Salivary Nerve Growth Factor Reactivity to Acute Psychosocial Stress: A New Frontier for Stress Research

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Objective: Neurotrophins such as nerve growth factor (NGF) may represent a stress-responsive system complementing the better known neuroendocrine (hypothalamic-pituitary-adrenal axis) and autonomic nervous system, but there is little evidence for NGF response to acute stress in humans because noninvasive measures have not been available. We investigated salivary NGF (sNGF) in 40 healthy young adults confronting a romantic conflict stressor. **Methods:** Five saliva samples—two collected before and three after the conflict—were assayed for sNGF, cortisol (hypothalamic-pituitary-adrenal marker), and α-amylase (sAA; ANS marker). In addition, a control group ($n = 20$) gave saliva samples at the same time intervals to determine whether sNGF changes were specific to the conflict stressor. **Results:** sNGF showed significant reactivity from entry to the first poststress sample among study participants ($\beta = .13$, $p = .001$), with nonsignificant change across poststress samples. Control participants showed no change in sNGF across the same period. Within-person changes in sNGF were generally aligned with both cortisol ($\beta = .17$, $p = .003$) and sAA ($\beta = .17$, $p = .021$) responses. Preconflict negative emotion predicted lower sNGF reactivity ($\beta = -.08$, $p = .009$) and less alignment with sAA ($\beta = -.09$, $p = .040$), whereas positive emotion predicted less alignment with cortisol ($\beta = -.10$, $p = .019$). **Conclusions:** This study is the first to document sNGF as a marker that responds to stress in humans. **Key words:** nerve growth factor, stress, cortisol, α-amylase, HPA, ANS.

HPA = hypothalamic-pituitary-adrenal axis; **ANS** = autonomic nervous system; **sNGF** = salivary nerve growth factor; **sAA** = salivary α-amylase.

INTRODUCTION

Well-characterized facets of the human stress response—the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic branch of the autonomic nervous system (ANS)—are thought to contribute to physiological wear and tear via allostatic load (1). Neurotrophins including nerve growth factor (NGF) may play a complementary role buffering these effects to protect the organism against stress-related damage (2). A very few studies have isolated NGF in human saliva (sNGF) and suggested that it relates to other stress system outputs (3–5), but no available evidence demonstrates acute stress-reactive properties of sNGF. Discovering a stress-responsive neurotrophic marker would expand conceptualization of the human stress response and resilience at a basic level. In turn, support for a noninvasive methodology to assess this response could open the door for important biomedical, psychiatric, and psychological research.

NGF release in the brain and periphery promotes neural growth and plasticity, in addition to regulating endocrine and immune cell activity (6). Whereas brain NGF directly affects neural growth, NGF released by the salivary glands is thought to affect peripheral targets through blood circulation. Primary sites of NGF expression and action—the hippocampus, hypothalamus, and adrenal gland—support its role in modulating the stress response at both central and peripheral levels (7). Available knowledge concerning NGF reactivity to acute stress comes from rodent models, which demonstrate increased blood and brain levels in mice after social stress. Intermale fighting and defensive behavior on the part of lactating females have been associated with NGF increases, whereas physical stressors such as immobilization and foot shock have not (8). Increased NGF in the blood, which seems to depend on salivary gland release, is found within minutes after fighting and peaks approximately 3 hours later (9).

In humans, only two studies have addressed (blood) NGF reactivity to stress, yielding mixed results. Whereas elevated NGF was found in parachutists both anticipating and after their first jump, compared with control participants (between-participant difference) (10), a within-participant study of response to a public speaking presentation failed to show elevated NGF, compared with a control day (11). Neither study demonstrated acute within-person NGF reactivity, perhaps because of design limitations. Both involved small, all-male samples responding to a stressor that did not closely resemble the type implicated in animal models (i.e., interpersonal dominance stressors). Inadequate measures of the stress response trajectory—likely dictated by reliance on blood sampling—further limited conclusions. An appreciation for how NGF does or does not respond to social stress in humans necessitates more comprehensive, minimally invasive, prestress and poststress measurement of NGF.

