, multiple infection via synapses does not simply lower treatment susceptibility. Without further experimental investigations, one cannot conclude that synaptic transmission provides an additional mechanism for the virus to persist at low levels during anti-viral therapy.

The dynamics between human immunodeficiency virus (HIV) and its target cells have been subject to much research, both experimentally and mathematically[1–4]. A relatively recent development in the field is the realization that direct cell-to-cell transmission via formation of virological synapses might contribute significantly to virus spread *in vivo*[5–11]. In fact, it has been argued that synaptic transmission might play a more important role than free virus transmission. This notion derived from the observed high efficiency of synaptic virus transmission on a per cell basis[5]. That is, a source cell has been observed to transfer tens to hundreds of viruses directly to its target cells. While certainly not all of the transferred viruses successfully infect the target cells, synaptic transmission is thought to be a major factor in the infection of cells with multiple copies of the virus. Multiple infection of cells is readily observed in tissue compartments[12], while cells in the blood tend to be infected with only one viral copy[13]. The reason for this difference is likely to be a reduced ability of cells to form virological synapses in the blood where cells mix better and are less packed.

Synaptic transmission and the consequent multiple infection of cells can have important consequences for the dynamics of the infection[14–25]. Possible effects on the response to drug treatment have been explored and the argument has been put forward that synaptic transmission and multiple infection render the virus population less susceptible to anti-viral drugs and could contribute to the maintenance of residual virus during treatment[26]. Based on data and some mathematical arguments[26], it has been suggested that multiple transfer of susceptible viruses simply makes it statistically more likely to infect a given target cell in the presence of the drug (for each virus particle there is some small chance that the drug does not bind), thus reducing the effect of anti-viral drugs to prevent infection of the cell. Here we use a new mathematical approach in which we explicitly include both free-virus and synaptic transmission into previously established models of virus dynamics. Analysis of this model shows that the relationship between synaptic transmission and drug susceptibility is rather complex and depends on the relative probability for a virus particle to infect a target cell during free virus and synaptic transmission. If this infection probability is higher for free virus transmission, then the lowest susceptibility to anti-viral drugs is observed if a single virus particle is transferred per synapse. Transferring a greater number of viruses increases susceptibility to drugs. On the other hand, if the probability for a virion to infect a cell is greater during synaptic transmission, then an intermediate number of viruses transferred per synapse minimizes susceptibility to anti-viral drugs. Both a lower and a higher number of transferred viruses increases susceptibility. Interestingly, in this case, the number of viruses transferred per synapse that minimizes treatment susceptibility does not coincide with

the maximal basic reproductive ratio of the virus. These results suggest that without further experimental work, it cannot be concluded that synaptic transmission contributes to continued low level replication of the virus during therapy.

Results

The model. *Population dynamics of infection*. We introduce synaptic transmission into ordinary differential equation models that have been used to study virus dynamics in the literature[1,2,27–30]. This is a different approach compared to other studies that examined cell-to-cell transmission in different contexts[29,31]. We have the following equations:

$$\begin{aligned}
\dot{x}_0 &= \lambda - dx_0 - \tilde{\beta} x_0 v - x_0 S \sum_{m=1}^{N} x_m \sum_{j=1}^{N} \gamma^{(m)}{}_j \ , \\
\dot{x}_i &= \tilde{\beta} x_{i-1} v - \tilde{\beta} x_i v + S \sum_{m=1}^{N} x_m \left(\sum_{j=1}^{i} \gamma^{(m)}{}_j \ x_{i-j} - x_i \sum_{j=1}^{N-i} \gamma^{(m)}{}_j \right) - \\
&\quad a^{(i)} x_i, \ 0 < i < N, \\
\dot{x}_N &= \tilde{\beta} x_{N-1} + S \sum_{m=1}^{N} x_m \sum_{j=1}^{N} \gamma^{(m)}{}_j \ x_{N-j} - a^{(N)} x_N, \\
\dot{v} &= \sum_{m=1}^{N} k^{free,(m)} x_m - uv.
\end{aligned} \tag{1}$$

Here x_i denotes the number of cells infected by i viruses; we will say that such cells have the multiplicity of infection i. The maximum multiplicity of infection is denoted by N. Uninfected cells are denoted by x_0, and v is the population of free virus. Target cell production and death rates are given by λ and d. Infected cells die with a rate a_i. It is assumed that a fraction of the viruses produced by a cell is transmitted via the synaptic (cell-cell) pathway. The remaining fraction is transmitted via the free-virus pathway. The free-virus pathway is represented by terms multiplying parameter $\tilde{\beta}$. For this pathway, virus is produced by infected cells at rate k_m^{free}, which in general can be a function of the cell's multiplicity of infection. Free virus decays with rate u. The cell-cell transmission pathway is represented by terms multiplying S, the rate of synapse formation. The coefficients γ_j^m are the probabilities for a cell with multiplicity of infection m to successfully transmit j copies of virus per synapse.

In the general system (1), kinetic parameters such as virus production and cell death can depend on the cells' multiplicity of infection (MOI). The effects of the MOI dependence are explored in[32]. In this paper however we will assume that the kinetic parameters are independent of the MOI, since there is currently no evidence to the contrary. In this case, we have $a_i = a$, $\gamma_j^m = \gamma_j$, $k_m^{free} = k^{free}$, and system (1) simplifies to a two-equation model,

$$\begin{aligned}
\dot{x} &= \lambda - dx - (\beta^{syn} + \beta^{free}) xy, \\
\dot{y} &= (\beta^{syn} + \beta^{free}) xy - ay,
\end{aligned} \tag{2}$$

where x denotes the number of uninfected cells and y the total number of infected cells. Note that in the derivation of system (2) we used a quasi-equilibrium approximation for the number of free viruses, see Supplementary Information for details. Denoting $r^{free} = \tilde{\beta}/u$, we can write the rates of infection for the two pathways as $\beta^{free} = k^{free} r^{free}$ and $\beta^{syn} = S \sum_{j=1}^{N} \gamma_j$.

