Short Report

Mood, Behavior, Testosterone, Cortisol, and Interleukin-6 in Adults During Immune Activation: A Pilot Study to Assess Sickness Behaviors in Humans

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Objectives: Sickness behavior, a suite of behavioral changes subsequent to infection that includes depression, decreased social behaviors, and sleep disturbances, has been well described in model organisms. The phenomenon is relatively unexplored in humans due to methodological difficulties, and hormonal correlates of sickness behavior have not been studied. We therefore attempted to use a vaccine to elicit sickness behaviors outside of a clinical setting and uncover any correlations among testosterone, cortisol, and sickness behavior.

Methods: Eleven participants (five male, six female, mean age 22.8 years) naïve to the rabies vaccine were recruited from the School of Veterinary Medicine at Purdue University. Participants provided daily saliva and urine samples and completed questionnaires to assess mood and social behaviors for a period of 6 weeks. Saliva samples were assayed for cortisol and testosterone. Urine samples were assayed for interleukin-6 and creatinine.

Results: Analysis revealed an expected decrease in testosterone and an increase in cortisol. While mood did not differ, other behaviors, such as physical activity and hours slept, showed expected changes following vaccination. However, none of these results achieved statistical significance.

Conclusion: Our results, while generally confirming previous research on sickness behavior and hormone changes during infection, are suggestive, but not statistically significant and so neither confirm nor contradict our hypotheses. We attribute this lack of significance to both the small sample size, as well as possible confounding factors, including the psychosocial stress of entering an intensive study program. Am. J. Hum. Biol. 00:000–000, 2014.

Sickness behavior is defined as an organized, adaptive suite of behavioral changes that occurs during infection in human and nonhuman animals (Hart, 1988). These behavioral changes include lethargy, decreased intake of food and water, anhedonia, decreased exploration, cognitive and sleep disturbances, and decreased libido and social behavior (Larson, 2002). It is believed that sickness behavior functions to conserve energy and direct it towards an immune response at the expense of other behaviors (Hart, 1988).

While it is known that the proinflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) drive sickness behavior (Dantzer et al., 1991), the degree to which other classic hormones may modulate sickness behavior has only been addressed relatively recently. For instance, both testosterone and cortisol have well known associations with the immune system (Muehlenbein and Bribiescas, 2005), mood (Booth et al., 1999), and social behavior (Booth et al., 2006). Both of these hormones have been shown to affect sickness behavior responses in experiments with birds (Ashley et al., 2009) and rodents (Johnson et al., 1996).

While sickness behavior has been previously studied in humans (e.g., Brydon et al., 2009; Eisenberger et al., 2010; Janicki-Deverts et al., 2007; Vollmer-Conna et al., 2004), it has not been quantified to the same degree as in other animals, largely due to the complexities of studying illness and immunity in humans outside of a clinical setting. Finally, hormone correlates with sickness behavior have not been examined in humans.

This pilot study is an initial step toward refining and expanding the study of sickness behavior in humans, and uses the rabies vaccine to elicit an immunological response. We hypothesized that vaccination would elicit sickness behavior, and that levels of testosterone would correlate with measures of affiliative behaviors, while IL-6 and cortisol would correlate with stress. We therefore predicted a decrease in social behaviors, positive mood, and energy expenditure in participants after vaccination. We also predicted a general decrease in testosterone levels and an increase in IL-6 and cortisol.

METHODS

Participants

Eleven first-year veterinary medicine students (six female, five male, mean age 22.8 years, range 21–27) receiving the rabies vaccine were recruited from the campus of Purdue University, West Lafayette, IN, using a within-subjects study design whereby each individual acted as his or her own control. All participants were recruited before receiving the first of three rabies shots, though five of the 11 participants were recruited the morning of their first dose. All participants were followed for a minimum of 5 weeks, while those recruited the day of vaccination were followed for an additional week (Fig. 1). This extra week was used to generate a baseline for subjects recruited the day of the first dose. In order to make valid comparisons given this disparity in baseline week dates, we compared the day immediately following vaccination (day +1) and day +6, using the latter as a baseline.

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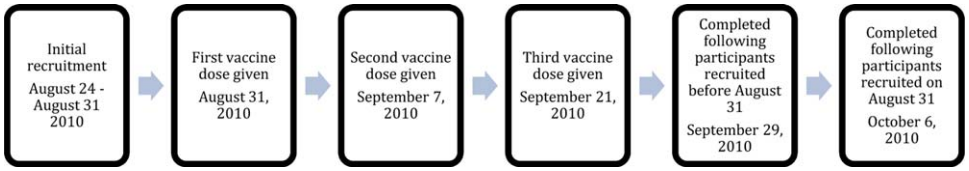

Fig. 1. Timing of Participant Recruitment.

Vaccine

Participants were inoculated with the Imovax® Rabies Vaccine (Sanofi Pasteur, SA) by Purdue University nurses and according to manufacturer's instructions. The vaccine was administered in a series of three injections, on days 0, +7, and +21, per protocol.

Biological samples

Subjects collected urine and saliva samples upon waking each morning. Saliva was collected prior to eating or drinking anything, and first morning void urine was collected following established clean catch procedure. Baseline samples were collected, on average, at 6:00 AM (range: 5:30–6:40), while samples following vaccination were collected at 6:50 AM (range: 6:05–7:25).