To understand NGF as part of a larger stress response, its alignment with other stress systems must also be elucidated. There is experimental evidence for bidirectional links—usually, but not always, potentiating—between NGF and both the HPA axis and sympathetic division of ANS (12–15), but as yet, no evidence has been presented based on naturalistic (in vivo) stress responses. The human studies cited above concluded that there was no association between NGF and cortisol responses, but null findings may be caused by study limitations, especially the paucity of within-person measures. It is also possible that the degree of cross-system alignment is more variable in humans and depends on psychological factors not measured in previous research. No human studies have addressed associations with ANS response to stress.

Finally, to fully interpret NGF's role in human stress response, relations with individual differences in well-being must

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be examined. A rise in central and/or peripheral neurotrophins has been associated with successful treatment of depression (16,17) and anxiety (18), as well as falling in love (19). On the other hand, increased neurotrophins have been found in animal models of depression and anxiety-like behavior (20,21). Clearly, further information is needed to determine how NGF during acute stress—both NGF response itself and its links with other stress systems—relates to emotional states in humans.

In sum, animal research supports a role of NGF in the stress response and suggests associations with other stress systems, but there are no studies, to our knowledge, addressing acute stress response trajectories in humans. sNGF may represent a particularly useful (noninvasive) biological measure, yet it has not been studied at all in response to stress. The present study takes a major step in human stress research by investigating the following questions:

1. Does sNGF show acute reactivity to a well-validated psychosocial stress task?
2. Does sNGF response relate to HPA and/or ANS response?
3. Do the above vary across people, and are differences related to emotional states when confronting the stressor?

METHODS

Sample and Procedures

Participants for this study were 40 (17 male, 23 female) healthy young adults (mean [M; standard deviation {SD}] age = 21.56 [5.89] years), drawn from a larger study of romantic couples. Data collection for this study occurred between March 2011 and December 2012. All procedures were approved by the University of Wyoming institutional review board, and participants gave informed consent to all study procedures. During a 2-hour laboratory session, participants confronted a validated psychosocial stressor—discussing an unresolved conflict with their romantic partner—shown to induce physiological (HPA) reactivity, with individual differences related to psychosocial adjustment (22–26). They also gave a series of saliva samples to index physiological stress trajectories.

All sessions began at 4 PM to control for diurnal variations in stress systems. After a set of questions to determine compliance with study conditions—that is, no current illness, no smoking or other drug use that day, no heavy exercise or brushing teeth past 3 hours, and no eating/drinking past hour—participants gave the first saliva sample (entry). Twenty minutes after receiving a vivid description of the conflict task highlighting the fact that this discussion could take the form of a fight or argument, the second sample was collected to measure stress anticipation (approximately 25 minutes after the entry sample and shortly before the discussion). Each partner nominated an unresolved issue that had caused an argument or fight recently, and one was selected by coin toss. Participants were given 15 minutes to discuss and attempt to resolve the selected conflict. Three poststress samples were collected 10, 25, and 40 minutes after conclusion of the discussion. Whole unstimulated saliva samples were collected using passive drool; all five samples in the series were assayed for sNGF and cortisol, and the first 4 were assayed for α-amylase (sAA).

In addition, saliva samples of 20 control participants (6 male, 14 female; M [SD] age = 20.8 [2.5] years) were collected using the same recruitment and saliva sampling procedures, including time of day and intervals between samples, in March 2013. However, these participants did not complete the conflict task; instead, they engaged in a quiet, noninteractive activity (such as reading) during the time between samples.

Measures

Salivary NGF

Saliva samples were assayed for NGF in triplicate using a commercially available enzyme immunoassay kit (NGF Emax immunoassay system Catalog No. G7631; Promega, Madison, WI) modified for use with saliva. All saliva samples were diluted 1:4 before testing. The assay standard curve range is 3.9 to 250 pg/ml; average intra-assay coefficient of variation, 13.5%; and average interassay coefficient of variation, 9.7%. Method accuracy was determined by spike recovery averaged 95.3%, and linearity by serial dilution ranged from 82.3% to 27.2%. Sex differences in sNGF were nonsignificant, as were associations with salivary flow rate (in milliliters per minute), so these variables were not included in model testing. sNGF values above the assay's sensitivity range (17%) were assigned the upper limit of the range (1000 pg/ml); deleting these cases yielded essentially unchanged results, although the association between mean sAA and sNGF levels became significant. In the absence of substantial skewness (<1.5), raw scores were used in analyses.

