Social Stress Alters Immune Response and Results in Higher Viral Load During Acute SIV Infection in a Pigtailed Macaque Model of HIV

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Abstract:

While social distancing is a key response to viral pandemics, social stress has been implicated in adverse health outcomes. A comprehensive understanding of the direct physiologic effects of social stress on viral pathophysiology is needed. To determine the effect of social stress on HIV pathogenesis during acute viral infection without sociobehavioral confounders inherent in human cohorts, we compared commonly measured parameters of HIV progression between singly and socially housed SIV-infected pigtailed macaques (*Macaca nemestrina)*. Singly housed macaques demonstrated lower CD4/CD8 ratios, greater CD4 T cell declines and greater T cell activation compared to socially housed macaques during acute SIV infection. Singly housed macaques furthermore had higher viral loads in the plasma and cerebrospinal fluid throughout acute infection. These data demonstrate that social stress directly impacts the pathogenesis of acute lentiviral infection and imply that social stress has a direct effect on the pathogenesis of HIV and potentially other viral infections.

One Sentence Summary:

Social stress impacts the pathogenesis of acute HIV infection, leading to immune system dysregulation and higher viral load.

Main Text:

Social stress is an environmental factor that has a measurable impact on general health outcomes (reviewed in *1*). Today, social stress has taken on greater societal significance, with social distancing practices vital to slow the SARS-CoV-2 pandemic contributing to increased psychological distress in physicians and health care workers caring for COVID-19 patients (*2–4*). The logical next question of how social stress

impacts the body's responses to pathogens, such as viral infections, remains incompletely understood. While data is currently unavailable for analysis in the COVID-19 pandemic, other viral pandemics, such as human immunodeficiency virus (HIV), may provide insight. HIV infection remains widespread and, while people with HIV (PWH) treated with antiretroviral therapy (ART) today live relatively normal lives compared to untreated individuals, outcomes for PWH vary greatly and depend upon a number of factors, including social stress. PWH are at higher risk for social stress due to stigma associated with infection and marginalization of people from demographics that are considered at higher risk of infection (*5*). Not surprisingly, PWH report decreased physical and mental well-being compared to the general population (*6*), and worse mental and physical quality of life than individuals with other chronic illnesses (*7*).

It has been difficult to determine the direct physiologic effects of social stress on viral pathogenesis and mechanisms through which such stress affects outcomes due to the multifaceted nature of social stress and sociobehavioral confounders that have the potential to limit access to effective care or otherwise compromise health. Animal models have historically been beneficial for parsing out biological mechanisms that can be oversimplified with *in vitro* studies but may be muddled in studies with human cohorts. Simian immunodeficiency virus (SIV) infection in macaques recapitulates many aspects of HIV pathogenesis, including decline in circulating CD4 T cells following infection and persistence of virus in latent reservoirs despite ART (*8*). The present study aims to demonstrate the direct effect of social stress on HIV pathogenesis by comparing how single versus social housing affects the progression of acute SIV infection in pigtailed macaques (*Macaca nemestrina*). We hypothesized acute SIV infection would

have greater impact on macaques housed individually prior to and following viral inoculation compared to macaques housed in pairs or trios. To test this hypothesis, we conducted a retrospective analysis of acute infection data from the pigtailed macaque model of HIV infection dual-inoculated with SIV/17E-Fr and SIV/DeltaB670 (*9*), and compared macaques housed singly with those housed socially with a compatible conspecific. We demonstrated that housing macaques individually directly affects CD4/CD8 ratios, CD4 T cell decline, T cell activation and SIV viral load during acute infection. Social stress thus directly influences disease progression in the SIV-infected macaque model of HIV and social stress is likely to have direct pathophysiologic impact on health outcomes of PWH and potentially on the pathogenesis of other viral infections.

