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Elevated testosterone and reduced 5-HIAA concentrations are associated with wounding and hantavirus infection in male Norway rats

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Abstract

Among rodents that carry hantaviruses, males are more likely to engage in aggression and to be infected than females. One mode of hantavirus transmission is via the passage of virus in saliva during wounding. The extent to which hantaviruses cause physiological changes in their rodent host that increase aggression and, therefore, virus transmission has not been fully documented. To assess whether steroid hormones and neurotransmitters contribute to the correlation between aggression and Seoul virus infection, Norway rats were trapped in Baltimore, Maryland and wounding, infection status, steroid hormones, and concentrations of neurotransmitters, including norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenol acetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindole-3-acetic acid (5-HIAA) in select brain regions were examined. Older males and males with high-grade wounds were more likely to have anti-Seoul virus IgG and viral RNA in organs than either juveniles or adult males with less severe wounds. Wounded males had higher circulating testosterone, lower hypothalamic 5-HIAA, and lower NE in the amygdala than males with no wounds. Infected males had higher concentrations of testosterone, corticosterone, NE in the hypothalamus, and DOPAC in the amygdala than uninfected males, regardless of wounding status. In the present study, wounded males that were infected with Seoul virus had elevated testosterone and reduced 5-HIAA concentrations, suggesting that these neuroendocrine mechanisms may contribute to aggression and the likelihood of transmission of hantavirus in natural populations of male Norway rats.

Keywords

aggression; corticosterone; hemorrhagic fever virus; host-parasite coevolution; Seoul virus; serotonin; stress

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Introduction

Within the central nervous system (CNS), the limbic system mediates the expression of social behaviors, including aggression (Kruk et al., 1998; Papez, 1937). Laboratory studies reveal that the neural circuit for intermale aggression involves several brain regions, including the amygdala, hippocampus, anterior hypothalamus, septum, anterior cingulate cortex, insular cortex, prefrontal cortex, ventral striatum, and interconnections (Nelson and Chiavegatto, 2001). Both steroid hormone and neurotransmitter receptors are densely populated on neurons in these brain regions and contribute to the expression of aggression in vertebrates (Simon et al., 1998).

Testosterone facilitates the expression of intermale aggression and this effect is caused by testosterone and its metabolites (e.g., dihydrotestosterone) binding to androgen receptors or by testosterone being aromatized to estrogens and binding to estrogen receptors (Nelson and Chiavegatto, 2001; Simon and Lu, 2005; Trainor et al., 2007). Among all major neurotransmitters, serotonin (5-HT) is most often associated with the expression of intermale aggression (Miczek and Fish, 2005; Nelson and Chiavegatto, 2001; Simon et al., 1998). Reduced 5-HT activity facilitates, whereas increased 5-HT neurotransmission decreases, intermale aggression (Olivier and Mos, 1990; Simon et al., 1998). Enhancement of aggression by reduced 5-HT activity is well-conserved across vertebrates, including humans. For example, in humans and non-human primates, reduced concentrations of the 5-HT metabolite, 5-hydroxyindole-3-acetic acid (5-HIAA), is associated with increased aggressive and violent behaviors (George et al., 2001; Higley et al., 1996). The precise relationship among hormonal, neurochemical, and neuroanatomical correlates of aggression is still under study; however, testosterone is hypothesized to facilitate aggression by suppressing 5-HT activity in the CNS (Higley et al., 1996; Simon et al., 1998). The overlapping distribution of sex steroid and 5-HT receptors in the CNS provides a functional framework for these interactions (Simon et al., 1998; Simon and Lu, 2005). In addition to reduced 5-HT neurotransmission, norepinephrine (NE) and dopamine (DA) activity have been linked to the expression of aggression (Miczek and Fish, 2005; van Erp and Miczek, 2000).