Kinetics of infection. The next layer of modeling relates the cells' rates of infection with their transmission strategies. We denote by s the mean number of viral particles that a source cell attempts to transmit to its target (per synapse). We will refer to the quantity s as the cell's "strategy". The parameter γ_j denotes the probability to successfully transmit j viruses per synapse, that is, the probability that j viruses get incorporated into the genome of the target cell per synapse. The parameter γ_j depends on the cell's strategy, and also on the infectivity per virus particle (we assume that the virions' success of infection is independent from each other). The latter quantity is the probability for an individual virus particle transmitted to survive and successfully infect a target cell; we denote this quantity by r. An example of the probability distribution γ_j for a fixed strategy s and for different values of r is given in figure 1(a). There, we made the simplifying assumption that an infected cell attempts to transfer s viruses to the target cells with probability σ, or it transfers 0 viruses with probability $1 - \sigma$, which corresponds to the following expression:

$$\gamma_j = \sigma \binom{s}{j} r^j (1-r)^{s-j}. \tag{3}$$

This model allows us to gain analytical insights into the process of coinfection, when examining the costs and benefits of the different synaptic transmission strategies (see Supplementary Information for

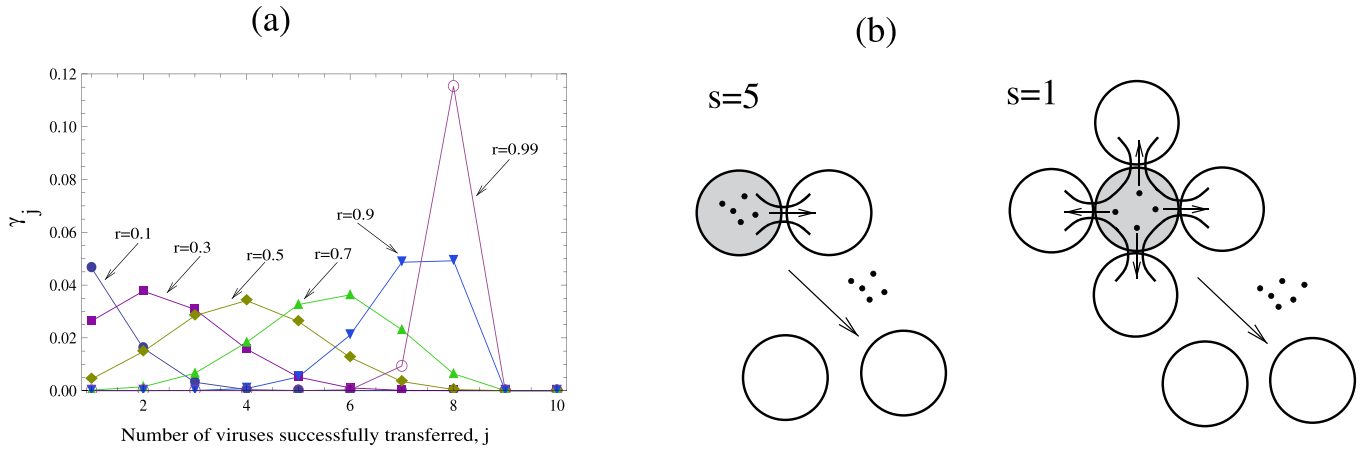

Figure 1 | The functions γ_j, the probability to successfully transmit j viruses, given strategy $s = 8$, for different values of the infectivity parameter, r. We have $\gamma_j = \sigma \binom{s}{j} r^j (1-r)^{s-j}$ and $\sigma = 0.125$. (b) A schematic illustrating the model of synaptic and free-virus transmission. For illustration purposes, we assume that $k^{tot} = 10$ viruses per time-unit. With $s = 5$ (that is, 5 viruses transferred per synapse), and one synapse formed per time-unit ($S = 1$), we have $k^{syn} = 5$ and the remaining viruses are transmitted by the free-virus pathway: $k^{free} = 10 - 5 = 5$. With $s = 1$, assuming that no more that 4 synapses can be formed per time-unit ($S = 4$), we have $k^{syn} = 4$ and $k^{free} = 10 - 4 = 6$ remaining viruses are transferred through free-virus transmission.

a more general case). The overall replication rate of the virus in the synaptic mode depends on the rate of synapse formation, S, on the number of viruses transferred per synapse, and on the probability for individual viruses to infect the target cell:

$$\beta^{syn} = S\sigma(1-(1-r)^s).$$

Virus transmission and the rate of synapse formation. We have to establish a relationship between the processes of virus production and synapse formation. Let us denote by k^{tot} the rate with which viruses are produced within an infected cell and transferred to target cells. The rate at which viruses are transferred to target cells via synapses is given by $k^{syn} = S\sigma s$. The rate at which viruses are transferred to target cells as free viruses is given by k^{free}, and we have

$$k^{tot} = k^{syn} + k^{free}.$$

In the simplest case, the synapse formation rate is inversely proportional to the number of viruses transferred per synapse (i.e. the viral strategy), such that $S = Q/(\sigma s)$. This means that if s is small, then a cell attempts to pass a small number of particles to many cells by forming many synapses. If s is large, then the cell's strategy is to transfer many viral particles to a few cells, by forming few synapses. If fewer viruses are transferred per synapse (lower s), the cell has to form a larger number of synapses to transfer the same number of viruses during its life-span. This may pose a problem if very few viruses are transferred per synapse because in this case, the cell would have to establish an unrealistically large number of synaptic connections during its life-span. A more realistic assumption is that there is a limited number of synapses a cell can form during its life-span because of time-constraints involved in the processes of synapse formation, virus transmission, and spatial constraints limiting the ability to find new target cells. Thus, for relatively low amounts of transferred viruses (low s), it is not possible to form enough synapses to transfer all the viruses produced, and this cap can be expressed mathematically by e.g., $S = \frac{Q}{\sigma(s+z)}$, where z is a parameter. Combining all the expressions, we obtain the case-study model for the rate of synaptic transmission (corresponding to formula (3) for successful transmission probabilities) that is used in this paper:

$$\beta^{syn} = \frac{Q(1-(1-r)^s)}{s+z}.$$

We further assume that the viruses that are not transferred via synapse leave the cell as free viruses. As a consequence, for low numbers of viruses transferred per synapse (low s), there will be a higher rate of free-virus transfer. The total rate with which viruses are produced within an infected cell and transferred to target cells, k^{tot}, is assumed to be independent of the viral strategy. These concepts are illustrated in figure 1(b). We note that this formulation can also be interpreted to correspond to spatial restrictions in synapse formation, where the ability of a source cell to connect to a target cell is limited to the local neighborhood.

