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Effects of morphine on behavioral task performance in SIV-infected Rhesus macaques

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Abstract

The abuse of opiates such as morphine in synergy with HIV infection not only exacerbates neuropathogenesis but significantly impacts behavioral attributes in HIV infected subjects. Thus, the goal of the current study was to characterize behavioral perturbations in rhesus macaques subjected to chronic morphine and SIV infection.

Specifically, we assessed three behavioral tasks: motor skill (MS), forelimb force (FFT) and progressive ratio (PR) tasks. After collecting baseline control data (44 weeks) and data during the morphine-only dependency period (26 weeks), a subset of animals were productively infected with neurovirulent strains of SIVmac (R71/E17) for an additional 33 weeks. A general pattern in the results is that behavioral decline occurred with high CSF viral loads but not necessarily with high plasma viral loads. Compared to saline controls, all treated animals showed significant decreases in performance on all three behavioral tasks during the morphine-only dependency period. During the post infection period, only the morphine plus SIV group showed a significant further decline and this only occurred for the MS task. Taken together, these data demonstrate a clear effect of morphine to produce behavioral deficits and also suggest that morphine can act synergistically with SIV/HIV to exacerbate behavioral deficits.

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Keywords

SIV; AIDS; morphine; motor skill; forelimb force; progressive ratio; behavior

INTRODUCTION

Chronic Human immunodeficiency virus (HIV) infection of the central nervous system (CNS) has been attributed to a spectrum of cognitive neuropathologies collectively referred to as HIV associated neurocognitive disorders (HAND) (Crews et al., 2009; Shapshak et al., 2011). These spectra range from asymptomatic neurocognitive impairment (ANI), minor cognitive motor disorder (MCMD) to the most severe HIV encephalitis (HIVE) (Woods et al., 2009). Although the advent of combined anti-retroviral therapy (cART) has significantly reduced the prevalence and mortality of HIVE, incidence of other forms especially ANI and MCMD are on a rise significantly impacting the quality of life in HIV+ subjects (Dore et al., 1999; Masliah et al., 2000; Gray et al., 2003). Adding a further layer of complexity is the aspect of drug abuse by these subjects thus increasing comorbidity. Opioids are the major addictive drugs of choice of which heroin and its active metabolite morphine are widely abused by HIV+ subjects. While chronic dependence on opioids leads to dependence, cessation leads to withdrawal symptoms (Rahim et al., 2004). In terms of central nervous system dysfunction associated with HIV infection, previous studies have documented significant evidence for opioids in exacerbating HIV neuropathogenesis. HIV+ subjects dependent on opioids show severe neuropathology compared to infected non-drug users (Bell et al., 1996; Bell et al., 2006; Anthony et al., 2008; Banerjee et al., 2011; Hauser et al., 2012).

While detailed mechanisms elucidating the role of morphine in exacerbating HAND is still under investigation, studies have documented evidence for increased activity of glial cells such as microglia and astrocytes in the presence of morphine and HIV proteins (Bruce-Keller et al., 2008; Turchan-Cholewo et al., 2009). In addition, opioid-mediated injury in synergy with HIV infection has documented a role for μ -opioid receptors (MOR) in an *in vitro* model to further enhance morphine-Tat mediated neurotoxicity (Turchan-Cholewo et al., 2008). Also, there is mounting evidence from previous studies that elevated amounts of opioids in circulation augment progression to AIDS in HIV+ subjects (Donahoe and Vlahov, 1998; Bell et al., 2006). Despite these emerging mechanisms associated with HIV and morphine synergy in exacerbating HAND, a significant gap exists in understanding the behavioral deficits associated with this synergy. The current study using a non-human primate model was designed to determine whether chronic administration of morphine to simian immunodeficiency virus (SIV)-infected rhesus macaques would lead to behavioral perturbations. Specifically, we assessed three behavioral tasks: motor skill (MS), forelimb force (FFT) and progressive ratio (PR) tasks. While the morphine alone treated animals showed significant decreases in performance on the three behavioral tasks during the control period compared to the saline controls, during the post infection period, morphine in conjunction with SIV showed a significant decline in the MS task that was greater than that from morphine or virus alone. Taken together, these data suggest an important role for

morphine in producing behavioral deficits and accelerating deficits in synergy with SIV/HIV infection.