Parasites, including viruses, can exploit the proximate mechanisms that modulate social behaviors to increase the likelihood of transmission (Moore, 2002; Vyas et al., 2007). Because social behaviors facilitate interactions between conspecifics, these behaviors may increase the transmission of parasites from infected to susceptible individuals. Infection with viruses, including rabies virus and hantaviruses, increases aggression and, presumably, transmission within host populations (Klein, 2003). Parasites may alter the behavior of vertebrates by infecting and killing neurons in the brain (Dietzschold et al., 2001), altering neurotransmission (Ceccaldi, et al., 1993; Lancaster et al., 2007), or influencing the synthesis of steroids in peripheral organs (Larralde et al., 1995).

Hantaviruses are negative sense RNA viruses that cause two diseases in humans, hantavirus pulmonary syndrome (HPS) or hemorrhagic fever with renal syndrome (HFRS). Transmission both between rodents and from rodents to humans occurs through contact with infected rodent reservoirs. Each of the more than 40 species of hantaviruses has coevolved with a specific rodent host species (Plyusnin and Morzunov, 2001). Rodents infected with their species-specific hantavirus remain persistently infected in the absence of any overt disease (Botten et al., 2003). Virus is shed in saliva, urine, and feces, so transmission of hantaviruses most likely occurs from inhalation of aerosolized virus in rodent excreta or through the introduction of infectious virus in a bite wound (Glass et al., 1988; Hinson et al., 2004; Hjelle and Yates, 2001).

Approximately 50% of the male Norway rats (*Rattus norvegicus*) in Baltimore, Maryland are infected with Seoul virus (i.e., the hantavirus that infects Norway rats) (Easterbrook et al., 2007; Glass et al., 1988). Studies with marked and released Norway rats in Baltimore demonstrate that male rats wounded between captures are more likely to become infected with Seoul virus upon recapture than are males that were not wounded between captures (Glass et al., 1988). Additionally, infection occurs more frequently in males than females (Glass et al., 1988). Among natural populations of Norway rats, males with more severe wounds also are more likely to shed Seoul virus and have viral RNA present in target tissues, such as the lungs, than are males with less severe or no wounds (Hinson et al., 2004). Thus, engaging in aggressive behavior may increase Seoul virus transmission among male Norway rats.

Whether engaging in aggressive behavior increases exposure to hantaviruses (i.e., host-mediated hypothesis) or whether infection increases the propensity to engage in aggression (i.e., parasite-mediated hypothesis) remains unclear. Laboratory studies of male Norway rats infected with Seoul virus reveal that males with more virus present in lung, kidney, and testis tissues engage in aggression for a longer duration of time than do males with less virus (Klein et al., 2004). Neither viral RNA nor protein is present in the brains of infected males (Botten et al., 2000; Hinson et al., 2004; Klein et al., 2004). Male Norway rats that are infected with Seoul virus have higher circulating testosterone concentrations than do uninfected males (Hinson et al., 2004). Thus, the changes in host aggressive behavior may be caused by elevated virus replication in peripheral target tissues, such as the testes, and may be communicated to the CNS via hormonal signals. The effect of hantaviruses on neurotransmission in the CNS has not been reported.

The goal of this study was to identify potential neuroendocrine mediators of the relationship between wounding and Seoul virus infection in natural populations of Norway rats. We report, for the first time in wild-caught rats, that males with more severe wounds are more likely to be infected with Seoul virus, to have higher circulating testosterone concentrations, and to have reduced 5-HT neurotransmission in the hypothalamus than male rats with less severe or no wounds. Infection with Seoul virus also is correlated with elevated synthesis of corticosterone and higher NE content in the hypothalamus, suggesting that infection may be a stressor for Norway rats. These data have important implications for understanding how viral infection mechanistically alters host behavior through effects on the nervous and endocrine systems.