Effects of the drug treatment. The effect of drug treatment is modeled by lowering the probability with which individual viruses infect a cell: $r \to r/f$, $r^{free} \to r^{free}/f$, where $f > 1$ is some number that characterizes the strength/effectiveness of the drug. In our modeling framework we make the following assumptions. Furthermore, we assume that the

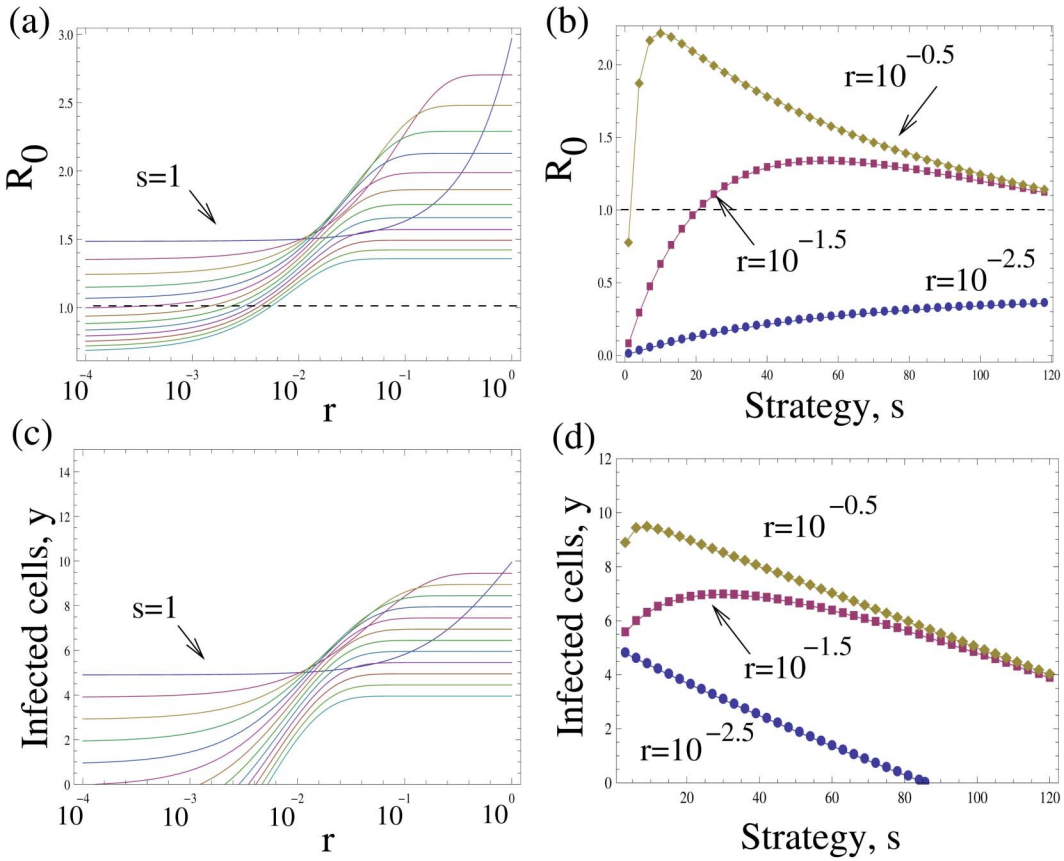

Figure 2 | Basic virus dynamics in the presence of cell-cell transmission. The basic reproductive ratio R_0 (a,b) and the total number of infected cells y (c,d), are plotted as functions of the infectivity r, and strategy s. The horizontal dashed line in (a) corresponds to the infection threshold, $R_0 = 1$. In panels (a,c) the curves correspond to strategies for increasing values of s, starting from $s = 1$ to $s = 121$, with increment 10. Panels (b, d) plot the number of infected cells as a function of strategy, s, for three fixed values of r. Other parameters are: $z = 100$, $Q = 1$, $\lambda = 15$, $a = 1$, $d = 0.1$, $r^{free} = 0.01$, $k^{tot} = 1$.

drugs affect the viruses identically, regardless of the pathway of transmission. This certainly applies to reverse transcriptase inhibitors. Protease inhibitors reduce the number of infectious offspring virus produced by the cell. If non-infectious offspring viruses are more likely to enter synapses than the extracellular environment, the two transmission pathways could be disproportionally affected. This was not studied here, since there is no indication that this occurs.

In what follows, we consider different synaptic transmission strategies, depending on the number of viruses that are transferred per synapse, which can range from $s = 1$ to arbitrarily high numbers. We will examine which strategies are more susceptible to treatment.

Basic virus dynamics. Before focusing on the relationship between synaptic transmission and anti-viral treatment, we will briefly review the basic virus dynamics described by system (2), and how they are influenced by the number of viruses transferred per synapse. Figure 2 shows graphs of the basic reproductive ratio of the virus (top graphs) and the equilibrium viral load, y (bottom graphs), as functions of virus parameters. From equation (2) we know that $y = \frac{\lambda}{a} - \frac{d}{\beta}$ and $R_0 = \frac{\lambda\beta}{ad}$. In (a) and (c), the quantities R_0 and y are plotted as functions of the parameter r, the probability for a virus particle in a synaptic transfer to infect a cell. The parameter r^{free}, the probability of a free virus particle to infect a target cell, remains fixed at 10^{-2}. Graphs are presented for different synaptic strategies, i.e. for different numbers of viruses transferred per synapse, s. Not surprisingly, the basic reproductive ratio and the equilibrium viral load are increasing functions of r. One striking observation is that the shape of all curves changes as the parameter r crosses the threshold value $r > r^{free}$. This can be seen better in figures 2(b,d), where the same quantities R_0 and y are presented as functions of the number of viruses transferred per synapse, s, for several fixed values of r. For $r < r^{free}$, the optimal number of transferred viruses (both in terms of the highest R_0 and the highest viral load) corresponds to s = 1, i.e. the transfer of a single virus particle per synapse. On the contrary, for $r > r^{free}$, there is an intermediate number of viruses transferred per synapse, s, that maximizes R_0 and equilibrium viral load.

These observations are explained as follows. As outlined above, if the number of viruses transferred per synapse is low (s = 1 in the limit), the cell cannot form enough synapses to transmit all offspring virus during its life-span, which are consequently released as free viruses. If the infectivity per virus particle is greater for free virus compared to synaptic transmission ($r < r^{free}$), then $s = 1$ becomes the best strategy because it maximizes the amount of virus released into the extra-cellular environment. On the other hand, if the infectivity per virus particle is greater during synaptic transmission ($r > r^{free}$), the $s = 1$ strategy becomes disadvantageous. Increasing the number of viruses transferred per synapse leads to an increase in the basic reproductive ratio of the virus, R_0. If the number of viruses transferred per synapse becomes too large, however, the value of R_0 declines again. The reason is that in this case a source cell transfers more viruses than are statistically needed to infect the target cell, and this excess virus is essentially wasted, as it could potentially be distributed to cells that are so far uninfected. Hence, there is an optimal number of viruses transferred per synapse, s, that maximizes R_0.