Cortisol

Samples were assayed for cortisol in duplicate by enzyme immunoassay without modification to the manufacturers' recommended protocol (Salimetrics, State College, PA). The test uses 25 μl of saliva and has a lower limit of sensitivity 0.007 μg/dl and a range of 0.007 to 3.0 μg/dl. The average intra-assay and interassay coefficients of variation are less than 5% and less than 10%, respectively.

α-Amylase

Samples were assayed for sAA in singlet using commercially available kinetic reaction assays (Salimetrics). The assay uses a chromagenic substrate, 2-chloro-4-nitrophenol, linked to maltotriose. The enzymatic action of sAA on this substrate yields 2-chloro-*p*-nitrophenol, which can be spectrophotometrically measured at 405 nm using a standard laboratory plate reader. The amount of sAA activity present in the sample is directly proportional to the increase (over a 2-minute period) in absorbance at 405 nm. Results are computed in units per milliliter of sAA. Intra-assay variation computed for the mean of 30 replicate tests was less than 7.5%. Interassay variation computed for the mean of average duplicates for 16 separate runs was less than 6%.

Positive and Negative Emotion

Directly before the conflict task (or after the second saliva sample for the control group), participants rated their emotion states using the Positive and Negative Affect Scale (27). A positive emotion score (mean rating of 10 positive emotion adjectives, Cronbach α = .89; M = 2.90 [SD] = 0.82 for study and M [SD] = 2.43 [0.60] for control participants) and a negative emotion score (mean rating of 10 negative emotion adjectives, Cronbach α = .87; M [SD] = 1.61 [0.60] for study and M [SD] = 1.26 [0.38] for control participants) were computed for each participant. Prestress positive and negative emotions were unrelated to one another (r = 0.08, not significant).

Analytic Strategy

Hierarchical linear modeling (28) was used to model sNGF response trajectories and associations with other biological markers related to stress. This approach separates within-person variability in sNGF over time (Level 1) from between-person differences in sNGF response (Level 2). A piecewise growth model estimated each participant's sNGF reactivity slope (from Sample 1–3), poststress sNGF level (Sample 3 intercept), and recovery slope (from Sample 3–5) at Level 1. At Level 2, the participant's positive and negative emotion scores were used to predict differences in each of these response components (i.e., slopes and intercepts).

First, sNGF trajectories were examined in both study and control participants to confirm that any sNGF changes were specific to the conflict stressor and did not represent a time-of-day or repeated sampling effect. Next, models tested associations across stress systems in the study participants. At Level 1, sNGF variability was modeled with an intercept and time-varying stress covariate (i.e., cortisol or sAA) centered around that participant's own mean; this captured within-person synchrony or alignment of responses across systems. At Level 2, the participant's mean cortisol or sAA (centered around the grand mean for the sample) was entered as a predictor of the sNGF intercept; this showed whether between-person differences in sNGF and cortisol/sAA levels were related. Finally, positive/negative emotion scores were entered as predictors of the Level 1 stress

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covariate effect to determine whether within-person alignment of stress systems varied by emotion state. Examples of the two-level equations explaining a) sNGF response trajectories and b) sNGF alignment with another stress system are given below:

sNGF response trajectories
Level 1

$$\text{sNGF} = \beta_0(\text{intercept}) + \beta_1(\text{slope 1}) + \beta_2(\text{slope 2}) + \text{error}$$

Level 2

$$\beta_0 = \gamma_{00} + \gamma_{01}(\text{positive emotion}) + \gamma_{02}(\text{negative emotion}) + \text{error}$$

$$\beta_1 = \gamma_{10} + \gamma_{11}(\text{positive emotion}) + \gamma_{12}(\text{negative emotion}) + \text{error}$$

$$\beta_2 = \gamma_{20} + \gamma_{21}(\text{positive emotion}) + \gamma_{22}(\text{negative emotion}) + \text{error}$$

sNGF alignment with cortisol
Level 1

$$\text{sNGF} = \beta_0(\text{intercept}) + \beta_1(\text{cortisol}) + \text{error}$$

Level 2

$$\beta_0 = \gamma_{00} + \gamma_{01}(\text{positive emotion}) + \gamma_{02}(\text{negative emotion}) + \gamma_{03}(\text{ mean cortisol}) + \text{error}$$

$$\beta_1 = \gamma_{10} + \gamma_{11}(\text{positive emotion}) + \gamma_{12}(\text{negative emotion}) + \text{error}$$