Results:

Social Stress Increases Immune Impact of Acute SIV Infection

To examine the effect of social stress on immune system parameters commonly measured while monitoring the progression of HIV infection, we completed a retrospective comparison of numbers of circulating CD4 and CD8 T cells and lymphocytes in singly and socially housed pigtailed macaques using linear mixed effects regression models. The CD4 to CD8 T cell ratio was examined first, as a lower ratio is associated with negative outcomes in PWH (*10*). CD4/CD8 ratio was 18% lower in singly housed animals at day 14 following inoculation compared to socially housed animals ($p=0.04$), though ratios decreased as expected for both singly and socially housed following infection (Fig.1A, $p=0.0004$). Difference in CD4/CD8 between the

groups was partly driven by a 38.5% greater decline in CD4 T cell number in singly housed compared to socially housed animals (Fig.1B; p=0.0001), in conjunction with a 34.1% greater decline in CD8 T cells (Fig.1C; p=0.0002) and a 44.4% greater decline in lymphocytes (Fig.1D; p<0.0001). These differential declines originated pre-inoculation, during which CD4 T cell numbers were 27.8% higher (Fig.1B, p=0.007), CD8 T cells were 25.9% higher (Fig.1C, p=0.0006) and lymphocytes were 28% higher (Fig.1D, p=0.0004) in singly housed compared to socially housed animals. Greater recovery in CD8 T cell (Fig.1C; p=0.0003) and lymphocyte number (Fig.1D; p=0.01) at day 14 in singly housed compared to socially housed macaques additionally contributed to lower CD4/CD8 in singly housed animals.

Social Stress Increases T cell Activation During Acute SIV Infection

To further determine the effect of social stress on immune dysregulation in response to SIV infection, we examined CD8 and CD4 T cell activation. As expected, all macaques had heightened CD8 T cell activation during acute infection, demonstrated by increased percentage of cells expressing CD69, with peak activation on day 7 (Fig.2A; p=0.001). However, singly housed animals had 68% more CD69+ CD8 T cells at peak activation compared to socially housed (p=0.0007). Notably, singly housed macaques had 9.2-fold more CD69+ CD4 T cells at peak activation in acute infection compared to pre-inoculation values (Fig.2B; p<0.0001) whereas socially housed animals demonstrated negligible activation.

Social Stress Results in Elevated Plasma and CSF Viral Loads During Acute SIV Infection

To determine if social stress and relative immunosuppression coupled with immune dysregulation in singly housed macaques impacts viral load, we compared plasma and cerebrospinal fluid (CSF) viral loads between singly and socially housed macaques throughout acute infection. Singly housed macaques had higher viral loads in plasma than socially housed counterparts throughout acute infection (by 6.1%, 6.5% and 21% on days 7, 10 and 14, respectively) (Fig.3A; $p<0.0001$). In the CSF, singly housed macaques had even higher viral loads than socially housed macaques (13%, 12% and 18%, respectively, at the same timepoints) (Fig.3B; $p<0.0001$). The magnitude of the difference in viral loads between the singly and socially housed macaques compounded over time in the plasma ($p=0.0007$) but not the CSF.

Social Stress Increases the Variability of the Data Produced by SIV-Infected Macaques

Reduction of exogenous stress through the introduction of stress-reducing refinements can reduce the variability of data in macaque models (*11*). To evaluate if social stress similarly impacts data variability in this SIV-infected pigtailed macaque model, we compared the standard deviations of the post-inoculation data reported herein for socially and singly housed animals. The data from singly housed animals was more variable than the data from socially housed animals (Fig.4 and S1; $p=0.02$).

Discussion:

Our data demonstrates that reducing social stress by providing social housing with a compatible conspecific lessens the immune impact of acute SIV infection in a pigtailed macaque model of HIV infection, resulting in a lesser reduction in CD4/CD8

ratio, less marked decline in circulating CD4 T cells, reduction in CD4 and CD8 T cell activation, and lower plasma and central nervous system (CNS) viral loads. This effect is observed while all aspects of infection and care remained consistent between these groups (Table S1), effectively removing the sociobehavioral confounders that affect access to care and adherence to treatment inherent in PWH. Thus, this study demonstrates that there is a direct pathophysiologic effect of social stress on pathogenesis of lentiviral infection and that social stress may have implications for pathogenesis of other viral infections, including SARS-CoV-2.