METHODS AND MATERIALS

Subjects

Sixteen male rhesus monkeys (*Macaca mulatta*) of Indian origin were used in this study. All monkeys were herpes B virus negative, specific pathogen-free, from the same colony, approximately the same age (6-7 years), and raised under the same conditions. Animals were individually housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility of the University of Kansas Medical Center (KUMC). All sixteen macaques were behaviorally trained to perform 3 different behavioral tasks: 1) motor skill (MS), 2) forelimb force (FFT), and 3) progressive ratio (PR). Animals were randomly assigned to three groups: (M) morphine treatment only (n=5); (V) SIV infected (n=5) and (MV) morphine treated plus SIV infected (n=6).

Morphine Injection & viral infection

Morphine injection and viral infection regimens used in the current study have been reported in our previous work (Marcario et al., 2008; Riazi et al., 2009). Our morphine regimen was based on a protocol introduced by (Seever MH, 1963) and used by others (Aceto et al., 1977; Aceto et al., 1981) for establishing dependence. They concluded that 3 mg/kg sc given at 6 hour intervals (4 /day) produced maximal morphine dependence. We used a similar protocol but with a maximal dose of 2.5 mg/kg sc given at 6 hour intervals. Although this daily dose level (10 mg/kg/day) is lower than that used in some previous studies, there is no doubt that it produced robust drug dependence. For example, Becker et al., used a similar dose (11.2 mg/kg/day) to study morphine withdrawal in rhesus macaques (Becker et al., 2008). To confirm morphine dependence with our dosing protocol, withholding morphine in an animal that was part of a different cohort precipitated severe withdrawal symptoms.

Our study consisted of three analysis periods: period 1 - a baseline control period where no treatments were given; period 2 - morphine administration with established dependence; period 3 - viral inoculation with productive infection. The animals in groups M and VM received morphine injections (2.5 mg/kg) administered IM four times daily at 6-h intervals via a 1-ml syringe with a 27.5G needle during the morphine and viral infection time blocks. All animals in group V were given sham injections of saline equal in volume to the 2.5 mg/kg of morphine. Injection sites were rotated, so that the left and right legs each received 2 injections a day, 12 hours apart. To establish dependence, the morphine dose was increased in small increments from 1 mg/kg in week 1 to 2.5 mg/kg in week 17. The 2.5 mg/kg dose was then maintained for the duration of the experiment. After a 26-week period of treatment with morphine and morphine-only data collection, the animals in Groups VM and V were inoculated in the bone marrow with SIVmacR71/17E. Morphine treatment was continued in the M and VM animals throughout the study period. After inoculation, weights were taken every 2 weeks, and the morphine and saline doses were adjusted accordingly. Over the course of the study, only two monkeys lost weight (6% and 7%). All other monkeys gained weight. The animals were monitored for progression of infection for a 33-week observation

period. The institutional animal care and use committee at KUMC approved all experimental protocols. Information on the course of disease progression post SIV infection along with changes in viral load, CD4, CD8 including changes in the periphery have been outlined in detail in a previous publication (Marcario et al., 2008).

Behavioral Tasks

Motor Skill Task (MS)—This task is detailed in our previous work (Marcario, et al., 1999). It provides a sensitive measure of distal and proximal movement skill, reaching accuracy, and hand-eye coordination. Successful performance required the monkey to retrieve a food pellet from a cup in a rotating Plexiglas disk. The monkey was tested for 10 trials at each speed, incrementing the speed by 5 RPM after each set. The speed at which the monkey quit working or had a success rate less than 50% was recorded as the maximum speed for the session.

Progressive Ratio Task (PR)—This task measures the reinforcing efficacy of the behavioral reward (food pellets), level of motivation, and the attention span of the subject. A large cyan target was presented in the middle of a computer touch screen. The monkey had to touch the target once, then twice, then three times, etc. (arithmetic progression), to receive a single food pellet as a reward after each sequence of touches. The number of touches the monkey made before receiving a reward was recorded. If the monkey did not touch the screen for 3 minutes, the task aborted. Each session was 10 minutes long.

Forelimb Force Task (FFT)—An isometric force transducer, was integrated into the behavioral apparatus as a “button” mounted on a vertical panel, and was used to measure force generated by the forelimb. Trials were self-initiated by the monkey and required depressing the “button” for a hold time of 4 seconds within a pre-determined force range. The monkey received auditory feedback (a tone) and visual feedback (a yellow light) when he was in the target force range. Pressing the button outside the allowed force range did not initiate a tone. In addition, a green light illuminated at the start of each trial and when too little force was used, and a red light illuminated if too much force was used. In either case, the trial aborted without a reward. Correct trials were rewarded with a food pellet. Task performance was quantified as the number of successful trials in a test session. This task required precision of visuo-motor coordination, like the motor skill task, but differed in its emphasis on accurate sustained tonic force generation as opposed to the rapid ballistic type movements required for the motor skill task.

