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Psychoneuroimmunology and tattooing

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Abstract: Though it injures the body in many ways, tattooing may also prepare it for later dermal stress through psychoneuroimmunological means. To test this, we examined salivary endocrine (cortisol), immune (secretory immunoglobulin A), and inflammatory (C-reactive protein) responses to receiving a new tattoo relative to previous tattoo experience among 48 adults attending a tattoo festival. We found no effect of previous tattoo experience on preposttest cortisol but a significant main effect of extent of body tattooed on secretory immunoglobulin A and significant interaction among extent of body tattooed, hours tattooed, and years since first tattoo on C-reactive protein. These findings suggest that the positive psychological evaluation of tattooing as eustress may contribute to biochemical adaptation through tattooing.

One-Sentence Summary: The psychological and physical experience of being tattooed contributes to physiological adaptations that prepare the skin for other injury.

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Despite a century of pathologizing tattooing (Angel, 2017), one in three adults in the United States has at least one tattoo, and it is even more common in those under 30 years of age (Blanton, 2015). Scholars have long suggested that tattooing may have health benefits (Alter-Muri, 2020; Ghosh, 2020; Hambly, 2009; Krutak, 2013; Tuttle & Vale, 1989), a motivation that has been underexplored. Tattooing is a purposely experienced stressor on the mind and body that may also produce a psychoneuroimmunological benefit. The skin is the body's largest organ, and the mind-body interactions vis-a-vis skin are particularly influential on overall health (Lugović Mihić, 2019; Tausk et al., 2008; Yan, 2016).

Acute negative stress and chronic stress are generally associated with immunosuppression, which likely results from trade-offs involved in fight-or-flight responses and the release of glucocorticoids. Depending on the type and duration of stressor, glucocorticoids can stimulate or inhibit immune responses (Sapolsky, 2002). For example, the fear of pain or anxiety about getting a tattoo may produce a glucocorticoid response (cortisol in humans) in anticipation of the experience. However, that cortisol increase may subside during and after each successful tattoo experience because of the positive cognitive evaluation of the circumstances. The skin is closely associated with psychological states. A number of skin disorders positively correlate with mental issues (Beltraminelli & Itin, 2008), suggesting the opposite may also be true—skin health and immune function may benefit mental health. A rewarding tattoo experience may produce a positive feedback effect that both motivates people to get more tattoos and improves the immune system through training the mind and body to handle pain and heal more quickly from subsequent tattoos and other dermal injuries.

There is little research examining physiological benefits of tattooing, but the many psychological motivations and advantages are clear (Swami, 2012). Physical exercise, another form of cultural eustress (good stress), provides a model for assessing health impacts of tattooing (Kohut, 2019). Following research in exercise science, we investigated the rheostatic mechanisms of endocrine and immune functions to tattooing. Rheostasis refers to the functions of biochemical gradients in regulating allostasis and homeostasis, such that increases and decreases of these homeostats have myriad effects within systems, as opposed to binary on/off functions (Boulos & Rosenwasser, 2004). For instance, cortisol is generally assumed to function primarily as a stress hormone but also plays roles in metabolism and immune regulation (Engeland et al., 2019). In exercise research, cortisol production appears to be linearly related to intensity or duration of effort (Tauler et al., 2014).

Immunoglobulins play major roles in mucosal defense and vary in response to exercise, likely due to differences in exercise duration and intensity (Bishop & Gleeson, 2009; Tauler et al., 2014). Secretory immunoglobulin A is considered one such agent of adaptive immunity (Engeland et al., 2019). C-reactive protein is a marker of inflammation under transcriptional control of interleukin-6 that works synergistically with glucocorticoids and other elements in acute responses (Tauler et al., 2014; Volanakis, 2001). By examining changes in salivary cortisol (CORT), secretory immunoglobulin A (sIgA), and C-reactive protein (CRP) relative to previous tattoo experience, we tested the prediction that immunological adaptations like those observed through physical exercise also occur from tattooing.

Materials and Methods

Participants and Study Site

We collected all data at the two-day 2018 Northwest Tatau Festival in Puyallup, Washington. We were invited to this specific tattoo festival by one of the hosts, who we had met while conducting a smaller study of tattooing and health in American Samoa the previous year (Lynn et al., 2019).