Drug-mediated reduction of R_0 below one. The most desirable effect of drug treatment is to reduce the basic reproductive ratio of the virus, R_0, below one. In our model, this leads to extinction of the infection. However, in vivo, this corresponds to maximal viral suppression because the virus persists due to factors not taken into account in the model, such as the presence of reservoirs. From equation (2) we see that infection can be stably maintained at level $y = \frac{\lambda}{a} - \frac{d}{\beta}$ if $R_0 = \frac{\lambda\beta}{ad} > 1$. Otherwise we have $y = 0$ and no infection can be established. The effect of anti-viral drugs in HIV is to reduce the value of R_0 by lowering the rate at which new cells become infected. Figure 3 shows the effect of the drug on the extinction of the virus population in the model assuming different viral strategies, i.e. different numbers of viruses transferred per synapse, s.

First, consider the scenario where $r > r^{free}$, i.e. the infectivity per virus particle is greater for synaptic than for free virus transmission (Figure 3 a,b). Each curve in figure 3(a) corresponds to a particular value of drug strength, f, and shows the basic reproductive ratio, R_0, as a function of the number of transferred viruses, s. The top curve with the highest R_0 can be interpreted to correspond to the absence of treatment. When a drug is applied, r and r^{free} decrease f-fold and the basic reproductive ratio also declines. As the strength of the drug, f, increases, fewer and fewer strategies remain viable. For larger values of f, a smaller part of the R_0 curve appears above the line $R_0 = 1$. Virus types characterized by values of s for which $R_0 < 1$ are eliminated by treatment in the model, practically meaning maximal viral suppression in vivo. As the strength of the drug, f, continues to grow (more effective treatment), only a few strategies remain viable (that is, they still correspond to $R_0 > 1$). The viral strategy that is the hardest to eliminate (for the parameter choice of figure 3) corresponds to about $s = 38$. The corresponding virus type is most resistant to treatment and requires the largest value of f to be eliminated. Figure 3(b) shows the threshold value of drug strength, f, above which the R_0 for viruses with different synaptic strategies, s, becomes less than one. The maximum of this curve corresponds to the strategy with $s = 38$. Note that the numbers quoted in this example are arbitrary and only serve the purpose of illustrating the result that the transfer of intermediate numbers of viruses per synapse minimizes susceptibility to anti-viral drugs. The kinetics of synaptic transmission cannot be currently parameterized based on available information.

It is interesting that the synaptic strategy that is the hardest to treat is not the same as the strategy that has the largest R_0 and the largest viral load in the absence of treatment. The latter strategy can be found in figure 3(a) as the one corresponding to the maximum of the top curve (the curve without treatment, s ≈ 5 for this parameter combination). This value of s is smaller than the value $s = 38$, which corresponds to the strategy that is the hardest to treat (the maximum of the bottom curve).

This effect is not observed if $r^{free} > r$, i.e. if the infectivity per virus particle is greater for free virus transmission (figure 3(c,d)). In this case, the strategy that is the hardest to treat always corresponds to $s = 1$, and it is also the strategy corresponding to the highest viral load.

To summarize, in the regime where synaptic transmission leads to a higher infectivity per particle than free virus transmission, we find that viruses characterized by the transfer of an intermediate number of viruses per synapse are the hardest to eliminate by treatment in the model (i.e. to maximally suppress in practice). As was demonstrated in the previous section, lower and higher numbers of transferred viruses are less efficient for virus spread, and it is not surprising that they are easier to eliminate. An interesting and somewhat counter-intuitive fact is that the viral transmission strategies which are the hardest to eliminate are not the same as the ones producing the largest viral load in the absence of treatment.

Treatment and the transmission index. Here we consider situations in which maximal viral suppression in not achieved, i.e. therapy does not reduce the basic reproductive ratio of the virus below one. In this case, the effectiveness of the drug can be measured by the so-called transmission index[26]. This quantity, T_x, evaluates the ratio between the viral load in the presence of the drug and the viral load in the absence of the drug. The lower the transmission index, the more effective the drug.

In the context of the drug transmission index, the behavior of the system again is defined by the relative magnitude of the infectivity per virus particle in the two transmission pathways (r vs r^{free}). In

figure 4 we show the graphs of $T_x(s)$, as function of the number of viruses transferred per synapse. Each of the curves is plotted for a fixed pair (r, r^{free}), and these two parameters differ from graph to graph. From left to right, the parameter r^{free} increases, and from bottom to top, parameter r increases. The graphs in the top left are characterized by $r > r^{free}$, and the graphs in the bottom right correspond to the opposite inequality.

We can see that in the region where $r > r^{free}$, the curve $T_x(s)$ is is characterized by one maximum. That is, the transfer of an intermediate number of virus particles per synapse leads to the lowest susceptibility to anti-viral drug treatment. Transferring a lower or higher number of viruses typically increases susceptibility to treatment. As in the case of complete virus elimination, the viral strategies that are the hardest to treat do not necessarily have the highest R_0 and viral loads (not shown).

In the opposite case, where $r < r^{free}$ (bottom right graphs of figure 4), the lowest susceptibility to treatment is observed if a single virus particle is transferred per synapse (s = 1). This is intuitively clear, as such strategies transfer the largest number of viral particles as free viruses, thus utilizing the strategy with the minimal losses (under the assumption that $r < r^{free}$).

The effect of drug saturation. In this section, we will consider the effect of saturating the drug when the virus enters the cell following different transmission strategies. This applies to drugs that act by preventing infection of the cell in question, most notably reverse transcriptase inhibitors. It is reasonable to assume that with certain drugs, only a limited number of drug particles are available in each cell. These drug particles act upon individual viruses by effectively reducing their probability of successfully infecting the cell.