Statistical methods

The *p* values for statistical tests were calculated using SAS. A type I error of 5% was used for determining statistical significance. Wilcoxon–Mann–Whitney tests conducted with SPSS were used to determine if there were group (VM, V) differences on outcomes of interest as well as within group changes from the baseline to the morphine period (Table 1). For the analysis of the effects of morphine on task performance, the VM and M animals were combined because the VM animals had not yet been infected and were essentially the same as the M animals. Similarly, the V animals were not yet infected and served as untreated controls.

For the analysis of SIV effects on task performance and its interaction with morphine, the M, VM and V animals were treated as separate groups. For this analysis, Kruskal–Wallis tests were used, and once again the significance of the effect of group was of primary interest. For questions involving trends over time, mixed models were evaluated using SAS Proc Mixed with an effect for group and an effect for time. For questions involving trends over time, mixed models were evaluated using SAS Proc Mixed with an effect for group and an effect for time. A summary of statistical comparisons for the behavioral analysis is presented in Table 1.

A separate correlational analysis was performed using Pearson's product-moment correlation to test for relationships between plasma and CSF viral load versus decrements in behavioral performance. Behavioral tasks and treatment group (VM and V) were analyzed separately. Terminal viral load and associated terminal behavioral performance were used for the analysis. Terminal behavioral performance typically included averaging data over the final 3-7 weeks of behavioral performance. The percent decrement in performance compared to the pretreatment baseline was calculated and correlated with plasma and CSF viral load.

RESULTS

Behavioral task performance during the morphine period

Task performance during the morphine dependency period (period 2) was compared to the control period (period 1) for animals that received morphine (groups M and VM, $n=11$) versus the saline control animals (group V, $n=5$). Difference scores from task measures were calculated using the average of the last five weeks of the morphine period minus the average of the last five weeks of the baseline period. Raw data plotted over time across all the analysis periods for the motor skill task, progressive ratio task and forelimb force task are shown in Figures 1, 2, and 3 respectively. Mann-Whitney tests for two independent samples were used to compare difference scores of animals in the morphine condition to animals in the saline condition (Table 1). In all 3 tasks (MS, PR and FFT), the morphine group had greater performance declines than animals receiving only saline, however, this difference only reached statistical significances for the PR task ($p = 0.002$)

Performance during the morphine dependency period versus the control period was also analyzed within groups (Table 1). The last five weeks of both periods were averaged and used as the test variables. Wilcoxon Signed Ranks tests were used to determine significant differences. There were no significant differences for the MS task. However, there were significant performance declines for groups VM and M on the PR ($p = 0.003$) and FFT ($p = 0.012$) tasks.

Behavior task performance during the SIV infection period

Task performance during the post-infection period (period 3) was analyzed by comparing across all three groups (VM, V, M) based on four dependent variables for each of the three tasks: the average of weeks 1 and 2 in period 3; the average of weeks 3 and 4 in period 3; the average of the last 3 weeks in period 3; the last data point in period 3 (Table 1). Kruskal-Wallis tests for k-independent samples were run to test each of the four independent

variables on each of the three tasks. Significance does not necessarily mean that all groups were different from each other but only that the median for one group differs from the median of at least one other group. Mann-Whitney analysis was then used to test differences in pairwise comparisons between groups M and V, groups M and VM, and groups V and VM for all three behavioral tasks where significance was obtained on the Kruskal-Wallis test (Group Comparison columns in Table 1).

For the MS task, there was a significant difference between groups V and VM for the final 3 weeks in period 3 ($p = 0.006$) and the final data point ($p = 0.006$) where the motor skill score for V animals was 2.4 times (better than) that of the VM animals in both cases. There were no significant differences between groups M and VM, although the average of the last 3 weeks came very close to achieving significance with the score for M animals at 2 times (better) that for VM animals. There were no significant differences between Groups M and V for any of the analysis periods examined.