Methods

Trapping and processing of wild-caught rats

Male *R. norvegicus* were live-trapped (Tomahawk Trap Co., Tomahawk, WI) in East Baltimore, Maryland from April 2005 to April 2006. All trapping locations were in urban areas in alleys behind residential dwellings. Traps were baited with peanut butter and set at locations approximately 1–2 h before sundown. Details about sampling procedures have been previously described (Glass et al., 1988). All rats remained in traps overnight and were processed immediately the next morning. Rats were euthanized using CO₂, weighed, sexed, and bled by cardiac puncture. Serum was used to measure concentrations of anti-Seoul virus IgG and testosterone. Age was determined based on body mass and animals were classified as juveniles (≤ 199 g), young adults (200 – 399 g), or adults (≥ 400 g) (Childs et al., 1988; Glass et al., 1988; Hinson et al., 2004). Rats were examined for wounds by combing the fur to uncover scars, abscesses, or scabs that corresponded with wounding (e.g. bites on the rump and neck; Blanchard et al., 1975). Wounds originally were scored into five classes: 0 = no wounds; 1 = minor tail wounds; 2 = larger tail wounds and small wounds (< 0.25 cm) on the rump; 3 = larger wounds (0.25–0.5 cm) on the body; and 4 = many extensive wounds on the body and tail (Glass et al., 1988; Hinson et al., 2004). For subsequent analyses, wounding categories were partitioned into high-grade (score = 3–4), low-grade (score = 1–2), or no wounds. Feces

and lung tissue were collected and stored at -80°C. Fecal samples were used to measure corticosterone and lung tissue was used to assess Seoul virus RNA. The Johns Hopkins Animal Care and Use Committee (protocol no. RA05H6) and the Johns Hopkins Office of Health, Safety, and Environment (registration no. P9902030108) approved all procedures described.

Enzyme-linked immunosorbent assay (ELISA) for Seoul virus antibody

Anti-Seoul virus IgG was measured by ELISA as previously described (Klein et al., 2001). Microtiter plates were coated overnight with lysate from Vero E6 cells infected with Seoul virus or from uninfected Vero E6 cells. Sera from experimental and control rats were diluted 1:100 and added to plates in duplicate. Secondary antibody (alkaline phosphatase-conjugated anti-rat IgG [Kirkegaard and Perry Laboratories, Gaithersburg, MD]) was added and developed with *p*-nitrophenylphosphate substrate buffer. Optical density (OD) was measured at 405 nm and the average OD for each set of uninfected Vero E6 duplicates was subtracted from the average OD for each set of infected Vero E6 duplicates. Samples were considered positive if the average adjusted OD was ≥ 0.100 .

Real-time RT – PCR for Seoul virus RNA

RNA was isolated from the lungs using Trizol LS and the manufacturer's protocol as previously described (Invitrogen, Carlsbad, CA) (Klein et al., 2004). First strand Seoul virus cDNA was synthesized using a gene specific primer (S-segment coordinate-828-TGTTGCCTGAGGGCTTGAA), Invitrogen Superscript III First Strand Synthesis reagents, and the manufacturer's protocol. An 81 bp nucleotide sequence of the negative strand of the S segment of Seoul virus was amplified using primers (forward: S-segment coordinate-1006-GTCGGAGGGATGGCTGAAT; reverse: coordinate-1087-AATCATGGCTTCAAAAAGTGTGG), probe (coordinate-1042-ATACTTCAGGATATGAGGAAC) and Platinum Quantitative PCR Supermix-UDG with ROX (Invitrogen) as described previously (Easterbrook et al., in review). A standard curve ranging from 10^6 to 10 copies of SR-11 S segment cloned into the plasmid pWRG7077 was run on each plate.

High performance liquid chromatography (HPLC)

Immediately following euthanasia, brains were rapidly removed, placed on ice, and the hypothalamus, hippocampus, and amygdala were dissected, frozen on dry ice, and stored at -80°C. HPLC with electrochemical detection was used to measure concentrations of NE, DA, DOPAC, 5-HT, and 5-HIAA in the dissected brain regions as previously described (Pletnikov et al., 2000). Briefly, tissues were homogenized on ice by sonication in 0.1 M perchloric acid. The internal standard, dihydroxybenzylamine (DHBA), was added to determine extraction efficiency. The extracts were centrifuged at 14,000 rpm for 5 min and aliquots (15 μ l) of supernatant were injected into the chromatographic column (μ Bondpack™ C18 125A, 10 μ m 3.9×300 mm²). The mobile phase contained sodium acetate (90 mM), heptane sulfonic acid (5.3 mM), EDTA (0.4 mM), and acetonitrile (pH 4.0). The monoamine peaks in chromatograms of samples were identified by their retention times.