This analysis has implications beyond primary infection for PWH. The CD4/CD8 ratio was lower and CD4 decline greater in singly housed compared to socially housed animals. Lower CD4/CD8 ratio is a predictor of non-AIDS related morbidity and mortality (*10*) and age-associated disease in PWH (*12*), while low CD4 counts are a direct prognostic indicator of disease progression throughout infection (*13*). Our observation of these negative prognostic indicators in the context of social stress provides additional evidence that social stress directly contributes to negative outcomes. Additionally, latent reservoirs are established during acute infection, remain the major barrier to cure, and are especially difficult to eliminate from sanctuary sites such as the brain (*14*, *15*). Our findings of higher viral loads in the plasma and CSF in the context of social stress have long-term implications for PWH. These higher viral loads may ultimately originate from the increased CD4 activation that we observed in the singly housed animals, as activated CD4 T cells simultaneously serve as permissive cells for the virus to infect and produce more virus if infected, thus potentiating viral spread and reservoir seeding (*16*). Thus, this work provides important evidence that social stress may have a direct

adverse effect on the immune response to HIV, viral production, the establishment of latent reservoirs, viral load, and disease outcomes.

Due to the retrospective nature of our analysis we did not evaluate the mechanism underlying the effect of social stress on SIV/HIV pathogenesis, yet work on the biology of stress responses of people with and without HIV provides insight. In uninfected people, chronic stress dysregulates immune function (*17*), leading to a suppression in antibody production (*18*), a suppression in leukocyte proliferation (*19*), and a reduction in virus-specific T cell and NK cell activity (*20*). Social isolation is associated with under-expression of proinflammatory genes, including those involved in innate antiviral resistance through type I interferon responses (*25, 31*), and could directly affect immune response and viral control. Compounding these effects, PWH have increased levels of plasma cortisol compared to uninfected controls, and inflammatory cytokine interleukin-1 (IL-1) and HIV gp120 are implicated in mediating this increased level of cortisol by increasing levels of corticotropin-releasing hormone. Clinical studies demonstrate that glucocorticoid receptors of PWH have decreased affinity for cortisol, resulting in glucocorticoid insufficiency despite increased circulating cortisol. Viral suppression with ART does not fully attenuate the dysfunction in the physiologic stress response in PWH, illustrating the complexity of the interaction between HIV and the stress response (reviewed in (*21*). Interestingly, social support mitigates the immunosuppressive impact of glucocorticoid release in uninfected individuals (*22*). However, the findings from studies on the effect of social support on PWH have been equivocal, and any impact of social stress on disease progression and health outcomes in PWH is compounded by the fact that social isolation is more

prevalent in PWH compared to uninfected controls (*23*). In sum, social stressors affect immune functioning and thus HIV progression, and robust, controlled studies are needed to better understand the pathophysiologic mechanisms underpinning these effects on PWH.

Though this study was retrospective, we were able to control for the majority of variables that could have affected our study outcomes (Table S1). Variables that were not controlled for in our study design were examined in multivariate post-hoc statistical analyses and found to not influence the conclusions. Importantly to note, there are limitations to the extent that these data can be taken as a representative model for the general population, as only data from juvenile male pigtailed macaques were available. For example, there is substantial literature documenting sex differences in the immune and stress responses and in HIV-associated disorders, such as neurocognitive impairment (*24*).