For the PR task, there was a significant difference between groups for all 4 analyses (Table 1; period 3 average of weeks 1 & 2, average of weeks 3 & 4, average of last 3 weeks and final data point). Pairwise comparisons showed a significant difference between groups V and VM for all four analyses ($p = 0.003$; 0.049; 0.009; 0.037, respectively in the order listed above). The performance scores of the V animals were respectively 2.4, 1.6, 2.1 and 1.7 times (better than) that of the VM animals. There were also significant differences between groups M and V for all four analyses ($p = 0.002$; 0.003; 0.006; 0.004, respectively). In this case, the respective performance score differences for V animals were 2.5, 2.6, 2.2 and 2.4 times that of the M animals. There were no significant differences between groups M and VM for any analysis ($p = 0.418$; 0.186; 0.433; 0.246, respectively).

For the FFT task, there was a significant difference between groups M and V for the average of weeks 1 & 2 ($p = 0.006$) and weeks 3 & 4 ($p = 0.006$) where V animal scores were 2.2 and 2.4 times (better than) those of the M animals. There was also a significant different difference between groups V and VM for the average of weeks 1 & 2 ($p = 0.006$) where V animal scores were 2.2 times those of VM animals. There were no significant differences between groups M and VM for any of the analysis periods examined.

Synergistic effects of morphine + SIV on behavioral performance

Table 2 gives the percent decline in performance on each task for each group, based on the difference between performance in period 1 (control period) vs. period 3 (post-infection period). Negative percentages reflect declines in task performance in period 3 relative to period 1. In the MS task, changes in performance were: group M, 0.2%; group V, -8.4% and group VM, -26.8%. In the PR task, changes in performance were: group M, -71.5%; group V, -18.0% and group VM, -71.9%. In the FFT task, changes in performance were: group M, -39.8%; group V, -26.0% and group VM, -46.9%. These results suggest a synergistic effect between morphine and SIV for the MS task in that the sum of the performance declines for the morphine and virus treatments alone are much less than the combined treatment. Performance declines in the FFT task were substantially greater in VM animals than either V or M animals but did not exceed the sum of declines for the V and M animals. In the PR task, M and VM animals showed a similar level of decline that far exceeded the decline in V

animals suggesting that in this task performance declines could be attributed largely to the effects of morphine.

Correlations between viral load and behavioral performance

Data for the VM and V groups were analyzed separately for each behavioral task. Correlations obtained for CSF viral load and performance decrements in the PR and PPT tasks were significantly different from 0 (Table 3). The correlation coefficients for other comparisons, particularly those with CSF viral load, were not significantly different from 0 but the limited number of data points constrained the statistical analysis. To increase the number of data points, we collapsed the data together across behavioral tasks to compare behavioral decrements regardless of task type to load levels. In this analysis, correlations between behavioral decrements and CSF viral load were significantly different from 0 for both the V and VM groups. Plasma viral load was related to behavioral decrement for the V group but not the VM group. The results establish a relationship between SIV load, particularly CSF viral load, and decline in behavioral performance. A general pattern in the results is that behavioral decline was associated with high CSF viral loads but not necessarily with high plasma viral loads.

DISCUSSION

Neurological complications such as increased cognitive deficits and behavioral impairments have been shown in HIV-infected subjects dependent on opiates compared to HIV-infected non-drug abusers (Bell et al., 1996; Chiesi et al., 1996; Bell et al., 1998; Goodkin et al., 1998; Nath et al., 2001; Bell et al., 2006; Anthony et al., 2008; Hellmuth et al., 2014) and in SIV infected macaques (Marcario et al., 1999a; Marcario et al., 1999b; Fox et al., 2000; Weed and Gold, 2001; Weed et al., 2003; Weed et al., 2004; Cheney et al., 2008). Previous studies from our group have shown that morphine potentiates neuropathogenesis in macaques infected with SIV mac R71/17E (Marcario et al., 2008; Bokhari et al., 2011). Such morphine mediated viral pathogenesis also resulted in alterations in brain stem responses and visual evoked potentials (Raymond et al., 1998; Cheney et al., 2008; Riazi et al., 2009). In the current study we assessed behavioral task performance using three groups of Indian rhesus macaques chronically treated with morphine to establish dependence and then infected with SIVmac R71/17E.

When comparing the control period (period 1) to the morphine dependency period (period 2), in all 3 tasks (MS, PR and FFT), the animals receiving morphine showed greater performance declines than the animals receiving saline. However, this difference only achieved statistical significance for the PR task ($p = 0.02$). This finding is consistent with other studies showing preferential performance declines from morphine on tasks relying on motivation, such as the PR task, as well as tasks dependent on learning and time perception (Schulze and Paule, 1991).