We collected data from 56 clients of 38 artists at the festival. Three of the artists administered tattoos using non-electric, hand-tapping methods, whereas the other 35 artists used contemporary electric tattoo machines to administer all tattoos. Four clients receiving electric-machine tattoos did not provide enough data for analysis and were therefore excluded. We removed four others whose saliva sample volumes were too low to complete all biomarker assays. Analysis included data from 15 women and 33 men aged 18-60 (mean \pm SD = 34.2 \pm 11.12). Participants self-identified mostly as Pacific Islander (46%), White (27%), or Asian (15%) but included Black (6%), Hispanic (4%), and American Indian (2%). Six percent of the participants reported their highest level of education as a graduate degree, 33% as an undergraduate degree, 29% as some college, 29% as a high school diploma, and 2% as some high school. Forty-four percent of participants were engaged or married, 19% were in a committed relationship, 2% were in a casual relationship, 31% were single, and 2% indicated another undescribed relationship status.

We obtained written consent from every participant after a verbal explanation of the study procedures.

Procedures and Measures

We received verbal permission from all tattoo artists on the day preceding the festival to recruit their clients for the study. After an artist stenciled or drew a tattoo pattern on a participant (but before they began tattooing) a member of our research team would walk the participant to the booth we used for data collection. After receiving informed consent, we collected demographics (age, gender, ethnicity, civil status, hours worked, alcohol/tobacco/medication use) and information about possible confounds (socioeconomic status [Singh-Manoux et al., 2003], perceived stress [Cohen et al., 1983; Cohen & Williamson, 1988], tattoo artist, tattoo delivery method [hand-tap or electric], number of supporters there with participant during tattoo, recent illness, medical problems with previous tattoos) and tattoo experience (Lynn et al., 2019).

Twelve percent of participants had recently been sick. Only one participant reported any previous medical complications associated with a tattoo. We compared current health and lifestyle variables to pretest biomarkers to determine the relevance of these issues at baseline. Recent sickness was significantly associated with pretest CRP (r = .40, p = .01), confirming our use of CRP as an objective proxy of baseline health.

Six participants received hand-tapped tattoos, whereas 40 received tattoos administered by electric machines. We determined tattoo experience by asking participants to self-report the year they got their first tattoo (from which we calculated years tattooed), number of tattoo sessions, number of completed tattoos, number of hours for each tattoo session (from which we calculated total hours tattooed), and extent of body tattooed. For the latter measure, we provided frontal and rear outlines of male and female bodies with grid overlays and asked participants to indicate the number and location of squares to represent the coverage of their tattoos. We calculated extent of body covered as the percent of boxes filled or partially filled out of the total number of boxes in the grids (Lynn et al., 2019). However, because many tattoos are integrated into larger pieces, making it difficult to count tattoos by number, and because people often could not recall how many sessions were involved for larger or older pieces, we did not include these two variables in tattoo experience calculations.

We then collected weight and fat percentage using a bioimpedance analyzer (Tanita TFB 310) and height using a SECA stadiometer (Model 217) to calculate BMI (using CDC formula for pounds and inches) and a Detecto hand dynamometer (Model DHS 174) to measure handgrip strength of both hands (twice each, averaged together for one mean) as a control for neurocompetence (Innes, 1999). We asked participants to passively drool into a 1.8 mL cryovial

to the fill line and recorded the time (in seconds) that it took each participant to complete the sample (flow rate). One hour into the tattoo session, we collected a second sample, recorded the flow rate, and asked participants to indicate the pain of the tattoo they were receiving on a 10-point scale (1 = no pain, 10 = worst pain imaginable). Saliva samples were refrigerated overnight and shipped immediately after the festival to the Baylor University where they were stored in -80°C freezers until assayed.

All study procedures were approved by the Institutional Review Board of the University of Alabama (#17-OR-156-ME-R1).

Biomarker Analysis

Samples were thawed, centrifuged for 15 minutes at 1500rcf at room temperature, aliquoted to prevent repeatable freeze/thaw cycles, and assayed. Salivary cortisol, sIgA, and CRP were analyzed with commercially available ELISA kits (#3002, #1602, #2102) from Salimetrics, LLC (State College, PA). Sensitivity for these assays are < .007 μ g/dL, 2.5 μ g/mL and 9.72 pg/mL, respectively. Correlation coefficients for each standard curve were better than .999. Intraassay CVs (based on sample duplicates within plates) were 5.46%, 4.54% and 1.67%, respectively. Inter-assay CVs (based on high and low control duplicates between plates) were 8.23%, 10.04%, and 3.96%, respectively.