These data also have implications for the translational value and reproducibility of HIV research with macaque models. Macaque models are instrumental in achieving scientific advancement for treatment of PWH and continue to be at the forefront of discovery in HIV and in other viral pathogenesis research (*9*, *25*–*32*). However, an animal model's value is only as great as the translational value of its data, and how we keep animals in research settings can impact translational value and the reproducibility of generated data (*33*). Specifically, excessive physiologic stress as a result of housing conditions such as single housing has the potential to reduce the translational value of animal models. Single housing of mouse and rat models of epilepsy led to an increase in corticosterone and adrenocorticotropic hormone levels, a decrease in brain-derived

neurotrophic factor levels and exacerbated the epilepsy phenotype including seizure frequency (*34*, *35*), leading to the conclusion that social isolation of social species adds an additional variable that can alter the interpretation of the data. Macaques are social animals, and housing macaques individually similarly results in physiologic stress. Otherwise healthy macaques relocated from social to single housing show modulated immune responses for weeks to months thereafter, as demonstrated by lower CD4/CD8 ratios and less robust proinflammatory cytokine responses (*36*). Such changes to the immune system driven by social stress have the potential to introduce a confounder into infectious disease studies. Certain psychosocial variables such as social stability lead to an increased risk of mortality from SIV infection (*37*). Our data complements the existing literature by demonstrating differences in the immune response and viral loads in singly compared to socially housed SIV-infected macaques. Though we cannot directly demonstrate improvements in translation and reproducibility through this work, the magnitude of the decline in CD4 T cell counts that we observed in socially housed macaques during acute infection (two-fold) is more analogous to that seen in humans during primary HIV infection (*38*) than the three-fold change observed in singly housed macaques. Further, the decrease in variability of the data generated by our socially housed macaques has implications for study reproducibility. Social housing of macaques is therefore an important refinement that may improve the translational value and reproducibility of data obtained from working with macaque models of HIV infection, and caution must be taken when comparing the data from studies completed in singly and socially housed macaques.

In summary, we demonstrate that social stress in the form of single housing of macaques has a direct negative impact on the pathogenesis of acute SIV infection, leading to lower CD4/CD8 ratios, more pronounced decline in circulating CD4 T cells, increase in T cell activation, and elevated peripheral and CNS viral loads. These data indicate that social support for PWH can have a direct positive impact on disease progression beyond the positive psychobehavioral effects and practical benefits, and provides evidence supporting a direct pathophysiologic effect of this social support on outcomes for PWH. Further research is needed into the implications of social isolation on the pathogenesis of other viral infections. In particular, the findings of our study have high relevance for the potential impact of social isolation practices widely advocated during the current SARS-CoV-2 pandemic. These data should furthermore be considered as evidence for the direct effect that social housing has on this vital animal model of HIV infection, as refinements such as social housing have the potential to improve the reproducibility and translational value of biomedical research conducted with animal models.

References and Notes:

1. G. Miller, E. Chen, S. W. Cole, Health psychology: developing biologically plausible models linking the social world and physical health. *Annu. Rev. Psychol.* **60**, 501–524 (2009).

2. J. Lai *et al.*, Factors associated with mental health outcomes among healh care workers exposed to covonavirus disease 2019. *JAMA Netw Open*. **3** (2020).

3. P. Bansal *et al.*, Clinician Wellness During the COVID-19 Pandemic: Extraordinary Times and Unusual Challenges for the Allergist/Immunologist. *J Allergy Clin Immunol Pract* (2020), doi:10.1016/j.jaip.2020.04.001.

4. H. Xiao, Y. Zhang, D. Kong, S. Li, N. Yang, The Effects of Social Support on Sleep Quality of Medical Staff Treating Patients with Coronavirus Disease 2019 (COVID-19) in January and February 2020 in China. *Med. Sci. Monit.* **26**, e923549 (2020).

5. J. A. Pellowski, S. C. Kalichman, K. A. Matthews, N. Adler, A pandemic of the poor: social disadvantage and the U.S. HIV epidemic. *Am. Psychol.* **68**, 197–209 (2013).