Analysis of behavioral performance during the infection period revealed significant performance losses on all three tasks and in all time periods of comparison in the VM animals compared to the V animals. The only exception was the final data point analysis in the FFT task where the p value just missed significance (0.051). There were no significant

differences in comparisons of the VM and M animals. The M animals showed significant performance losses compared to the V animals on all behavioral tasks and at all time periods tested for the PR and FFT tasks. It was somewhat surprising that the M and V animals were not different on the MS task. This may reflect the greater need for sustained attention to perform the PR and FFT tasks compared to the MS task.

It might be argued that all these findings could be explained by the effects of morphine on attention and motivation. However, the performance of each group during the infection period compared to the control period shows some interesting results. First, all three groups (V, M, VM) showed performance declines on all three tasks with the exception of the M group on the MS task. On the PR and FFT tasks, the M group monkeys showed behavioral performance losses of 71.5% and 39.8% respectively. The V group monkeys showed performance decrements ranging from 8.4 – 26% depending on the task and the MV group showed declines of 26.8 – 71.9%. Is there evidence of a synergistic interaction of morphine with SIV? In most cases, the behavioral performance losses of the MV group could not be described as synergistic. However, on the MS task, the MV group decline was 26.8%, which was much greater than the loss on the same task of the M and V monkeys combined. Hence it seems reasonable to conclude that in this case there is a synergistic interaction between morphine and SIV resulting in a greater decline in performance than would have been expected from the behavioral decrements associated with virus or morphine alone. However, we acknowledge that this conclusion must be tempered by the relatively small sample size and the lack of dose-response curves.

The behavioral deficits presented in this paper complement earlier work that looking at physiological mechanisms underlying the synergy between HIV/SIV infection and morphine dependence. Some of these include alterations in HIV-1 co-receptors and chemokines. For example activation of CC-chemokine receptor 5 (CCR5) by its ligand CCL5 regulates the pathogenesis of HIV/SIV infection (Sasseville et al., 1996; El-Hage et al., 2008). Earlier studies have shown an increase in CCL5 expression in astrocytes treated with the viral protein Tat (El-Hage et al., 2005) (59) as well as increase in CCR5 expression in the astrocytes by morphine (Mahajan et al., 2005) (49). Such changes in chemokines and their receptors by morphine have been shown to lead to aberrant signaling mechanisms (Malik et al., 2011) as well as inducing high rates of apoptosis (Nair et al., 1997; Reddy et al., 2012) further exacerbated by HIV (Hu et al., 2005; Hauser et al., 2009; Malik et al., 2011). The consequence of these changes in HAND subjects dependent on morphine is enhanced neuropathogenesis and subsequent behavioral deficits. In conclusion, while morphine alone produces significant behavioral decrements, morphine dependence intersects with SIV infection to produce more severe behavioral impairments in some domains than either morphine or virus alone. The results support our previous findings suggesting a significant detrimental effect of SIV/HIV infection in the setting of opiate dependence. Targeting such physiological mechanisms could further help develop therapeutic interventions to treat behavioral impairments associated with the HIV/SIV and morphine synergy.

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Figure 1. Motor Skill (MS) task performance for individual monkeys across all experimental groups. This task required the monkey to retrieve a food pellet from a cup in a rotating Plexiglas disk. The speed at which the monkey quit working or had a success rate less than 50% was recorded as the maximum speed for the session. Each data point represents the average of one week's worth of data. The black vertical line in each plot indicates the beginning of morphine administration; the red vertical line indicates the date of inoculation with SIV. Individual monkeys are identified by numbers given below each graph.

Figure 2. Progressive Ratio (PR) task performance for individual monkeys across all experimental groups. This task required the monkey to touch the target once, then twice, then three times, etc. (arithmetic progression), to receive a single food pellet as a reward after each sequence of touches. The number of touches the monkey made before receiving a reward was recorded. Each data point represents the average of one week's worth of data. The black vertical line in each plot indicates the beginning of morphine administration; the red vertical line indicates the date of inoculation with SIV. Individual monkeys are identified by numbers given below each graph.

Figure 3. Forelimb Force (FFT) task performance for individual monkeys across all experimental groups.. This task required the monkey to depress a force sensitive button for a hold time of 4 seconds and maintain it within a pre-determined force range. Failure to maintain force in the prescribed range resulted in the trial being aborted without a reward. Task performance was quantified as the number of successful trials in a test session. Each data point represents the average of one week's worth of data. The black vertical line in each plot indicates the beginning of morphine administration; the red vertical line indicates the date of inoculation with SIV. Individual monkeys are identified by numbers given below each graph.