RESULTS

Repeated-measures analyses of variance with follow-up contrasts were used to initially examine overall changes in sNGF and other stress measures across measurement points. Study participants showed significant change across samples in all three biological measures ($F(4) = 5.75$, $p < .001$ for sNGF; $F(4) = 15.00$, $p < .001$ for cortisol; $F(3) = 6.04$, $p = .001$ for sAA). sNGF increased significantly from entry to anticipation ($F(1) = 7.02$, $p = .012$) and from anticipation to the first poststress sample ($F(1) = 5.99$, $p = .019$). This suggests that sNGF responds to both anticipatory stress and to the experience of conflict stress itself. Differences among poststress levels were nonsignificant ($F(1) = .70$, $p = .41$ from Sample 3–4; $F(1) = 1.89$, $p = .18$ from Sample 4–5). Significant increases in cortisol also occurred between entry and anticipation, with sustained elevation immediately following the stressor ($F(1) = 27.47$, $p < .001$ from Sample 1–2; $F(1) = 1.06$, $p = .31$ from Sample 2–3). Cortisol recovery occurred after the task ($F(1) = 10.74$, $p = .002$ from Sample 3–4; $F(1) = 11.46$, $p = .002$ from Sample 4–5). sAA increased from entry to anticipation ($F(1) = 5.31$, $p = .027$) and continued increasing to the poststress sample ($F(1) = 4.25$, $p = .046$) before showing recovery ($F(1) = 5.39$, $p = .026$ from Sample 3–4). By contrast, the control participants showed no significant changes in sNGF across samples ($F(4) = 0.12$, $p = .98$). Figure 1A shows mean sNGF across samples for study participants, and Figure 1B shows these values for

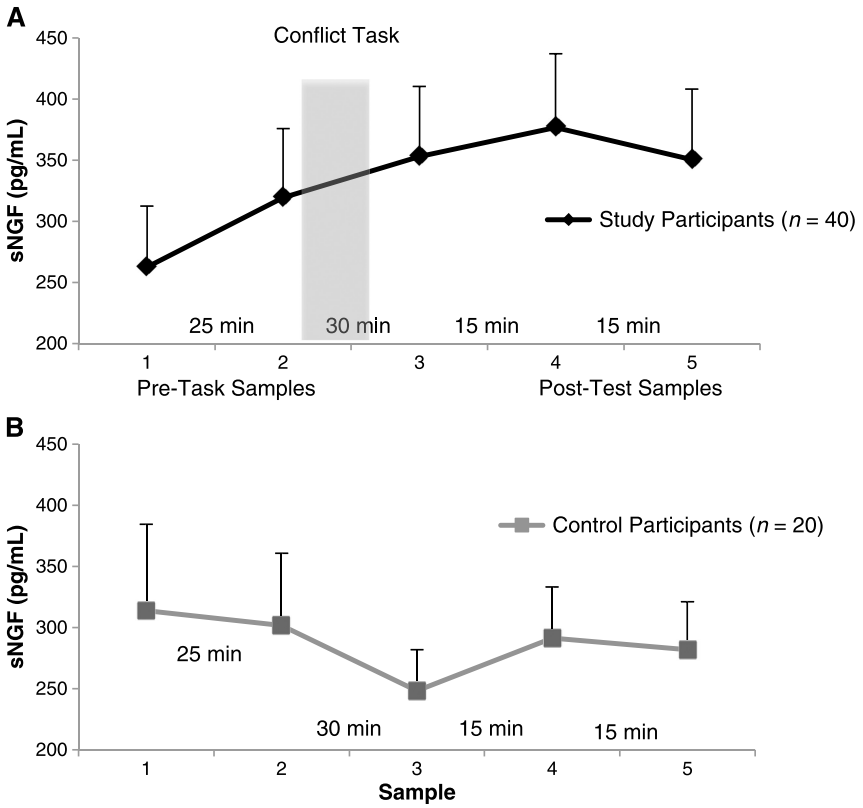

Figure 1. A, Mean sNGF values across samples for study participants (bars represent standard errors). B, Mean sNGF values across samples for control participants (bars represent standard errors). sNGF = salivary nerve growth factor.