Statistical Analysis

Statistical analysis and plotting of data were performed using SPSS Version 27 (IBM Corp., Armonk, NY). We calculated means, standard deviations (SD), and range (min—max) of study variables and used student's *t*-tests to compare pre-posttest means of biomarkers (Table 1). Since all three biomarkers for pre- and posttests derive from the same saliva sample, we checked biomarker independence using bivariate analysis.

To investigate the impacts of tattooing on endocrine, immune, and inflammatory functions, we used hierarchical analysis of covariance (ANCOVA) with posttest CORT, sIgA, and CRP as dependent variables, respectively. In the first block, we included the pretest measure of the respective dependent variable and the pre-posttest difference of the other two biomarkers in the model as covariates, along with gender, age, and BMI. Other covariates were selected if they significantly correlated with the dependent variable (Table 2). Possible covariates included ethnicity, education, civil status, socioeconomic status, perceived stress, tattoo artist, tattoo delivery method (hand-tap or electric), handgrip strength, fat percentage, number of supporters, pain rating, medical complications from previous tattooing, recent sickness, alcohol use (past 24 hours), tobacco use (cigarettes, vaping, or loose leaf; past week), marijuana use (past 24 hours), medication use, and hours worked (past week). All variables were standardized using Z-scores. Tattoo experience variables were entered in the second block, and interaction terms created as products of standardized tattoo experience variables were entered in the third block.

Results

During a two-day tattoo festival in Puyallup, Washington, we queried 15 women and 33 men about previous tattoo experience. We also collected their saliva samples before (pretest) and one-hour into (posttest) their session. Sample descriptives are outlined in Table 1.

Table 1. Sample characteristics

	$Mean \pm SD$	Min—Max
Age	34.19 ± 11.108	18—60
BMI	30.93 ± 5.886	17—43

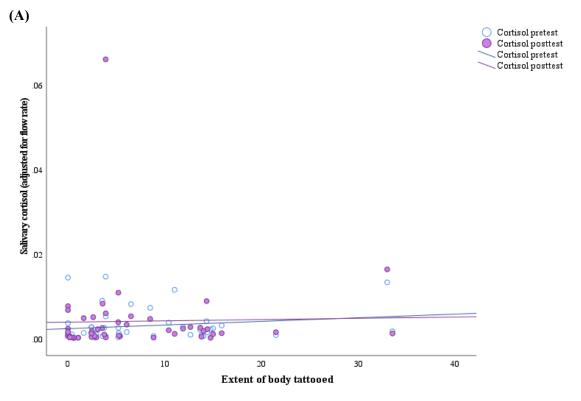
Socioeconomic status		7.25 ± 1.525	4—10
Perceived stress		7.02 ± 2.899	4—16
Supporters Handgrip strength		2.23 ± 2.070	0—7
		41.33 ± 11.234	20—66
Tattoo	Years since first tattoo	15.76 ± 10.973	0—52
experience	Extent of body tattooed	7.27 ± 7.778	0—34
	Hours tattooed	14.81 ± 18.376	0—85
Pain rating		4.63 ± 2.053	1—9

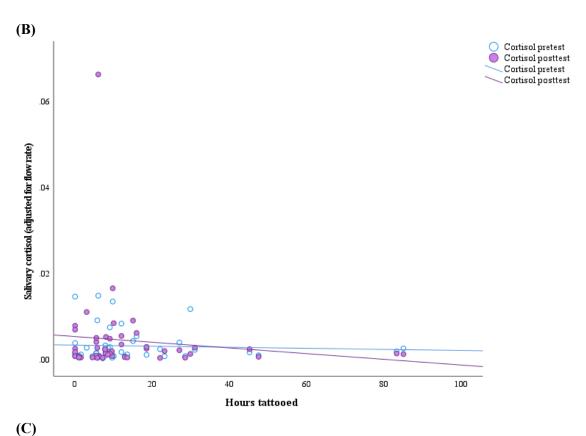
Cortisol increased from pre-posttest, but, contrary to prediction, the change was not statistically significant. Similarly, apparent decreases sIgA and CRP suggest pre-posttest immunosuppression, but the decreases were also not significant (Table 2).