Let us consider an individual synaptic transmission strategy. If the number of viruses transmitted via a synapse is lower than the number of drug particles in the cell, then the model explored in the previous sections applies without change. If however the number of viruses sent through a synapse is larger than the number of drug particles, we expect to observe the effect of flooding. Let us suppose that n_1 drug particles are available in the cell. Then the first n_1 viruses will be bound to them, resulting in a low individual probability of infection per virus, r/f. If the number of viruses entering the cell by synapse, $s > n_1$, then the remaining $n_2 = s - n_1$ particles will have a higher probability of successfully infecting, given by r (the probability of infection in the absence of treatment). This leads to a different expression for the probability of successfully transmitting j viruses,

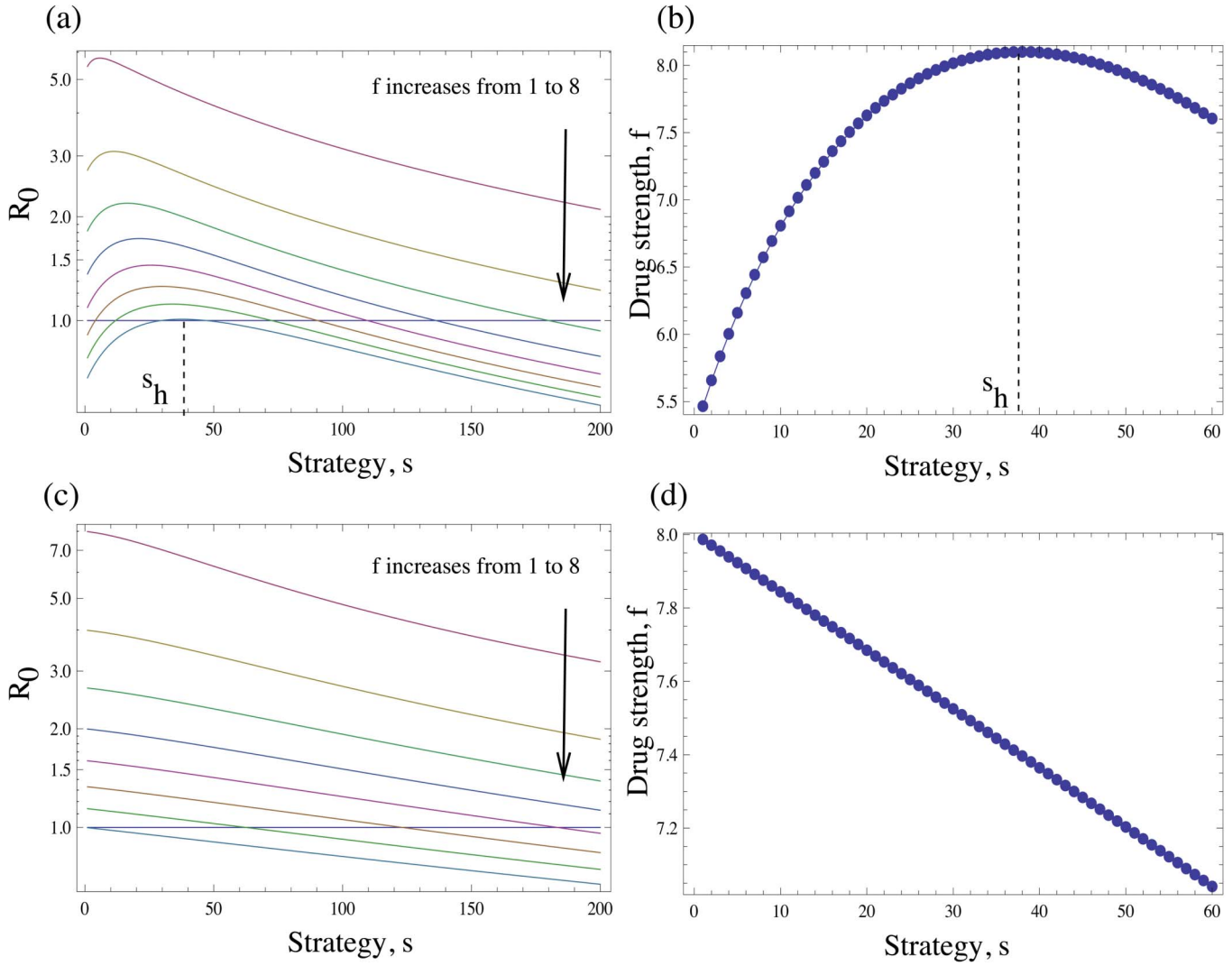

Figure 3 | The effect of increasing the drug strength. Top row: $r^{free} < r$. (a) The basic reproductive ratio, R_0, as a function of s, for several different values of drug strength, f. The value s_h corresponds to the strategy which remains viable for the highest drug strength, f. (b) The value, f, above which a strategy becomes non-viable. Parameters are $z = 100$, $Q = 1$, $\lambda = 10.5$, $a = 1$, $d = 0.1$, $r^{free} = 0.05$, $r = 0.25$, $k^{tot} = 1$. Bottom row: $r^{free} > r$. (c–d) The same as (a,b), except $r = 0.04$, $\lambda = 16$.