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TABLE 1. Correlations Among Biological Stress Measures and Prestress Emotions

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1. Resting cortisol (Sample 1)	—										
2. Poststress cortisol (Sample 3)	.67**	—									
3. Cortisol change (Sample 3-1)	.34*	.92**	—								
4. Resting sAA (Sample 1)	−.034	.21	.29	—							
5. Poststress sAA (Sample 3)	.008	.060	.072	.86**	—						
6. sAA change (Sample 3-1)	.056	−.15	−.22	.35*	.78**	—					
7. Resting sNGF (Sample 1)	−.17	−.26	−.24	−.011	.034	.075	—				
8. Poststress sNGF (Sample 3)	−.21	−.29	−.26	−.006	.081	.16	.89**	—			
9. sNGF change (Sample 3-1)	−.13	−.16	−.13	.006	.12	.20	.046	.49**	—		
10. Positive emotion	−.010	.060	.082	−.21	−.25	−.20	−.18	−.23	−.16	—	
11. Negative emotion	.043	.38*	.47**	.020	−.053	−.12	−.14	−.23	−.24	−.080	—

sAA = salivary α-amylase; sNGF = salivary nerve growth factor.
* $p < .05$, ** $p < .01$.

control participants. Table 1 shows correlations among observed resting levels (Sample 1), poststress levels (Sample 3) and change in (Sample 3–1) the three biological measures, as well as prestress emotion ratings.

Next, hierarchical linear models were fit to better understand individual stress trajectories and associations across stress systems. Piecewise growth models in the study participants demonstrated a significant sNGF reactivity slope from entry to the first poststress sample ($\beta = .13$, SE = 0.036) and a nonsignificant recovery slope from the first to third poststress sample ($\beta = .003$, SE = 0.039), confirming that sNGF typically rose as participants confronted the stress task but did not change during the poststress period. Significant between-person variability in each response component ($\chi^2(39) = 69.9–943.26$, $p < .001$) suggested that this pattern varied across participants. Negative emotion predicted less sNGF reactivity (i.e., shallower slopes) and lower poststress levels, explaining 15.4% of the variance in intercepts and 34.8% of the variance in slopes (Table 2, top). Positive emotion did not significantly predict sNGF trajectories.

TABLE 2. Prestress Emotion Related to sNGF Response and Within-Person Alignment of Stress Systems

Outcome	Positive Emotion		Negative Emotion	
	Standard Coefficient (β)	SE	Standard Coefficient (β)	SE
sNGF reactivity slope	−.042	.031	**−.083**	**.030**
Poststress sNGF level	−.288	.161	**−.289**	**.127**
sNGF recovery slope	−.067	.055	.069	.040
Cortisol-sNGF association	**−.104**	**.042**	−.062	.036
sAA-sNGF association	−.011	.061	**−.091**	**.042**

Multilevel regression analyses conducted using hierarchical linear modeling. Significant effects ($p < .05$) highlighted in bold.
sNGF = salivary nerve growth factor; SE = standard error; sAA = salivary α-amylase.

Again, control participants did not show significant changes in sNGF—either from Sample 1–3 (corresponding to study participant stress reactivity), from Sample 3–5 (corresponding to study participant poststress recovery), or across all five samples (β values = −.01 to −.02, not significant). Emotion ratings did not relate to control participants' sNGF levels.

Cortisol ($\beta = .38$, SE = 0.075) and sAA ($\beta = .16$, SE = 0.046) also showed significant reactivity slopes, and cortisol showed a significant recovery slope ($\beta = -.32$, SE = 0.056; a recovery slope could not be computed for sAA because of the limited number of poststress samples). Significant within-person positive effects of both cortisol ($\beta = .17$, SE = 0.053) and sAA ($\beta = .17$, SE = 0.072) in study participants confirmed that changes in sNGF were typically aligned with changes in other stress systems over time. However, between-person differences in mean sAA did not predict sNGF levels, and mean cortisol only marginally predicted lower sNGF levels ($\beta = -.11$, SE = 0.066).[1] Between-person variability in these stress covariates ($\chi^2(39) = 59.35$, $p = .015$ for cortisol; $\chi^2(39) = 51.74$, $p = .068$ for sAA) again suggested differences that could be explained by emotion predictors.