TABLE 1

Summary of Statistical Comparisons

Variables analyzed	Analysis Groups	Number of animals	p values ¹ (2 tailed)
Effects of Morphine on Task Performance			
Group Differences in Morphine Period			
- Motor Skill Task	(VM and M vs. V)	11, 5	0.487
- Progressive Ratio Task	(VM and M vs. V)	11, 5	0.002 *
- Forelimb Force Task	(VM and M vs. V)	11, 5	0.069
Within Group Change from Baseline to Morphine Period¹			
- Motor Skill Task	VM and M	11	0.833
- Progressive Ratio Task	VM and M	11	0.003 *
- Forelimb Force Task	VM and M	11	0.012 *
Effects of SIV on Task Performance			
		p values ²	Group Comparisons (p values ¹)
Motor Skills Group Comparisons		M-V	M-VM V-VM
- period 3 avg weeks 1 & 2	M, V, VM	5, 5, 6	0.057
- period 3 avg weeks 3 & 4	M, V, VM	5, 5, 6	0.122
- avg of the last 3 weeks	M, V, VM	5, 5, 6	0.049 * 0.264 0.052 0.006 *
- final data point	M, V, VM	5, 5, 6	0.044 * 0.171 0.072 0.006 *
Progressive Ratio Task Group Comparisons			
- period 3 avg weeks 1 & 2	M, V, VM	5, 5, 6	0.007 * 0.002 * 0.418 0.003 *
- period 3 avg weeks 3 & 4	M, V, VM	5, 5, 6	0.031 * 0.003 * 0.186 0.049 *
- avg of last 3 weeks	M, V, VM	5, 5, 6	0.025 * 0.006 * 0.433 0.009 *
- final data point	M, V, VM	5, 5, 6	0.039 * 0.004 * 0.246 0.037 *
Forelimb Force Task Group Comparison			
- period 3 avg weeks 1 & 2	M, V, VM	5, 5, 6	0.021 * 0.006 * 0.476 0.006 *
- period 3 avg weeks 3 & 4	M, V, VM	5, 5, 6	0.044 * 0.006 * 0.197 0.051
- avg of last 3 weeks	M, V, VM	5, 5, 6	0.310
- final data point	M, V, VM	5, 5, 6	0.897

* Statistically significant

¹ Wilcoxon-Mann-Whitney test

²Kruskal-Wallis test

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TABLE 2
Synergistic Effect of Morphine and SIV

Percent Decline for Each Group on Performance Tasks

	Group Means	Morphine	SIV	Morphine and SIV
Motor Skills	Period 1	125.2	129.2	127.7
	Period 3	125.4	118.4	93.5
	Difference	0.2	-10.8	-34.2
	% Difference	0.2%	-8.4%	-26.8%
Progressive Ratio	Period 1	22.8	22.8	25.8
	Period 3	6.5	18.7	7.3
	Difference	-16.3	-4.1	-18.6
	% Difference	-71.5%	-18.0%	-71.9%
Forelimb Force	Period 1	36.4	40.0	38.2
	Period 3	21.9	29.6	20.3
	Difference	-14.5	-10.4	-17.9
	% Difference	-39.8%	-26.0%	-46.9%

Table 3

Viral load behavioral performance correlations

VM group (n=6)		Correlation Coefficient	p-value
Motor skill task	vs plasma viral load	-0.045	0.933
Motor skill task	vs CSF viral load	0.532	0.227
FFT task	vs plasma viral load	-0.451	0.369
FFT task	vs CSF viral load	0.578	0.230
PR task	vs plasma viral load	-0.398	0.435
PR task	vs CSF viral load	0.712	0.113
V only group (n=5)			
Motor skill task	vs plasma viral load	0.765	0.132
Motor skill task	vs CSF viral load	0.527	0.361
FFT task	vs plasma viral load	0.108	0.863
*FFT task	vs CSF viral load	0.999	< 0.001
PR task	vs plasma viral load	0.768	0.129
*PR task	vs CSF viral load	0.985	0.002
VM, all tasks lumped	vs plasma viral load	-0.281	0.259
	*vs CSF viral load	0.543	0.020
V only, all tasks lumped	*vs plasma viral load	0.790	0.003
	*vs CSF viral load	0.825	< 0.001

* statistically significant correlations; all correlations based on Pearson's product-moment correlation analysis with $p=0.05$ as the significance level.

Viral load data from Marcario et al. (2008).