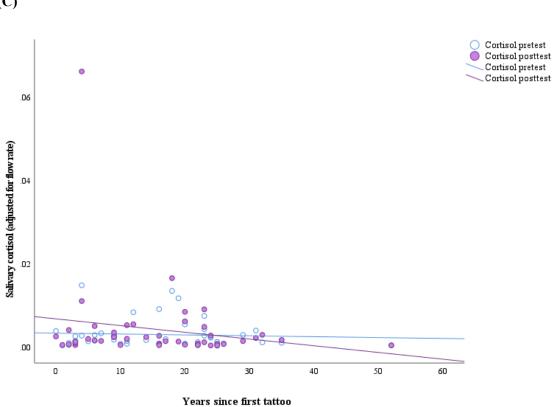
Table 2. Comparison of pre- and posttest cortisol (CORT [$\mu g/dL$]), secretory immunoglobulin A (sIgA [$\mu g/mL$]), and C-reactive protein (CRP [pg/mL]) (adjusted for flow rate) using student's *t*-test

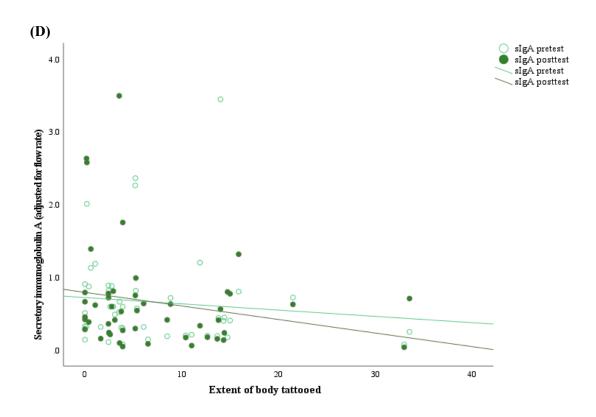
	Pretest		Posttest		
	$Mean \pm SD$	Min—Max	$Mean \pm SD$	Min—Max	P
CORT	$.0031 \pm .0038$.0002—.0148	$.0042 \pm .0097$.0003—.0662	.34
sIgA	$.6569 \pm .6603$.0737—3.4455	$.6554 \pm .6934$.0358—3.4939	.99
CRP	9.9366 ± 27.7058	.0000—141.2633	6.8177 ± 11.9910	.0495—55.8908	.34

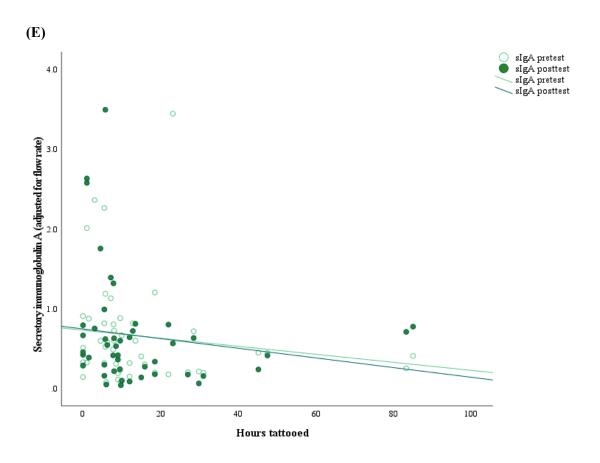
There were no significant correlations between dependent (CORT_{posttest}, sIgA_{posttest}, CRP_{posttest}) and independent variables (years tattooed, extent tattooed, hours tattooed). However, visualizations (Fig 1) reflect changes in CORT, sIgA, and CRP relative to these aspects of tattoo experience.



