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Salivary nerve growth factor response to stress related to resilience

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HIGHLIGHTS

- Nerve growth factor in saliva (sNGF) has been shown to respond to stress.
- We investigated how acute sNGF responses relate to markers of resilience.
- People with positive stress appraisals showed stronger sNGF reactivity and recovery.
- Agency and well-being are also related to dynamic sNGF reactivity and recovery.
- The sNGF response to stress may help explain differences in resilience.

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ABSTRACT

Salivary nerve growth factor (sNGF) has recently been shown to respond to psychosocial stress, but little is known about how individual differences in this neurotrophic marker relate to stress vulnerability vs. resilience. This study followed up on these initial findings by examining sNGF responses to interpersonal stress in relation to both well-being and state/trait factors that determine the way a person approaches and is impacted by stress. Young adults ($n = 40$) gave 5 saliva samples over the course of a laboratory session that involved an interpersonal conflict stressor, and all samples were assayed for sNGF. Participants also completed self-report measures of global well-being, stress appraisals before and following the conflict, and agency. Greater sNGF reactivity to conflict related to stronger appraisals of coping ability and agency. Post-conflict sNGF recovery related to lower anticipatory stress appraisals, and to higher agency and well-being. These results support the idea that dynamic sNGF responses are adaptive. Implications for the potential role of the neurotrophic system in stress resilience are discussed.

1. Introduction

Nerve growth factor measured in human saliva (sNGF) has recently been shown to respond to acute psychosocial stress, highlighting a neurotrophic component of the stress response system that may complement the more well-known sympathetic branch of the autonomic nervous system (ANS) and hypothalamic–pituitary–adrenal (HPA) axis [21]. However, little is known about how individual differences in neurotrophic response relate to stress vulnerability vs. resilience. The current investigation takes a critical step toward defining this system's adaptive value by relating young adults' sNGF responses to interpersonal stress to both well-being and state/trait variables known to reduce the negative impacts of stress.

Nerve growth factor is one of a larger class of neurotrophins that regulate neural differentiation and growth/plasticity [24]. NGF is expressed in both the brain and periphery, with the salivary glands representing the largest source of circulating NGF in rodent models [23]. To date, most of the evidence for NGF's acute stress-reactive properties comes from mice, which demonstrate brain and blood increases following social stress (e.g., [1,2,4]). The recent discovery that NGF measured in saliva responds to psychosocial (interpersonal conflict) stress, and that this response relates to both HPA axis and ANS responses, has opened the door for investigation of sNGF as part of the human stress response [21]. This initial study documented significant sNGF reactivity to a relationship conflict discussion, in contrast to nonsignificant changes in sNGF across the same time period for a control group of subjects not exposed to conflict stress. It further revealed significant associations between participants' sNGF response trajectories and both their cortisol (HPA marker) and salivary alpha-amylase (sAA; ANS marker) responses across the session, helping to validate this measure as part of a larger stress response. Finally, this research

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related sNGF reactivity to lower levels of negative emotion when confronting conflict stress, suggesting that it may be beneficial. The present study follows up on these early findings in the same dataset, moving from the basic question of whether sNGF responds to acute stress to the question of why this response matters—in particular, how does the sNGF response relate to more lasting markers of well-being?

There is a body of research relating blood and/or brain NGF levels to stress-related psychological difficulties, though these links are not straightforward. A neurotrophic deficit has been implicated in depression, which is reversed by successful antidepressant treatment (e.g., [18,25,36,41]). At the same time, increased neurotrophin (NGF and/or BDNF) levels were observed in animals subjected to early stress that later showed depression-like behaviors [7,10,11]. It seems that NGF does not directly influence mood, but rather plasticity and the ability to benefit from new learning experiences [9]. Humans in anxiety-inducing situations including a first parachute jump and caring for an ill spouse have also exhibited elevated blood NGF [3,15]. A study showing higher NGF among people in love [12] suggests that NGF may be increased by states of high arousal, rather than negative affect per se, consistent with an alternate interpretation of the parachute situation as inducing excitement (instead of or in addition to anxiety). Thus, there is evidence that both elevated and diminished NGF could underlie differences in well-being, but no information as yet about relations with acute stress responsiveness.

To understand how neurotrophic responses relate to stress adaptation, relations not only with the outcome of such adaptation (i.e., well-being), but also with individual difference factors driving adaptation, must be explored. Both state and trait differences in the ways people approach stress are known to contribute to well-being, with resilience depending on a host of cognitive and personality factors. For example, appraising a stressor as non-threatening (low primary appraisal) and oneself as having the power to control the situation (high secondary appraisal) reduces distress and promotes positive coping, which in turn protects against mental disorder [40]. At the dispositional level, people higher in agency—i.e., instrumental personality characteristics related to mastery and a strong, independent self—similarly show superior coping and mental health outcomes [16]. Although these factors are known to impact other aspects of stress physiology (i.e., the ANS and HPA axis; [13]; [42]; [37]), their role in neurotrophic responses to stress is unknown.