Steroid hormone enzyme immunoassays (EIA)

Fecal samples (50-100 mg) were homogenized in 1 ml of methanol to extract corticosterone. Samples were further diluted 1:100 in assay buffer (Cayman Chemicals, Ann Arbor, MI). Testosterone was extracted from 50 μ l of serum into 1 ml of diethyl ether. Ether was evaporated following incubation in a 42°C water bath for 45 min and samples were resuspended in 500 μ l of assay buffer (1:10 final dilution; Cayman Chemicals). Steroid concentrations were measured according to the manufacturer's protocol (Cayman Chemicals). Extraction efficiency was measured using a spiked standard (corticosterone: 0.640 ng/ml and testosterone: 0.125 ng/

ml) and was used to correct for steroid loss during extraction. To minimize inter- and intra-plate variability, a standard curve was run on each plate. For the testosterone EIA, sensitivity was 80% at 6 pg/ml and for the corticosterone EIA, sensitivity was 80% at 38 pg/ml.

Statistical analyses

Chi-squared analyses were used to evaluate the proportion of rats that were infected with Seoul virus based on wounding status and age. Steroid hormone and neurotransmitter concentrations were assessed using either a student's t-test or one-way analyses of variance (ANOVA). In cases where the data violated the assumptions of normality, nonparametric statistics were used. Significant interactions were further analyzed using the Tukey or Dunn method for pairwise multiple comparisons. Correlational analyses were assessed using the Pearson Product Moment. Mean differences were considered statistically significant if $P < 0.05$.

Results

Prevalence of Seoul virus among male Norway rats

During the study period, 104 male rats were trapped and 68% (71/104) were exposed to Seoul virus (i.e., either had Seoul virus RNA in lung tissue or circulating IgG antibody against Seoul virus). There was a very strong concordance between the presence of detectable levels of IgG antibodies to Seoul virus and the detection of Seoul virus RNA in tissue (81/104; Pearson Product Moment: $R = 0.55$, $P < 0.001$). Males that did not have anti-Seoul virus IgG, but that had detectable viral RNA in the lungs (13/104), likely represent a population that was recently infected. Males that had circulating anti-Seoul virus IgG, but no detectable viral RNA in the lungs (10/104) may represent a population of rats persistently infected with Seoul virus at levels below the limits of detection of our assay.

Age and Seoul virus infection

Of the male rats trapped in Baltimore, Maryland, 50% ($n = 51$) were adults (≥ 400 g), 30% ($n = 32$) were young adults (200 – 399 g), and 20% ($n = 21$) were juveniles (≤ 100 g). Adults were more likely to be infected with Seoul virus (84% [43/51] SEOV Ab+ and 94% [48/51] SEOV RNA+) than were young adults (38% [12/32] SEOV Ab+ and 44% [14/32] SEOV RNA+) and both were more likely to be infected than juveniles (14% [3/21] SEOV Ab+ and 0% [0/21] SEOV RNA+) (Figure 1A; $X^2 = 93.7$, $df = 5$, $P < 0.001$).