Whereas positive emotion predicted a weaker within-person association of cortisol and sNGF, negative emotion did so for sAA and sNGF (Table 2, bottom). According to region of significance calculations, changes in sNGF related significantly to changes in cortisol for participants with positive emotion 81st or greater percentile and to changes in sAA for participants with negative emotion 82nd or greater percentile. Examination of cortisol and sAA trajectories predicted by positive/negative emotion illustrated why this was the case; participants with high negative emotion showed greater dissociation between reactive sAA and nonreactive sNGF responses (Fig. 2), and those with

[1]This effect was significant when the within-person cortisol covariate was not included.

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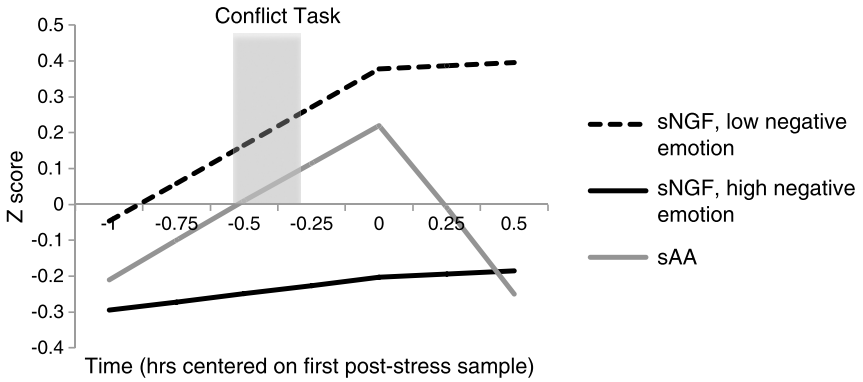

Figure 2. Negative emotion predicts sNGF response and alignment with sAA. Predicted trajectories shown at ±1 SD from the mean for PANAS negative or positive emotion. sNGF = salivary nerve growth factor; SE = standard error; sAA = salivary α-amylase; SD = standard deviation; PANAS = Positive and Negative Affect Scale.

high positive emotion showed greater dissociation between recovering cortisol and an ongoing sNGF responses (Fig. 3).

DISCUSSION

This study adds a new dimension to what can be considered the human stress response—not just shifts in energy use facilitated by the HPA and ANS, but also a complementary regenerative force. This is the first study to demonstrate sNGF reactivity to acute psychosocial stress in humans, suggesting that this is a useful marker of neurotrophic stress response. Moreover, we showed alignment between sNGF responses and other well-known facets of the stress response—that is, HPA and ANS—within people. Finally, we found differences in both sNGF response itself and in its relation to other stress systems according to prestress emotional states. Each of these findings lays an important piece of the foundation for understanding NGF as a part of human stress adaptation.

Repeated measurement of sNGF confirmed that it is responsive to psychosocial stress, rising in anticipation of and immediately after a conflict discussion. The observation that sNGF did not change during this same period among control participants strengthens the conclusion that observed sNGF reactivity was tied to the conflict task and not an artifact of diurnal rhythms or repeated sampling. Consistent with animal research, it did not show recovery to baseline in the 40 minutes after the stressor (unlike cortisol and sAA), although it also did not continue rising. Further assessment over a longer poststress period will be needed to determine under what conditions sNGF shows ongoing reactivity and when it typically recovers in humans. The constraints of the current study procedures did not allow continuing assessment of sNGF across the period required to observe recovery (which animal models suggest may last as long as 24 hours) or blood sampling of NGF to compare salivary and serum values, although these represent important directions for future research.

The present data support the idea that sNGF reactivity serves a useful function; people reporting lower negative emotion when confronting the stressor also showed greater sNGF reactivity and higher poststress levels. A neurotrophic response to acute stress—found at both central and peripheral levels—may have evolved as a resilience mechanism to protect the brain and other tissues against the effects of cortisol release. Although cortisol exposure is not always implicated in neural atrophy (29), there is substantial evidence that chronic stress exposure and associated HPA axis activity can play a role in reversible brain volume reductions, consistent with prior findings of reduced