6. I. R. Perez *et al.*, Health-related quality of life of patients with HIV: Impact of sociodemographic, clinical and psychosocial factors. *Qual. Life Res.* **14**, 1301–1310 (2005).

7. E. A. N. Engelhard *et al.*, Health-related quality of life of people with HIV: an assessment of patient related factors and comparison with other chronic diseases. *AIDS*. **32**, 103–112 (2018).

8. J. D. Deere, R. F. Schinazi, T. W. North, Simian immunodeficiency virus macaque models of HIV latency. *Curr Opin HIV AIDS*. **6**, 57–61 (2011).

9. C. M. Abreu *et al.*, Infectious Virus Persists in CD4+ T Cells and Macrophages in Antiretroviral Therapy-Suppressed Simian Immunodeficiency Virus-Infected Macaques. *J. Virol.* **93** (2019), doi:10.1128/JVI.00065-19.

10. S. Serrano-Villar *et al.*, Increased risk of serious non-AIDS-related events in HIV-infected subjects on antiretroviral therapy associated with a low CD4/CD8 ratio. *PLoS One*. **9**, e85798 (2014).

11. M. L. Graham *et al.*, Successful implementation of cooperative handling eliminates the need for restraint in a complex non-human primate disease model. *J Med Primatol*. **41**, 89–106 (2012).

12. S. Serrano-Villar *et al.*, The CD4:CD8 ratio is associated with markers of age-associated disease in virally suppressed HIV-infected patients with immunological recovery. *HIV Med*. **15**, 40–49 (2014).

13. A. A. Okoye, L. J. Picker, CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunol. Rev.* **254**, 54–64 (2013).

14. S. E. Queen *et al.*, Replication-competent simian immunodeficiency virus (SIV) Gag escape mutations archived in latent reservoirs during antiretroviral treatment of SIV-infected macaques. *J. Virol.* **85**, 9167–9175 (2011).

15. J. D. Siliciano, R. F. Siliciano, Recent developments in the search for a cure for HIV-1 infection: targeting the latent reservoir for HIV-1. *J. Allergy Clin. Immunol.* **134**, 12–19 (2014).

16. M. Stevenson, T. L. Stanwick, M. P. Dempsey, C. A. Lamonica, HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J.* **9**, 1551–1560 (1990).

17. C. J. Padro, V. M. Sanders, Neuroendocrine regulation of inflammation. *Semin. Immunol.* **26**, 357–368 (2014).

18. D. M. Silberman, M. R. Wald, A. M. Genaro, Acute and chronic stress exert opposing effects on antibody responses associated with changes in stress hormone regulation of T-lymphocyte reactivity. *J. Neuroimmunol.* **144**, 53–60 (2003).

19. R. W. Bartrop, E. Luckhurst, L. Lazarus, L. G. Kiloh, R. Penny, Depressed lymphocyte function after bereavement. *Lancet*, 834–836 (1977).

20. R. H. Bonneau, J. F. Sheridan, N. G. Feng, R. Glaser, Stress-induced suppression of herpes simplex virus (HSV)-specific cytotoxic T lymphocyte and natural killer cell activity and enhancement of acute pathogenesis following local HSV infection. *Brain Behav. Immun.* **5**, 170–192 (1991).

21. A. N. Valdez, L. H. Rubin, G. N. Neigh, Untangling the Gordian knot of HIV, stress, and cognitive impairment. *Neurobiol. Stress*. **4**, 44–54 (2016).

22. C. E. Hostinar, R. M. Sullivan, M. R. Gunnar, Psychobiological mechanisms underlying the social buffering of the hypothalamic-pituitary-adrenocortical axis: a review of animal models and human studies across development. *Psychol. Bull.* **140**, 256–282 (2014).

23. S. R. Greysen *et al.*, Does social isolation predict hospitalization and mortality among HIV+ and uninfected older veterans? *J. Am. Geriatr. Soc.* **61**, 1456–1463 (2013).