Saliva samples were assayed for testosterone and cortisol using commercially available enzyme immunoassay kits (Salimetrics #1–2402 and #1–3002). Urine was assayed for IL-6 and creatinine using commercially available enzyme immunoassay and colorimetric detection kits (Quantikine #HS600B and #ADI-907-030A). Sensitivities were 0.11 pg/ml and 0.042 mg/dl, respectively. Creatinine was measured to normalize IL-6 levels relative to urine concentration. All samples were assayed in duplicate. High- and low-level controls were included in each standard curve, and results for controls in each assay were within established confidence limits. Intra-assay coefficients of variation were assessed using the mean coefficients of variation of control duplicates. Intra-assay coefficients of variation were less than 5% for cortisol, 10% for IL-6, 3% for testosterone, and 1% for creatinine.

Behavioral data

Intake questionnaires surveyed demographics, relationship status, general health and vaccination history. The SF-36 (QualityMetric) health history questionnaire, Beck Depression Inventory (BDI), and the Perceived Stress Scale (PSS) were also included. Daily questionnaires, completed before participants went to bed, were comprised of a sociability scale (Cheek and Buss, 1981), the Big Five Inventory (Big 5), the expanded Positive and Negative Affect Schedule (PANAS-X), the Center for Epidemiologic Studies Depression Scale (CES-D), and the International Physical Activity Questionnaire (IPAQ). Participants also reported the number of social encounters experienced that day (in person and by telephone and text message), sleep duration and quality, food and drink consumed, and any medications taken.

Caloric intake was quantified using the online tool www.choosemyplate.gov. When quantities were not reported, the standard serving size given by the webpage was used. When type of preparation was not reported or apparent, the healthier option was preferred (i.e., baked vs. fried).

Statistical analyses

All data were assessed for normality using the Shapiro-Wilk test. Wilcoxon signed-rank tests were used to compare cytokine and hormone levels, as well as behavioral measures at baseline and after the first immunization dose. Correlations among all variables were made using Spearman's rank correlation test. All tests were two-tailed and significance was set at $P \leq 0.05$.

RESULTS

IL-6 and hormones

There were no significant differences between IL-6 ($z = -0.700$, $P = 0.484$), cortisol ($z = -0.840$, $P = 0.401$), or testosterone levels ($z = -1.260$, $P = 0.208$) at baseline and after immunization.

Cortisol levels following vaccination were positively correlated with both the number of people encountered ($r = 0.799$, $P = 0.017$) and number of encounters initiated ($r = 0.765$, $P = 0.027$) after vaccination.

Affect

There were no significant differences in measures of affect between baseline and following vaccination.

Sociability

Participants encountered six more people, on average, following vaccination ($z = -2.536$, $P = 0.011$) and initiated approximately four more of these encounters ($z = -2.094$, $P = 0.036$) compared to baseline. There were no significant differences with regard to phone or text conversations.

Sleep

Participants slept slightly (0.67 h) more following vaccination ($z = -2.02$, $P = 0.043$), and reported no major changes in sleep quality.

Diet

Calorie intake increased by an average of 752.6 calories following vaccination ($z = -2.395$, $P = 0.017$).

Physical activity

While IPAQ scores declined (mean scores of 120.91 at baseline and 71 postvaccination), this change was not statistically significant ($P = 0.759$).

DISCUSSION

There are several factors that clearly limit this study. The number of participants is quite small, making statistical power low. Because half the participants were recruited the morning of the first inoculation, we could not use our intended baseline measures and thus

compared day +1 and day +6 after vaccination. Finally, participants had regular weekly exams and often attended student club recruitment meetings during the duration of the study; we were unaware of this active schedule prior to the study. Academic examinations are known to be associated with increased perceived stress, higher cortisol levels, and changes in immune function (e.g., Glaser et al., 1994). Our findings of increased social contacts could be accounted for by club meetings, increased social behavior due to the beginning of the school year, or simply the fact that students attended classes, which gave them the opportunity to speak and interact with more people than they might otherwise. Similarly, the observed increased caloric intake could be due to a change in diet, rather than the consumption of more food; some participants did report going out to eat more often during the first few weeks of classes. All observations were made during the school week, so any changes in behavior or mood are not due to weekend activities.

Many findings do not conform to our predictions. While sickness behavior is characterized by decreases in social behavior and food intake, we found an increase in social contacts as well as caloric intake. Increased lethargy is also characteristic of sickness behavior, and participants did increase their sleep by approximately two-thirds of an hour. Changes in testosterone and cortisol did follow our predictions, and are consistent with previous reports. The decrease in IL-6 is clearly outside of expectations.

It is interesting to note that levels of cortisol after vaccination showed a positive correlation with both the number of in-person social contacts and the number of interactions initiated after vaccination. While this may be a spurious association, with class attendance acting as a confounding variable, it is also possible that higher levels of cortisol are associated with seeking social support (i.e., the tend and befriend hypothesis; Taylor et al., 2000).

We feel that refining this general study design will yield meaningful results. While a double-blind placebo experiment utilizing vaccines is clearly the strongest design for such a project, a within-subjects design is the most expeditious. Finally, we suggest that the use of vaccines outside of a clinical setting to elicit sickness behavior will allow for strong advances in the study of human sickness behavior.

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