Wounding and Seoul virus infection

Male rats were examined for wounds of the size, shape, and location that likely were caused by intermale aggression (Glass et al., 1988; Hinson et al., 2004). Of the males trapped, 18% (19/104) had no wounds, 29% (30/104) had low-grade wounds, and 53% (55/104) had high-grade wounds. As reported previously (Glass et al., 1988; Hinson et al., 2004), wounding frequency increased with age, with older/larger male rats having more severe wounds (40/51 with high-grade wounds, 11/51 with low-grade wounds, and 0/51 with no wounds) than either young adults (15/32 with high-grade wounds, 12/32 with low-grade wounds, and 5/32 with no wounds) or juveniles (0/21 had severe wounds, 7/21 had moderate wounds, and 14/21 had no wounds) ($X^2 = 48.3$, $df = 2$, $P < 0.001$). Males with high-grade wounds were more likely to have detectable antibody and viral RNA (73% [40/55] SEOV Ab+ and 84% [46/55] SEOV RNA+) than males with either low-grade (53.3% [16/30] SEOV Ab+ and 46.7% [14/30] SEOV RNA+) or no wounds (10.5% [2/19] SEOV Ab+ and 10.5% [2/19] SEOV RNA+) (Figure 1B; $X^2 = 55.6$, $df = 5$, $P < 0.001$). Because the prevalence of anti-Seoul virus IgG and Seoul virus RNA in lung tissue were correlated and similarly associated with wounding and age, subsequent analyses were conducted based on whether animals had been exposed to Seoul

virus (i.e., tested positive for either antibody or viral RNA; SEOV+) or not (i.e., tested negative for both antibody and viral RNA; SEOV-).

Steroid hormone concentrations

Males infected with Seoul virus had higher circulating concentrations of testosterone than did uninfected males (Figure 2A; t-test: $t = 2.10$, $df = 100$, $P = 0.038$). Males with either high- or low-grade wounds had higher circulating testosterone concentrations than males with no wounds (Figure 2B; Kruskal-Wallis ANOVA: $H = 6.1$, $df = 2$, $P = 0.05$). As expected, adult and young adult males had higher testosterone concentrations than juveniles (Kruskal-Wallis ANOVA: $H = 7.1$, $df = 2$, $P = 0.029$; data not shown).

Fecal corticosterone concentrations were higher in males infected with Seoul virus (3.66 ± 0.50 ng/mg) than uninfected males (2.21 ± 0.29 ng/mg; Mann-Whitney Rank Sum Test: $T = 1699$, $P = 0.027$). There was no relationship between corticosterone and wounding, age, or circulating testosterone concentrations ($P > 0.05$).

Wounding and neurotransmitter content in the brain

Hippocampus and amygdala samples were collected and analyzed from 104 males and hypothalamus samples were collected and analyzed for 64 males. Wounded males (i.e., animals with either high- or low-grade wounds) had lower 5-HIAA concentrations in the hypothalamus than did males with no wounds (Figure 3A; ANOVA: $F(2,63) = 4.08$, $P = 0.022$). Neither 5-HT concentrations nor 5-HT turnover (5-HIAA/5-HT) were affected by severity of wounding in males (Figure 3A; $P > 0.05$ in each case). Males with lower hypothalamic 5-HIAA concentrations had higher circulating testosterone concentrations ($R = -0.33$, $P = 0.007$). Similarly, 5-HT turnover was negatively correlated with testosterone concentrations in males ($R = -0.25$, $P = 0.049$).

Neither concentrations of DA nor DA turnover were affected by wounding status in the present study population (Figure 3B; $P > 0.05$ in each case). Wounded males, however, had moderately lower DOPAC concentrations in the hypothalamus than males with no wounds (Figure 3B; Kruskal-Wallis ANOVA: $H = 5.5$, $df = 2$, $P = 0.065$). Males with reduced hypothalamic DOPAC had high testosterone concentrations ($R = -0.27$, $P = 0.031$). There was no effect of wounding on NE content in the hypothalamus (Figure 3C; $P > 0.05$). Wounding was not associated with 5-HT, DA, or NE activity in the hippocampus (Table 1; $P > 0.05$ in each case). In the amygdala, males with moderate or severe wounds had lower concentrations of NE than did males with no wounds (Table 1; Kruskal-Wallis ANOVA: $H = 6.12$, $df = 2$, $P = 0.047$).