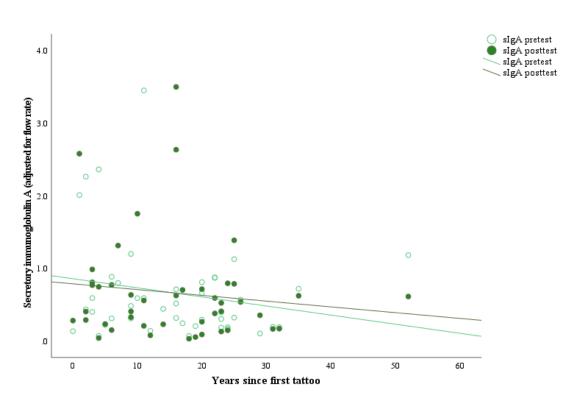


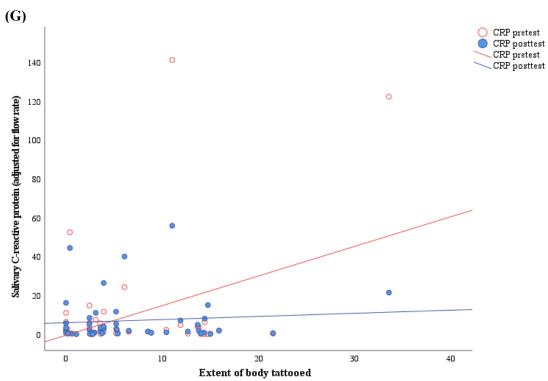


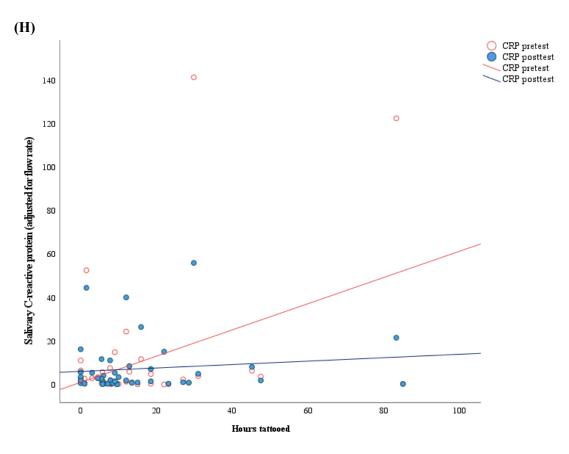












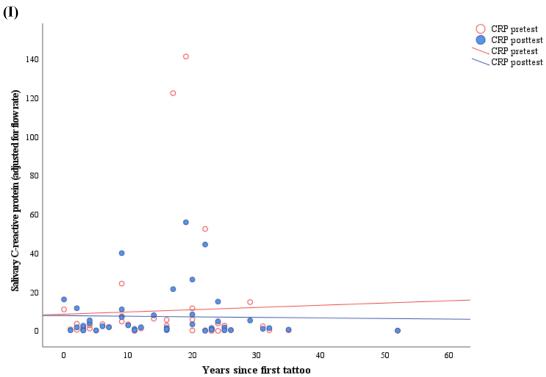


Fig 1. Pre- and posttest cortisol (CORT [μ g/dL]) (A-C), secretory immunoglobulin A (sIgA [μ g/mL]) (D-F), and C-reactive protein (CRP [μ g/mL]) (G-I) in relation to extent of body tattooed (A, D, G), hours tattooed (B, E, H), and years tattooed (C, F, I).

We investigated endocrine, immune, and inflammatory responses using posttest CORT, sIgA, and CRP as dependent variables, respectively, in separate hierarchical ANCOVA models. We included the pretest measure of each biomarker in the respective model, along with the preposttest difference of the other biomarkers to control for possible interactions with each other. We included age, gender, body mass index (BMI), tattoo delivery style (hand-tap or electric), and other covariates in the first block. We conducted bivariate correlations with dependent variables to determine other covariates for inclusion (Table 3). Handgrip strength significantly correlated with CORT_{posttest} (r = .31, p = .04), education with sIgA_{posttest} (r = .36, p = .01), and recent sickness (r = .39, p = .01) with CRP_{posttest}. We measured perceived stress to control for non-tattoo related anxiety, but there were no significant associations between perceived stress and dependent variables. We included tattoo experience variables in second blocks and interaction terms in the third.

Table 3. Bivariate correlations between potential covariates and dependent variables

	$CORT_{posttest}$	$sIgA_{posttest}$	CRP _{posttest}
Ethnicity	155	056	.239
Education	106	358*	020
Civil/marital status	152	.179	.183
Socioeconomic status (1-10)	162	001	062
Perceived stress	.025	.279	.016
Tattoo artist	033	.098	102
Tattooing method (hand-tap or electric)	.028	444**	.007
Handgrip strength	.306*	272	237
Fat percentage	252	.194	.194
Number of supporters	.167	.174	111
Pain rating (1-10)	.171	182	164
Problems with previous tattoos (no/yes)	029	090	.015
Recent sickness (no/yes)	050	027	.378**
Alcohol consumption (past 24 hours)	089	046	.032
Tobacco use (past week)	131	104	.269
Marijuana use (past 24 hours)	051	108	098
Medication use	061	.156	082
Hours worked (past week)	024	107	.024

^{*} p < .05 (2-tailed), ** p < .01 (2-tailed)

All models were significant, but there was no influence of previous tattooing on cortisol levels. However, both sIgA and CRP were significantly predicted by different aspects of tattoo experience, and the effect sizes were large. There was a significant main effect of the extent of the body tattooed on sIgA_{posttest} but no significant interactions. For CRP, there were no main tattoo experience effects, but both interaction variables (extent-by-time and extent-by-time/years) were significant, with notable increases in adjusted r^2 (Table 4).