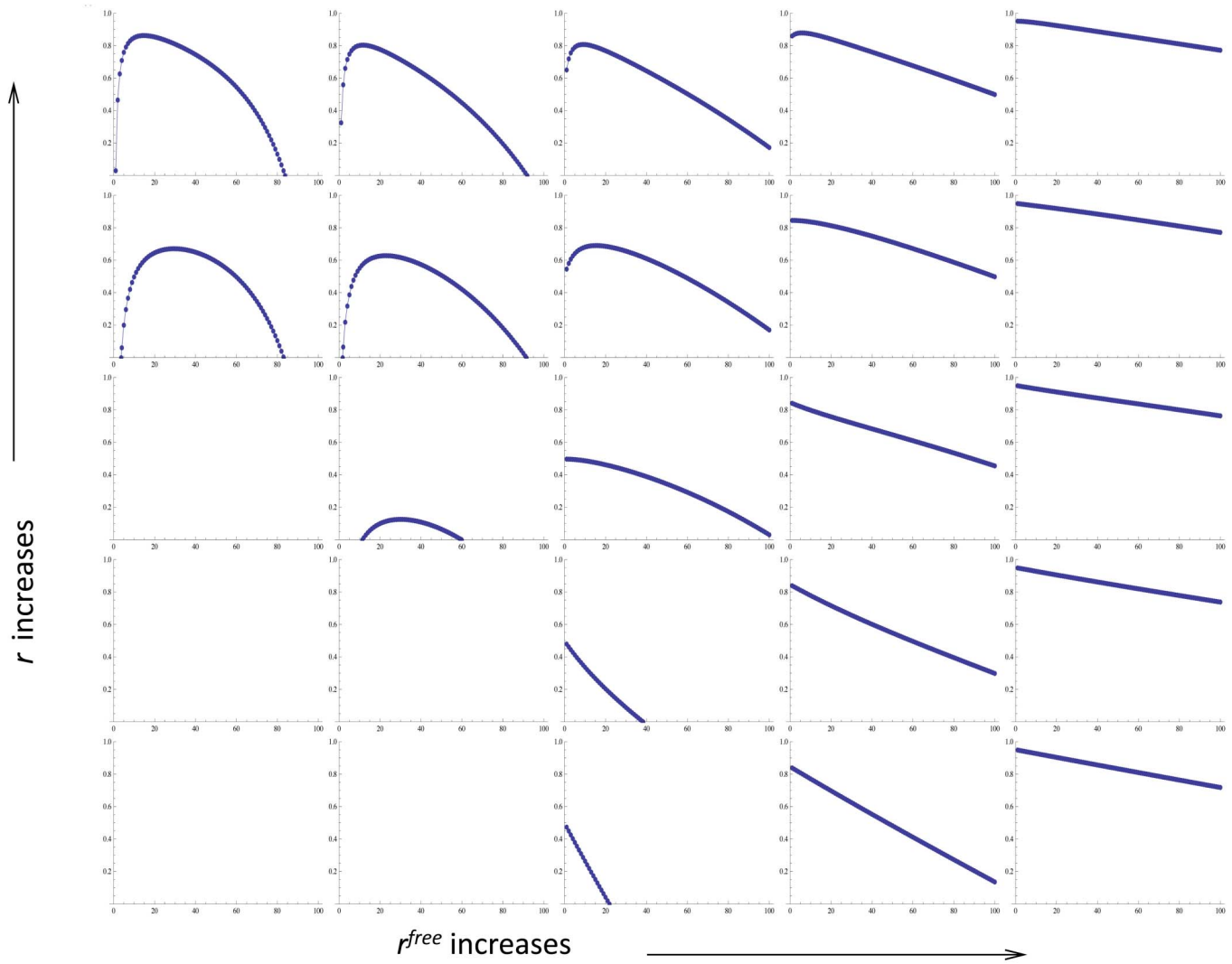

Figure 4 | The effect of drug treatment. Each graph plots the transmission index, T_x, as a function of strategy, s. The graphs differ from each other by the values of r^{free}, which increase from left to right from $10^{-2.2}$ to $10^{-0.2}$, with the exponent changing with a uniform step-size, and by the values of r, which increase from bottom to top in the same manner, from $10^{-2.2}$ to $10^{-0.2}$. Empty graphs indicate that $R_0 < 1$ for all values of s. Other parameters are: $z = 60$, $Q = 1$, $\lambda = 150$, $a = 0.5$, $d = 1$, $k^{tot} = 1$, $f = 10$.

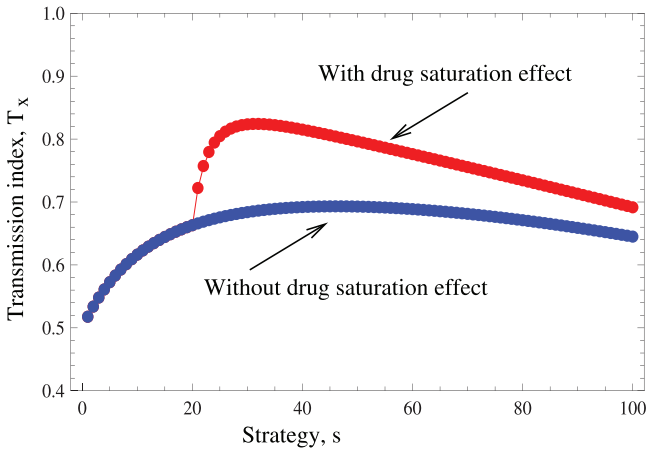

Figure 5 | The effect of drug saturation. Plotted is the transmission index as a function of s. Parameters are: $z = 60$, $Q = 1$, $\lambda = 150$, $a = 0.5$, $d = 1$, $r^{free} = 10^{-1.2}$, $r = 10^{-0.7}$, $f = 10$, $n_1 = 20$. For comparison, the same quantity is also presented in the absence of drug saturation ($n_1 \to \infty$).

γ_j. It then follows that an advantageous strategy is to transmit $s > n_1$ viruses, such that some of them will have a higher chance of infection. As a result, the basic reproductive ratio of the virus and the equilibrium virus load during treatment increases sharply as the number of virus particle transferred per synapse, s, rises above a certain threshold and begins to saturate the drug, see figure 5. In accordance with this trend, the transmission index increases drastically after this threshold in s, and the drug susceptibility decreases sharply. For even higher number of viruses transferred per synapse, s, R_0 and equilibrium virus load during treatment starts to decrease and the drug susceptibility increases. This is because of the general trend described above, where very high-s strategies are disadvantageous because they waste virus particles by transmitting "too many" of them per synapse.

The model described here can be refined by including more complexity. For example, in the present model it is assumed that all n_1 drug particles reduce the infection probability of n_1 viruses (if available). In reality it is possible that not all drug particles successfully bind the virions. In this case, the difference between strategies with $s < n_1$ and $s > n_1$ will be less sharp, but the qualitative conclusions will still hold. Related, a virus particle contains multiple reverse

transcriptase molecules, although only one of them performs the function. If further drug particles bind to the other reverse transcriptase molecules, the drug can be saturated with a lower number of viruses. Results would remain qualitatively identical, only changing the exact threshold number of transferred virus particles which is required to see this effect.

In the situation described here we have a subtle trade-off between "too few" and "too many" viruses transmitted. "Too few" virus particles cannot saturate the drug and may get eliminated by treatment. "Too many" may not comprise a sustainable infection. Depending on the parameters, there may be an intermediate optimum (from the virus point of view), which saturates the drug but still corresponds to a viable strategy. From the point of view of treatment, this strategy is the toughest to handle.

This phenomenon may not be observed for other parameter combinations, where strategies capable of flooding the drug (that is, strategies with s with $s > n_1$) correspond to $R_0 < 1$ in the absence of treatment and thus are not viable. In this case the mathematical analysis of the previous sections holds without change.