Seoul virus infection and neurotransmitter content in the brain

Infection with Seoul virus did not affect concentrations of 5-HT, DA, or DOPAC or turnover of these neurotransmitters in the hypothalamus (Figures 4A and B; $P > 0.05$ in each case). Seoul virus infected male rats tended to have lower concentrations of 5-HIAA in the hypothalamus than uninfected males (Figure 4A; t-test: $t = -1.8$, $df = 62$, $P = 0.086$). Males that were infected with Seoul virus had higher concentrations of NE in the hypothalamus than did uninfected males (Figure 4C; t-test: $t = 2.3$, $df = 62$, $P = 0.025$). In the amygdala, DOPAC was higher in males infected with Seoul virus than in uninfected males (Mann-Whitney Rank Sum Test: $T = 1610$, $P = 0.031$). This trend was similar in the hippocampus, but because of low innervation of DA releasing-neurons, DA metabolism to DOPAC was minimal (Mann-Whitney Rank Sum Test: $T = 1702$, $P = 0.108$). Animals with higher concentrations of DOPAC in the amygdala had higher levels of Seoul virus RNA in the lungs ($R = 0.33$, $P < 0.001$). NE content in the hippocampus was positively correlated corticosterone concentrations in male rats ($R = 0.40$, $P < 0.001$).

Discussion

During host-pathogen co-evolution, hosts evolve adaptations that enable them to avoid or resist infection, while pathogens evolve counter-adaptations that increase the likelihood of transmission from infected to susceptible (i.e. uninfected) individuals. One cornerstone of the parasite-manipulation hypothesis is that pathogens have evolved mechanisms that enable them to alter host behavior (Klein, 2003; Moore, 2002; Sapolsky, 2003). For example, animals infected with rabies virus exhibit elevated aggression (Macrae, 1973), which may be caused by neural cell death in regions associated with aggression (i.e. amygdala, hippocampus, and hypothalamus) as well as suppression of 5-HT neurotransmission (Ceccaldi et al., 1993; Dietzschold et al., 2001). Male mice infected with tick-borne encephalitis (TBE) virus have elevated concentrations of circulating testosterone and are more aggressive than uninfected mice (Moshkin et al., 2002). Seoul virus increases the testosterone concentrations and the expression of aggression in male rats (Hinson et al., 2004; Klein et al., 2004); whether Seoul virus suppresses 5-HT activity in the CNS, similar to rabies virus, requires further investigation. In the current study, wounded male rats were more likely to be infected with Seoul virus and to have elevated circulating testosterone and reduced 5-HT neurotransmission as compared with non-wounded males. Whether alterations in steroid and neurochemical communication underlie the relationship between aggression and Seoul virus transmission remains an intriguing possibility.

Older males were more likely to be wounded, be infected with Seoul virus, and have higher concentrations of testosterone than younger males; hypothalamic 5-HT neurotransmission, however, was not affected by age group. Age (i.e. as defined by body mass) is consistently recognized as an important predictor of Seoul virus infection (Glass et al., 1988; Hinson et al., 2004). Although age may predict who is most likely to be infected, wounding establishes how Seoul virus infection is acquired. Wounding appears to contribute to intermale transmission of hantaviruses, including Seoul virus, Sin Nombre virus, and Puumala virus among their respective rodent hosts (Escutenaire et al., 2002; Glass et al., 1988; Hinson et al., 2004; Mills et al., 1997).

Testosterone facilitates aggression in males (Simon and Lu, 2005) and male Norway rats that are infected with Seoul virus have higher concentrations of testosterone than uninfected males (Hinson et al., 2004). Because testosterone is immunosuppressive (Klein, 2000; Schuurs and Verheul, 1990), elevated concentrations may increase the potential of infection through effects on the immune system (i.e. susceptibility) and/or aggressive behavior (i.e. exposure). Testosterone has its biological effects by binding to specific steroid receptors in target tissues (Simon and Lu, 2005; Trainor et al., 2007). Whether Seoul virus alters the density and distribution of androgen receptors, estrogen receptors, or both remains to be determined.