Table 4. Hierarchical ANCOVA models for posttest secretory cortisol (CORT [μg/dL]), immunoglobulin A (sIgA [μg/mL]), and C-reactive protein (CRP [pg/mL]) by tattoo experience

(- If s))	•	Standardized β	P	Adjusted r ²
CORT				
	Extent	195	.232	
Tattoo experience	Hours	062	.707	.469
	Years	.004	.983	
Extent-by-hours		.168	.388	.465
Extent-by-hours/years		.096	.511	.460
sIgA				
	Extent	463	.004	
Tattoo experience	Hours	.111	.463	.429
	Years	179	.321	
Extent-by-hours		050	.799	.559
Extent-by-hours/years		030	.843	.545
CRP				
	Extent	183	.241	
Tattoo experience	Hours	081	.635	.604
	Years	103	.554	
Extent-by-hours		578	.002	.698
Extent-by-hours/years		516	.001	.724

Discussion

It appears that much tattooing does not meet the threshold load associated with cortisol increase and stress-induced immunosuppression (within the timeframe measured in the present study), which is equivalent to or greater than 60% VO_{2max} in exercise studies (Hill et al., 2008). By contrast, tattoo experience positively predicted posttest measures of both sIgA and CRP, indicating that tattoo experience buffered against stress-induced immunosuppression. Tattooing appears analogous to exercise and, similarly, variation in intensity and duration of tattooing has differential impacts on immune and inflammatory biomarkers.

For instance, in a study of ultra-endurance marathon runners, Tauler et al. (2014) found pre-posttest increases in CORT and CRP and decrease in sIgA. However, differences for marathon runners were significant, whereas differences for tattoo recipients in our study were not. Additionally, we found significant effects on sIgA for tattoo extent but not for other aspects of tattoo experience or for interactions among tattoo extent, hours, and years. For CRP, there were significant interaction effects of tattoo experience but no main effects.

"Moderate doses" of exercise have been associated with increases in post-exercise sIgA and lower rates of colds and other infections, whereas acute bouts of exercise have been associated with reduced sIgA (Klentrou et al., 2002; Trochimiak & Hübner-Wozniak, 2012). When we examined raw data in this and previous tattoo studies (Lynn et al., 2016; Lynn et al., 2019), it appears that sIgA decreases in response to tattooing as well, but when controlling for tattoo experience, it is clear this effect prevails for those with lower experience. We noted increases in CRP and sIgA among participants with higher tattoo experience, as with moderate exercise doses.

We found tattoo experience associated with reductions in CRP, which is consistent with other health activities (Engeland et al., 2019; Morgan et al., 2014; Yan, 2016). Salivary CRP

does not change readily with acute stress, suggesting our findings represent robust effects. Increased levels of CRP are associated with physical infection, childhood trauma, depression, obesity, and distress (Engeland et al., 2019; Yan, 2016). Higher salivary CRP is also associated with degradations in oral health that accompany poor overall health and age (Engeland et al., 2019). However, CRP has good utility when compared to changes from baseline in response to acute stressors (Engeland et al., 2019). Other studies suggest the emotional valence related to the stress may mediate CRP response, as negative emotionality has been found in relation to elevations in CRP, while effortful control of emotions is associated with lower CRP (Engeland et al., 2019).

We expected to see differences in baseline biomarkers relative to previous tattoo experience, but the differences were not statistically significant. The primary observed difference was a greater flexibility in sIgA response. Secretory IgA was influenced by extent and hours of previous tattooing, but CRP was only influenced by a combined effect of previous hours and extent of tattooing. This higher flexibility in sIgA may reflect greater sensitivity necessary for reactive rheostasis, rather than predictive regulation or "programmed rheostasis" (Boulos & Rosenwasser, 2004).

Our results support those from two limited previous studies of tattooing and health biomarkers (Lynn et al., 2016; Lynn et al., 2019) and demonstrate the influences positive psychological appraisal may have on endocrine and immune responses to stressors repeatedly experienced and over time. Future psychoneuroimmunological studies of tattooing can include pretest measures of stress and pain appraisal to compare with posttest ratings and biomarkers of endocrine and immune function. The benefit of positive evaluation of stress, based on our findings, is that immune response to tattoo stress is immediate, suggesting vigilance against bacterial infection of the new wound. Adding a salivary assay for bacteria killing activity (Demas et al., 2011) could test this hypothesis and confirm these health benefits of tattooing.

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Competing interests: Authors declare that they have no competing interests.

Data and materials availability: The materials that support the findings of this study are openly available in The University of Alabama Institutional Repository at [URL TBD].

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