24. L. H. Rubin *et al.*, Sex Differences in Neurocognitive Function in Adults with HIV: Patterns, Predictors, and Mechanisms. *Curr Psychiatry Rep*. **21**, 94 (2019).

25. D. H. Barouch *et al.*, Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). *Lancet*. **392**, 232–243 (2018).

26. S. G. Hansen *et al.*, A live-attenuated RhCMV/SIV vaccine shows long-term efficacy against heterologous SIV challenge. *Sci. Transl. Med.* **11** (2019), doi:10.1126/scitranslmed.aaw2607.

27. B. Moldt *et al.*, Neutralizing antibody affords comparable protection against vaginal and rectal simian/human immunodeficiency virus challenge in macaques. *AIDS*. **30**, 1543–1551 (2016).

28. M. A. Martins *et al.*, Vaccine protection against SIVmac239 acquisition. *Proc. Natl. Acad. Sci. USA*. **116**, 1739–1744 (2019).

29. A. A. Okoye *et al.*, Early antiretroviral therapy limits SIV reservoir establishment to delay or prevent post-treatment viral rebound. *Nat. Med.* **24**, 1430–1440 (2018).

30. N. Huot, P. Rascle, M. Müller-Trutwin, Contribution of animal models to HIV research. *Virologie (Montrouge)*. **23**, 229–240 (2019).

31. S. Lu *et al.*, Comparison of SARS-CoV-2 infections among 3 species of non-human primates. *BioRxiv* (2020).

32. B. Rockx *et al.*, Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science*, eabb7314 (2020).

33. D. Grimm, Are happy lab animals better for science? *Science* (2018), doi:10.1126/science.aat2326.

34. H. Manouze *et al.*, Effects of single cage housing on stress, cognitive, and seizure parameters in the rat and mouse pilocarpine models of epilepsy. *Eneuro*. **6** (2019), doi:10.1523/ENEURO.0179-18.2019.

35. P. Stern, Social isolation biases rodent data. *Science*. **365**, 653–654 (2019).

36. C. G. Benton, M. W. West, S. M. Hall, S. T. Marko, J. C. Johnson, Effect of short-term pair housing of juvenile rhesus macaques (Macaca mulatta) on immunologic parameters. *J. Am. Assoc. Lab. Anim. Sci.* **52**, 240–246 (2013).

37. J. T. Cacioppo, S. Cacioppo, J. P. Capitanio, S. W. Cole, The neuroendocrinology of social isolation. *Annu. Rev. Psychol.* **66**, 733–767 (2015).

38. E. W. Fiebig *et al.*, Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS*. **17**, 1871–1879 (2003).

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AI094189, P01 AI131306, K01 OD018244, RR00116, P40 OD013117 / U42 OD013117, R01 NS089482, NS097221, NS055651, MH61189, MH070306, NS36911, & RR019995, BSi, Blaustein Pain Foundation. **Author Contributions:** SMGM and KAMP designed this retrospective study, planned and oversaw all analyses and wrote manuscript. LHR planned analyses, generated linear mixed model and performed statistics. SMGM, LHR, ENS, SEQ, BWC, RJA, LG, JEC, JLM and KAMP provided input into the experimental design. SMGM, KMM, BWC, ENS and SEQ organized and analyzed data. ENS, SEQ, ML, BB, KAMP, RJA, LG, DRG, MCZ, JEC and JLM performed and analyzed the original studies that generated the data analyzed in this retrospective analysis. LG, DRG, MCZ, JEC and JLM designed the original studies and procured resources for them. **Competing Interests:** Authors declare no competing interests. **Data and materials availability:** All de-identified data will be provided upon request.