In the CNS, 5-HT activity also affects the expression of aggression. Reduced 5-HT neurotransmission (i.e. low 5-HIAA) is correlated with elevated aggression in humans and in rodents (George et al., 2001; Giotakos et al., 2004; Miczek and Fish, 2005). Concentrations of 5-HIAA in the hypothalamus were lower in wounded male Norway rats than in males with no wounds. Because the turnover of 5-HT was not lower in wounded males than unwounded males, metabolism of 5-HT to 5-HIAA likely was not reduced; rather, less neuronal 5-HT may have been presynaptically released in wounded males. These data support the hypothesis that reduced 5-HT neurotransmission is associated with elevated aggression. Testosterone-dependent intermale aggression is exacerbated by reduced 5-HT neurotransmission (George et al., 2001; Higley et al., 1996; Simon et al., 1998) and, in the current study, higher concentrations of testosterone were associated with lower hypothalamic 5-HIAA and 5-HT turnover in male rats. These data suggest that the elevated testosterone and reduced 5-HT neurotransmission may mediate the expression of intermale aggression in wild male rats.

Because engaging in aggressive behavior can alter testosterone and 5-HT activity (Summers et al., 2005; Wingfield et al., 1990), whether neuroendocrine alterations are a cause or consequence of aggression remains to be investigated.

Glucocorticoids produced by the adrenal gland, are associated with aggression in rats (Blanchard et al., 2007). There was no association between corticosterone concentrations and wounding in wild-caught male Norway rats. Male rats that were infected with Seoul virus, however, had higher concentrations of fecal corticosterone than uninfected males. Circulating concentrations of corticosterone typically are measured in serum, but are susceptible to immediate changes in response to stressors (e.g. animal handling or trapping procedures). Previous studies report no association between circulating corticosterone and Seoul virus infection or wounding, probably because corticosterone concentrations were excessively high due to trapping or animal handling (Hinson et al., 2004; Klein et al., 2002). Fecal concentrations of corticosterone are arguably a more accurate measure than serum concentrations for field studies (Cavigelli et al., 2005).

In addition to having elevated corticosterone, male rats that were infected with Seoul virus had elevated hypothalamic NE. Norepinephrine stimulates the hypothalamic-pituitary-adrenal axis and corticosterone can stimulate NE activity in several brain regions with high densities of glucocorticoid receptors (i.e. hypothalamus, hippocampus, and amygdala) (Gold et al., 2002). Hippocampal NE was positively correlated with corticosterone, illustrating the potential role of the glucocorticoid-noradrenergic system in response to Seoul virus infection. These data suggest that male rats may perceive Seoul virus as a stressor as indicated by their induced stress response. Because corticosterone also is a potent immunosuppressive steroid hormone, whether elevated corticosterone leads to increased susceptibility to Seoul virus or whether Seoul virus infection causes an elevation in corticosterone is currently under investigation.

Our data illustrate that elevated testosterone and reduced 5-HT neurotransmission are associated with increased aggression in natural populations of male Norway rats. Whether Seoul virus infection directly alters the interrelationships among steroid hormones, neurotransmitters, and aggression remains to be investigated in a laboratory setting. Assessing the outcomes of Seoul virus infection on these neuroendocrine systems contributes to better understanding behavioral transmission and maintenance of pathogens in their rodent reservoirs.

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Figure 1. Proportion of wild-caught male Norway rats infected with Seoul virus by age and wounding grade

Adult males (i.e. ≥ 400 g) were more likely to be infected with Seoul virus (i.e. as determined by the presence of circulating anti-Seoul virus IgG [SEOV Ab+] or Seoul virus RNA in the lungs [SEOV RNA+]) than either young adults (i.e. 200-399 g) or juveniles (i.e. ≤ 199 g) (A). Male rats with high-grade wounds were more likely to be infected with Seoul virus than males with low-grade or no wounds (B). Bars with different letters indicate statistically significant differences across groups ($P < 0.05$).