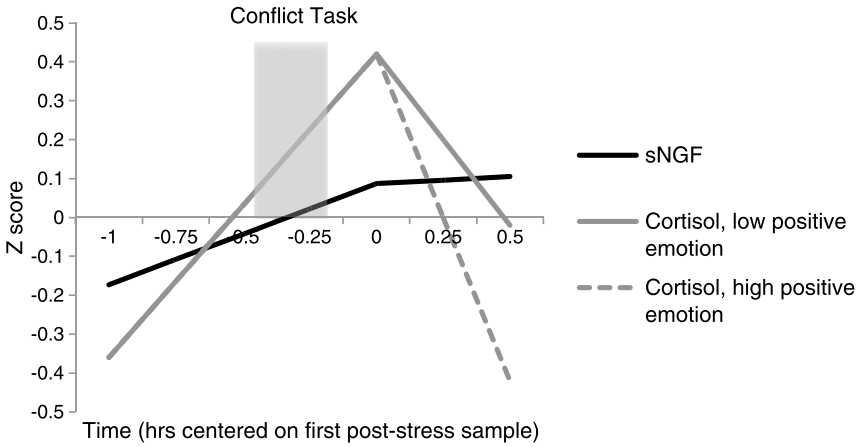

Figure 3. Positive emotion predicts sNGF alignment with cortisol. Predicted trajectories shown at ±1 SD from the mean for PANAS negative or positive emotion. sNGF = salivary nerve growth factor; SE = standard error; SD = standard deviation; PANAS = Positive and Negative Affect Scale.

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brain volume in stress-related mental disorders and recovery-related increases in NGF (16,17,30–32). At the same time, prolonged NGF elevation during chronic stress may remodel central (brain) and peripheral (adrenal) response mechanisms in a way that exacerbates stress responsiveness and ultimately leads to mental disorder (7,20,21). Analyses in the current sample (conducted by our group but not yet published) suggest that both sNGF reactivity and recovery relate to markers of psychological health, supporting the idea that transient but not chronic sNGF elevations are adaptive. Future research should probe the benefits of sNGF by relating responses to more lasting measures of emotional well-being, including depression and anxiety.

Tests of within-person synchrony across stress systems showed that sNGF response generally aligned with both cortisol and sAA responses. This fits with experimental (animal) research connecting activation across NGF, the HPA axis, and the ANS, although variability in the strength of cross-system alignment suggests a more nuanced relationship in humans that depends on psychological factors. Additional evidence that these stress systems are related but not redundant comes from the observation that the magnitude of sNGF response was unrelated to that of sAA and that people with higher absolute levels of cortisol actually tended to have lower sNGF. Previous research suggests a biphasic association between NGF and HPA activity, with elevated glucocorticoids also playing an inhibitory role in NGF release (33,34). People with chronically elevated HPA activity may eventually down-regulate NGF, a hypothesis that should be tested with developmental investigations of multisystemic stress responses.

These findings further suggest that it may be good for the sNGF response to be decoupled from cortisol, but coupled with sAA, across an acute stressor. Specifically, people reporting more positive emotion showed a greater dissociation between sNGF and cortisol responses (particularly during recovery), whereas those reporting less negative emotion showed a greater association between sNGF and sAA responses (particularly during reactivity). It seems that an adaptive response to conflict stress involves strong ANS and NGF coactivation, followed by HPA recovery. This is consistent with prior research linking sAA reactivity to emotional regulation (35), and delayed cortisol recovery to mood disturbance (36). Psychological factors fostering different cross-system stress profiles—for example, stress appraisals and coping—and effects on nervous system integrity should be explored to better characterize resilient responses.

Although this study takes a critical first step in sNGF stress research, it also raises important questions. As suggested above, future investigations should identify early developmental influences on sNGF response and associated mental/physical health outcomes. Greater knowledge of the types of stressors to which sNGF responds and the time course of reactivity and recovery are needed, and lagged (potentially bidirectional) effects across stress systems should be explored. Further experimental manipulations and naturalistic observation research will help to clarify under what conditions and to what degree responses are related in both animals and humans. An additional limitation of the current study was the singlet assay of sAA, which may have reduced the reliability of this measure and obscured further associations.

The current research offers important insights that could change the way adaptive stress responses are conceptualized and measured—not simply as activation in any one system but as a pattern of activation across multiple linked systems. It further spurs consideration of stress system flexibility/plasticity, given NGF's potential to promote stress-related learning in both healthy and unhealthy ways. sNGF represents an exciting new player on the stage of stress research. At present, it seems a benevolent foil to the HPA axis and sympathetic nervous system; Additional research using this measure will enable evaluation of salivary nerve growth factor reactivity and its biobehavioral correlates in psychosomatic medicine.

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