Supplementary Materials:

Materials and Methods

Tables S1-S4

Figure S1-S3

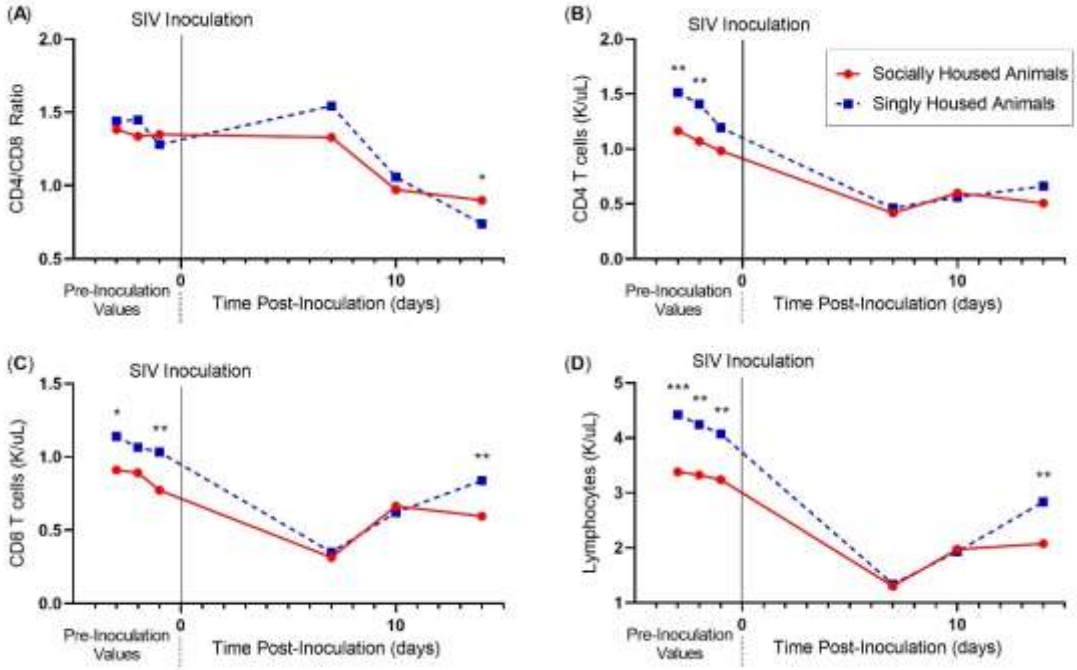

Figure 1. Social stress increases the immune impact of acute SIV-infection

CD4/CD8 T cell ratio (**A**), CD4 T cells (**B**), CD8 T cells (**C**) and lymphocyte count (**D**) in the peripheral blood of macaques pre- and post-inoculation with SIV. Linear mixed effects regression model. Statistics depicted in figure represent significance of difference between singly and socially housed animals at each time point. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$. Circles connected by a solid line (red) indicate socially housed animals while squares connected by a dashed line (blue) represent singly housed animals. Solid vertical line at day 0 indicates time point of SIV-inoculation. ART initiated on day 12 post-inoculation.