Figure 2. Circulating testosterone concentrations in wild-caught male Norway rats
 Male rats infected with Seoul virus (SEOV+) had higher circulating concentrations of testosterone than uninfected males (SEOV-) (A). Wounded males (i.e., animals with high- or low-grade wounds) had higher circulating testosterone than males with no wounds (B). Vertical bars indicate mean $\pm$ SEM and bars labeled with different letters are significantly different across groups ($P < 0.05$).

Figure 3. Concentrations of 5-HT, 5-HIAA, DA, DOPAC, and NE, as well as 5-HT (i.e. 5-HIAA/5-HT) and DA (i.e. DOPAC/DA) turnover, in the hypothalamus of wild-caught male rats stratified by wounding grade

Males with either high- or low-grade wounds had lower 5-HIAA (A) and moderately lower DOPAC (B) concentrations in the hypothalamus as compared with males that had no wounds. There was no association between NE in the hypothalamus and wounding (C). Vertical bars are mean $\pm$ SEM and bars with different letters are significantly different across groups ($P < 0.05$).

Figure 4. Hypothalamic concentrations of 5-HT, DA, and NE and turnover of 5-HT and DA in male Norway rats stratified by infection status

Infection with Seoul virus did not affect either 5-HT (A) or DA (B) neurotransmission in the hypothalamus. Norepinephrine concentrations were higher in the hypothalamus of Seoul virus-infected males (SEOV+) as compared with uninfected males (SEOV-) (C). Vertical bars are mean $\pm$ SEM and different letters signify significant differences across groups ($P < 0.05$).

Table 1

	Infection Status		Wounding Grade		
	SEOV+	SEOV-	no	low	high
Hippocampus	(pg/mg tissue)		(pg/mg tissue)		
5HT	327.7 ± 17.3	395.6 ± 34.7	412.5 ± 52.3	378.6 ± 36.4	317.3 ± 16.8
5HIAA	60.9 ± 6.1	57.0 ± 4.7	73.5 ± 14.8	50.6 ± 5.4	58.4 ± 6.7
5HIAA/5HT	0.18 ± 0.02	0.17 ± 0.03	0.22 ± 0.05	0.15 ± 0.02	0.18 ± 0.02
DA	462.9 ± 18.4	506.6 ± 23.0	487.0 ± 25.8	503.8 ± 27.3	461.6 ± 21.1
DOPAC	19.3 ± 5.9	8.9 ± 1.9	15.3 ± 4.1	10.1 ± 1.6	18.8 ± 7.2
DOPAC/DA	0.033 ± 0.007	0.019 ± 0.004	0.032 ± 0.009	0.022 ± 0.004	0.029 ± 0.009
NE	219.9 ± 9.5	204.2 ± 9.1	206.1 ± 10.3	226.4 ± 1.64	210.6 ± 8.9
Amygdala	(pg /mg tissue)		(pg /mg tissue)		
5HT	498.4 ± 25.3	502.8 ± 29.7	468.0 ± 49.6	507.8 ± 35.3	495.8 ± 24.7
5HIAA	368.1 ± 49.1	265.9 ± 29.8	255.3 ± 28.2	430.1 ± 103.3	306.3 ± 28.6
5HIAA/5HT	1.01 ± 0.15	0.67 ± 0.13	0.92 ± 0.33	0.80 ± 0.13	0.83 ± 0.13
DA	479.4 ± 16.3	469.5 ± 22.2	464.6 ± 27.4	514.4 ± 28.7	458.3 ± 16.6
DOPAC	115.4 ± 15.1 ^a	74.0 ± 11.6	115.4 ± 23.5	123.3 ± 25.9	82.7 ± 11.6
DOPAC/DA	0.24 ± 0.03	0.16 ± 0.02	0.26 ± 0.06	0.23 ± 0.04	0.18 ± 0.02
NE	377.6 ± 17.7	377.5 ± 20.7	430.5 ± 30.9	381.5 ± 20.4 ^b	356.8 ± 20.1 ^b

^a SEOV+ > SEOV-, $P = 0.031$

^b high- and low-grade wounds < no wounds, $P = 0.047$