The current study was designed to follow up on our initial discovery that sNGF responds to psychosocial stress in humans with tests of resilience-related individual differences in sNGF before and following interpersonal conflict stress. In particular, we examined relations between sNGF reactivity/recovery patterns and well-being, stress appraisals, and agency, in the same sample of young adults we reported on previously [21]. Based on indications from prior human and animal research involving circulating NGF levels, we hypothesized that resilience—evidenced by higher well-being and agency, as well as lower primary and higher secondary stress appraisals—would be associated with greater sNGF reactivity and higher post-stress levels. Absent previous research on post-stress dynamics, we made no hypotheses about sNGF recovery.

2. Method

2.1. Participants and procedures

Participants for this study were 40 (17 male, 23 female) healthy young adults (M age = 21.56, SD = 5.89), drawn from a larger study of romantic couples recruited from a departmental human subject pool and community fliers. All participants gave informed consent prior to completing the study, which was approved by the University of Wyoming Institutional Review Board. During a two-hour laboratory session, participants confronted a validated psychosocial stressor—discussing an unresolved conflict with their romantic partner—known to induce physiological (HPA) reactivity. In particular, the task was modeled after the task found by Kiecolt-Glaser and colleagues (e.g., [20]) to elicit both subjective and physiological stress responses, the magnitude and/or duration of which may vary according to individual differences in psychosocial adjustment (i.e., negative emotionality, attachment security, trauma symptoms—see [22,30,31]). 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.[1] Following a set of initial questions to determine compliance with study conditions—i.e., no current illness, no smoking or other drug use that day, no heavy exercise or brushing teeth in the past 3 h, no eating/drinking in the past hour—participants gave the first saliva sample (entry). The second sample, collected 20 min after receiving a vivid description of the conflict task and shortly before the discussion, measured stress anticipation. 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 min to discuss and attempt to resolve the selected conflict. Three post-stress samples were collected 10, 25, and 40 min after the conclusion of the discussion. Whole unstimulated saliva samples were collected using passive drool and stored at −20 °C prior to shipment on dry ice to Salimetrics for assay.

2.2. Measures

2.2.1. sNGF

As detailed in [21], all saliva samples were assayed for NGF in triplicate using a commercially available enzyme immunoassay kit (Promega NGF E_{max} Immunoassay System Cat.# G7631; Madison, WI) modified for use with saliva. The NGF salivary test method was developed by Salimetrics (State College, PA) using the commercially available Promega NGF E_{max} Immunoassay System. Coating buffers, sample diluent and wash buffer were developed and optimized for accurate and precise detection of NGF in saliva. The coating buffer is comprised of 27 mM carbonate–bicarbonate. Sample diluent is phosphate buffered saline with bovine serum albumin and a preservative. The wash buffer is phosphate buffered saline with 0.05% Tween-20.

Saliva samples with varying levels of NGF were used during validation to ensure accuracy and precision and lack of matrix effects. Method accuracy was assessed by measuring the recovery of exogenous NGF added to saliva, which was found to be 100.3% for recovery of 30 pg/mL and 97.6% for recovery of 100 pg/mL. Intra-assay precision was 16.5% (134.5 pg/mL) and 12.6% (36.9 pg/mL) as determined by running 20 replicates within one plate. Inter-assay precision was 11.9% (133.57 pg/mL) and 19% (20.98 pg/mL) as determined by the mean of average results of 6 runs. Linearity of dilution was used to assess matrix effects from saliva. Admixtures of a high (134.5 ng/mL) and low (37 pg/mL) NGF saliva samples were prepared and tested according to NCCLSEP6-A. The average recovery from across the range was 102.7% with a range of 82.3% to 127.2%. All saliva samples were assayed in the Salimetrics CLIA approved testing facility with trained operators and technicians. Saliva samples were tested for NGF in triplicate after being diluted 1:4 prior to testing. The assay standard curve range is 3.9 to 250 pg/mL. For this investigation, five samples were obtained from each subject and all were run on the same assay plate in triplicate.

Associations with salivary flow rate (mL/min) were nonsignificant, so flow rate was not included in model testing. sNGF values above the

[1] As reported previously [22], a control sample of 20 participants was recruited to give saliva samples at the same times as study participants, but without undergoing a stress task. Nonsignificant changes in control participants' sNGF suggested that there were no diurnal effects, at least in the late afternoon period during which the study occurred.