Discussion

Our analysis has shown that the relationship between synaptic transmission, multiple infection, and susceptibility to treatment is complex. The exact result depends on which transmission pathway is characterized by a higher infectivity per virus particle. If this is higher for free-virus transmission, then susceptibility to anti-viral drugs is minimized if a single virus is transferred per synapse, since this maximizes the release of free virus from infected cells. On the other hand, if the infectivity per virus particle is higher for synaptic transmission, then we found that an intermediate number of viruses transferred per synapse minimizes susceptibility to antiviral drugs. Transferring too few viruses per synapse reduces the efficiency of virus spread. This is because the infected cell would have to establish synaptic connections with an unrealistically high number of target cells in order to maintain overall synaptic output, which cannot happen. This consequently leads to the release of more free virus characterized by a lower infectivity per particle in this case. The transfer of too many viruses per synapse also reduces the efficiency of virus spread because many viruses that could be distributed among uninfected cells are passed to a cell that has already become infected by previously transferred viruses.

With this result in mind, one can ask whether free virus transmission is more or less susceptible to anti-viral drugs than the synaptic strategy. To answer this question one needs to compare parameters characterizing the kinetics of the two transmission modes. In particular, measurements of r and r^{free} are required, i.e. the probability that individual virus particles successfully infect cells. This contains the infectivity itself and also the decay rate of virions in the two scenarios. Unfortunately, the probability for a virus particle to successfully infect during free virus and synaptic transmission is currently not known. It is feasible that *in vivo*, the rate of virus loss in the extracellular environment significantly exceeds that occurring during synaptic transmission, even though a sizable amount of virus can be lost in the endocytic pathway during synaptic transmission[5]. Neutralizing antibodies can have a drastic impact on the survival of free virus. On the other hand, viruses passed through synapses could be less susceptible to antibody-mediated activity, although the effect of antibodies on synaptically transmitted viruses is currently controversial[5,8,33–35]. A better understanding of these processes will be crucial to determine which parameter regime in our model most likely applies to HIV.

A recent study has shown reduced susceptibility of HIV to reverse transcriptase inhibitors when the virus was grown in co-culture conditions (where synaptic transmission is thought to occur) compared to a situation where infection occurs with free virus only[26]. To explain this observation, it has been argued that multiple infection of cells through synaptic transmission always lowers susceptibility to anti-viral drugs and that this could contribute to long-term residual viral replication during the course of treatment, which can be considered a barrier to the ultimate goal of eradicating the virus from the patient. Such an effect would be highly relevant in the light of current efforts aimed at flushing out residual viral reservoirs during anti-viral treatment. The study suggested that transfer of more viruses to target cells monotonically reduces susceptibility to treatment due to a simple statistical effect: each virus particle has a low chance to infect a cell during treatment, and increasing the number of infection attempts per cell increases the likelihood of infection. However, this argument implicitly assumes that there is no limit to the burst size of the infected cell. In contrast, our model assumes a fixed burst size of infected cells such that the transfer of a larger number of viruses per synapse reduces the number of cells to which a source cell can potentially transmit its offspring. This has the consequence that for relatively large numbers of viruses transferred per synapse, the rate of viral growth slows down and susceptibility to treatment increases. Thus, increasing the number of virus particles per synapse does not simply reduce susceptibility to treatment.

Hence, without further investigation, the experimental observation that virus grown in co-culture conditions is less susceptible to anti-viral drugs cannot simply be explained by a large number of viruses transferred during synaptic transmission. If this experimental result is indeed correct, one can hypothesize, based on our model, that in the co-culture conditions the number of viruses transferred to the target cells lies around the intermediate value that maximizes the transmission index and thus minimizes susceptibility to treatment. In order to test this hypothesis, model parameters that determine the kinetics of synaptic and free virus transmission must be determined since they are mostly unknown. A more likely explanation for the reduced drug susceptibility in co-culture conditions might be the drug saturation scenario explored in our model. According to the model, the transmission index jumps significantly once the number of viruses transferred through synapses crosses a threshold. In this case, all available inhibitors become bound to viruses in the cell, and the rest of the transferred viruses can undergo reverse transcription unopposed. This could be tested experimentally by using reporter viruses labeled with different colors. Cells could be exposed to a certain amount of virus labeled with one color and to a reverse transcription inhibitor at the same time. Shortly after the first virus, a dose of the second virus should be added to the culture. If drug saturation plays a significant role, the first virus would be expected to saturate the drug, while most successful infection events will be done by the second reporter virus. Higher doses of the drug should diminish the saturation effect, while a larger inoculum of the first virus should restore saturation.

It is, however, not currently clear whether virus grown in co-culture conditions is indeed less susceptible to anti-viral drugs. A recent study[36] argues that the transmission index is comparable in the context of both transmission pathways, and therefore free virus and synaptic transmission are characterized roughly by the same susceptibility to anti-viral drugs. This study argues that the difference in results compared to the paper by Sigal et al.[26] is due to differences in methodology. They claim that for synaptic transmission, the assay used by Sigal et al.[26] indicates infection in a significant number of cells that do not become productively infected, thus overestimating the number of infection events during treatment in co-culture conditions.

An interesting outcome of our model is that the viral strategy (i.e the number of transferred viruses per cell) that minimizes the susceptibility to treatment does not coincide with the strategy that maximizes the basic reproductive ratio of the virus or the equilibrium virus load in the absence of treatment. Drug treatment not only reduces the basic reproductive ratio of the virus, but it also increases the synaptic strategy, s, that maximizes R_0 during treatment. The

stronger the drug, the lower the chance that a transferred virus will infect the cell, *r*. Thus, a larger number of viruses needs to be transferred before additional transfer of viruses leads to wasting of virus particles through the infection of an already infected cell, and thus to a decline of R_0. This argues against the notion that the basic reproductive ratio of the virus in the absence of treatment predicts the dose of the drug that is required to obtain maximal drug-mediated suppression of the virus population, as suggested by standard virus dynamics models that do not take into account different transmission modes and multiple infections.

Methods

In order to study the effect of synaptic transmission on treatment susceptibility, we developed a set of deterministic (ordinary differential equation) models. These equations describe viral dynamics in the presence of both free-virus and synaptic transmission. The properties of thes equations, and in particular their equilibrium solutions, their stability and parameter dependences are studied analytically. The details are provided in Supplementary Information.