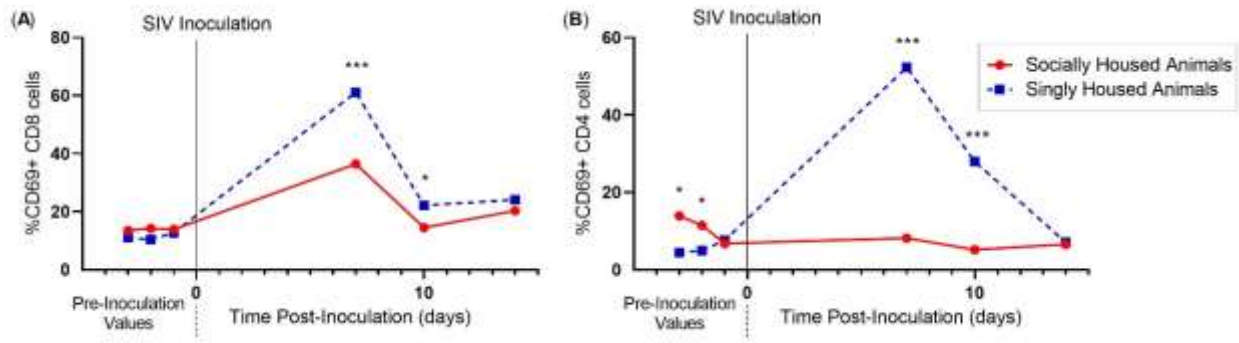

Figure 2. Social stress increases immune activation during acute SIV-infection

Percentage of CD8 T cells (**A**) and percentage of CD4 T cells (**B**) expressing CD69 in the peripheral blood of macaques pre- and post-inoculation with SIV. Linear mixed effects regression model. Statistics depicted in figure represent significance of difference between singly and socially housed animals at each time point. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$. Circles connected by a solid line (red) indicate socially housed animals while squares connected by a dashed line (blue) represent singly housed animals. Solid vertical line at day 0 indicates time point of SIV-inoculation. ART initiated on day 12 post-inoculation.

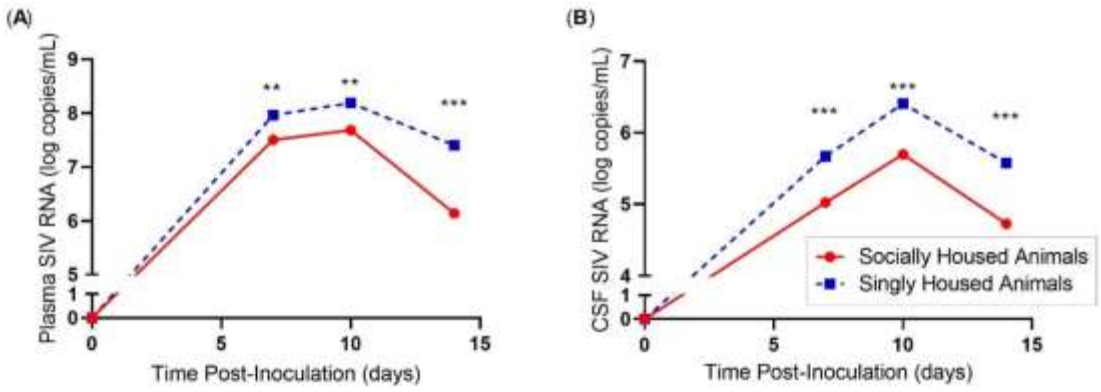

Figure 3. Social stress increases the plasma and CSF viral load in acute SIV-infection

SIV viral load in plasma (**A**) and cerebrospinal fluid (**B**) were quantified utilizing qRT-PCR. Linear mixed effects regression model. Statistics depicted in figure represent significance of difference between singly and socially housed animals at each time point. *p≤0.05, **p≤0.01 and ***p≤0.001. Circles connected by a solid line (red) indicate socially housed animals while squares connected by a dashed line (blue) represent singly housed animals. ART initiated on day 12 post-inoculation.

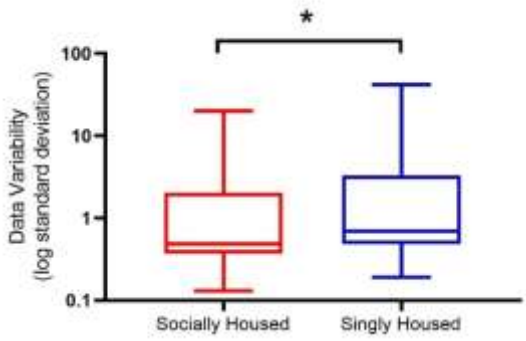

Figure 4: Social stress increases the variability of data produced by the SIV-infected macaque model during acute infection

Standard deviation for all data sets presented in Figures 1 through 3 were compared in a pair-wise fashion between socially and singly housed animals using a Wilcoxon matched pairs signed rank test. *p<0.05.