1. Nowak, M. A. & May, R. M. *Virus dynamics. Mathematical principles of immunology and virology.* (Oxford University Press, 2000).
2. Perelson, A. S. Modelling viral and immune system dynamics. *Nature Rev Immunol* **2**, 28–36 (2002).
3. Douek, D. C., Picker, L. J. & Koup, R. A. T cell dynamics in HIV-1 infection. *Annu Rev Immunol* **21**, 265–304 (2003).
4. Simon, V. & Ho, D. D. HIV-1 dynamics in vivo: implications for therapy. *Nat Rev Microbiol* **1**, 181–190 (2003).
5. Chen, P., Hubner, W., Spinelli, M. A. & Chen, B. K. Predominant mode of human immunodeficiency virus transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. *J Virol* **81**, 12582–12595 (2007).
6. Feldmann, J. & Schwartz, O. HIV-1 Virological Synapse: Live Imaging of Transmission. **2**, 1666–1680 (2010).
7. Hubner, W. *et al.* Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. *Science* **323**, 1743–1747 (2009).
8. Martin, N. & Sattentau, Q. Cell-to-cell HIV-1 spread and its implications for immune evasion. *Curr Opin HIV AIDS* **4**, 143–149 (2009).
9. Sattentau, Q. Avoiding the void: cell-to-cell spread of human viruses. *Nat Rev Microbiol* **6**, 815–826 (2008).
10. Sattentau, Q. J. Cell-to-Cell Spread of Retroviruses. **2**, 1306–1321 (2010).
11. Sourisseau, M., Sol-Foulon, N., Porrot, F., Blanchet, F. & Schwartz, O. Inefficient human immunodeficiency virus replication in mobile lymphocytes. *J Virol* **81**, 1000–1012 (2007).
12. Jung, A. *et al.* Multiply infected spleen cells in HIV patients. *Nature* **418**, 144 (2002).
13. Josefsson, L. *et al.* Majority of CD4+ T cells from peripheral blood of HIV-1-infected individuals contain only one HIV DNA molecule. *Proc Natl Acad Sci U S A* **108**, 11199–11204 (2011).
14. Bonhoeffer, S., Chappey, C., Parkin, N. T., Whitcomb, J. M. & Petropoulos, C. J. Evidence for positive epistasis in HIV-1. *Science* **306**, 1547–1550 (2004).
15. Fraser, C. HIV recombination: what is the impact on antiretroviral therapy? *J R Soc Interface* **2**, 489–503 (2005).
16. Vijay, N. N., Vasantika, Ajmani, R., Perelson, A. S. & Dixit, N. M. Recombination increases human immunodeficiency virus fitness, but not necessarily diversity. *The Journal of general virology* **89**, 1467–1477 (2008).
17. Althaus, C. L. & Bonhoeffer, S. Stochastic interplay between mutation and recombination during the acquisition of drug resistance mutations in human immunodeficiency virus type 1. *J Virol* **79**, 13572–13578 (2005).
18. Kouyos, R. D., Althaus, C. L. & Bonhoeffer, S. Stochastic or deterministic: what is the effective population size of HIV-1? *Trends Microbiol* **14**, 507–511 (2006).
19. Kouyos, R. D., Silander, O. K. & Bonhoeffer, S. Epistasis between deleterious mutations and the evolution of recombination. *Trends Ecol Evol* **22**, 308–315 (2007).
20. Gelderblom, H. C. *et al.* Viral complementation allows HIV-1 replication without integration. *Retrovirology* **5**, 60 (2008).
21. Iwabu, Y. *et al.* Superinfection of defective human immunodeficiency virus type 1 with different subtypes of wild-type virus efficiently produces infectious variants with the initial viral phenotypes by complementation followed by recombination. *Microbes Infect* **10**, 504–513 (2008).
22. Wodarz, D. & Levy, D. N. Human immunodeficiency virus evolution towards reduced replicative fitness in vivo and the development of AIDS. *Proc Biol Sci* **274**, 2481–2490 (2007).
23. Wodarz, D. & Levy, D. N. Multiple HIV-1 infection of cells and the evolutionary dynamics of cytotoxic T lymphocyte escape mutants. *Evolution* **63**, 2326–2339 (2009).
24. Wodarz, D. & Levy, D. N. Effect of different modes of viral spread on the dynamics of multiply infected cells in human immunodeficiency virus infection. *J R Soc Interface* **8**, 289–300 (2011).
25. Wodarz, D. & Levy, D. N. Effect of multiple infection of cells on the evolutionary dynamics of HIV in vivo: implications for host adaptation mechanisms. *Exp Biol Med (Maywood)* **236**, 926–937 (2011).
26. Sigal, A. *et al.* Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. *Nature* **477**, 95–98 (2011).
27. De Boer, R. J. & Perelson, A. S. Target cell limited and immune control models of HIV infection: a comparison. *J Theor Biol* **190**, 201–214 (1998).
28. Bonhoeffer, S., May, R. M., Shaw, G. M. & Nowak, M. A. Virus dynamics and drug therapy. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 6971–6976 (1997).
29. Dixit, N. M. & Perelson, A. S. Multiplicity of human immunodeficiency virus infections in lymphoid tissue. *J Virol* **78**, 8942–8945 (2004).
30. Dixit, N. M. & Perelson, A. S. HIV dynamics with multiple infections of target cells. *Proc Natl Acad Sci U S A* **102**, 8198–8203 (2005).
31. Dimitrov, D. S. *et al.* Quantitation of human immunodeficiency virus type 1 infection kinetics. *J Virol* **67**, 2182–2190 (1993).
32. Cummings, K. W., Levy, D. N. & Wodarz, D. Increased burst size in multiply infected cells can alter basic virus dynamics. *Biol Direct* **7**, 16 (2012).
33. Gupta, P., Balachandran, R., Ho, M., Enrico, A. & Rinaldo, C. Cell-to-cell transmission of human immunodeficiency virus type 1 in the presence of azidothymidine and neutralizing antibody. *J Virol* **63**, 2361–2365 (1989).
34. Martin, N. *et al.* Virological synapse-mediated spread of human immunodeficiency virus type 1 between T cells is sensitive to entry inhibition. *J Virol* **84**, 3516–3527 (2010).
35. Massanella, M. *et al.* Antigp41 antibodies fail to block early events of virological synapses but inhibit HIV spread between T cells. *AIDS* **23**, 183–188 (2009).
36. Permanyer, M. *et al.* Antiretroviral Agents Effectively Block HIV Replication after Cell-to-Cell Transfer. *J Virol* **86**, 8773–8780 (2012).

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Author contributions

N.K. and D.W. wrote the main manuscript text. N.K. performed the mathematical analysis. N.K., D.L. and D.W. generated and discussed the ideas and reviewed the manuscript.

Additional information

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