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ORIGINAL ARTICLE

Psychoneuroimmunology and Tattooing

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Abstract

Objective Though it injures the body in many ways, tattooing may also prepare it for later dermal stress through psychoneuroimmunological means.

Methods 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.

Results We found no effect of previous tattoo experience on pre-posttest cortisol but a significant main effect of extent of previous tattoo experience on pre-posttest cortisol and secretory immunoglobulin A and significant extent of body-by-hour tattooed interaction effect on C-reactive protein.

Conclusions These findings suggest that the positive psychological evaluation of tattooing as eustress may contribute to biochemical adaptation through tattooing.

Keywords Psychoneuroimmunology · Tattooing · Endocrine function · Immune function · Allostasis · Salivary biomarkers

In the 1980s, "homeostasis" or the concept of a self-regulating physiological equilibrium was reconceived as "allostasis" or stability through change (Sterling & Eyer, 1988). This change refers to physiological set points that permanently

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

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shift in response to environmental alterations, aging, and other factors. This reconceptualization allowed for the quantification of allostatic "load," which is a measurement of one's prior stress experience (Goldstein, 2004). Allostatic load is often treated as a continual process of accumulation that results in negative outcomes, but this phenomenon is better termed allostatic "overload" (McEwen & Wingfield, 2003). Allostatic *load* can be discharged through the return to equilibrium without accumulating as overload. Thus, allostatic load is as likely to be beneficial or neutral as negative.

Nevertheless, these life experiences in adulthood can have functional changes on stress physiology in ways that represent "ongoing development" (Ganzel et al., 2010, p. 135). Growth and development tend to get lumped together as something that culminates in full adult stature. However, this view limits our understanding of lifelong processes involving physiological changes. For instance, allostatic load accrued through experiences people usually try to avoid, such as the death of a loved one or job loss, can be associated with negative health consequences; by contrast, exercise is a stressor that we engage in on purpose and that we expect to build up our health, even though it doesn't feel good at the time. Nonetheless, both bereavement and working out can influence allostasis. The changing of allostatic set-points in response to environmental changes is called allostatic accommodation and is inherent to lifelong development (Sterling & Eyer, 1988).

Another lens through which we can view the mechanisms of allostatic accommodation is by examining physiological responses to tattooing. Tattoos essentially constitute hundreds to thousands of puncture wounds that leave a foreign substance in the body, are generally painful to receive, and can result in infections or other medical complications. The tattoo industry is the fastest growing in the United States (Ibisworld, 2017), and around 20–30% of people in Europe, Australia, and the United States have at least one tattoo (Harris, 2016; Kluger, 2015). Thus, like exercising, we pay a lot of money to engage in this stressor and generally hope to be more fit as a result (e.g., healthier or more attractive). In fact, some scholars have long indicated that tattoos have health benefits, though most suggest these benefits come through tattooing as means of coping with trauma or of altering identity (Ghosh, 2020; Hambly, 2009; Krutak, 2013; Tuttle & Vale, 1989). No one has explored the link between psychology and physiology that may be critical to what Sterling and Eyer (1988) term "allostatic accommodation."

Tattooing provides a novel avenue for investigating previously unexplored aspects of changes over the lifecourse. Two previous studies of tattooing and biomarkers of stress and immune response indicate that allostatic accommodation is likely taking place when being tattooed (Lynn et al., 2016, 2019). A stress response typically involves the release of glucocorticoids (cortisol in humans) to mobilize for fight-flight-or-freeze. 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 cortisol response in anticipation of the experience. People receiving their first tattoo in those previous studies tended to display elevated cortisol and a lowered immune response (Lynn et al., 2016, 2019). By contrast, those with more tattoo experience likely have less fear of being tattooed and may experience them as less painful or be able to generally ignore the

pain. They had less cortisol elevation and no suppression of the immunoglobulin A antibody; in fact, those with the most tattoo experience generally showed an immediate rise in immune function to address the new wound (Lynn et al., 2016, 2019), as well as higher baseline levels of immune biomarker than people receiving a first tattoo, suggesting the effect persists over time (Lynn et al., 2019). Those findings are considered preliminary because the studies' small samples sizes limited the statistical power of the models and generalizability of the interpretations.

Following research in exercise science, we expanded the investigation of tattooing and biomarkers to consider the rheostatic mechanisms of endocrine and immune functions (cortisol, immunoglobulin A, and C-reactive protein) in relation to tattooing at a large tattoo convention with many artists and attendees. 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, rather than 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). With tattooing, we expect cortisol to increase in relation to pain.

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 salivary C-reactive protein (CRP) relative to previous tattoo experience, we evaluated 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 tattoo festival by one of the hosts, who we had met while conducting a study of tattooing and health in American Samoa the previous year (Lynn et al., 2019). We collected data from 38 artists and 56 clients 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 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. Final analysis of clients included data from 15 women and 33 men aged 18–60. 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 ($r = 0.40$, $p = 0.01$), supporting the use of CRP as a rough proxy of health (cf. Pay & Shaw, 2019 for issues with using salivary CRP as indicator of systemic 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, heavily tattooed people had difficulty counting their tattoos by number because many are integrated into larger pieces and could not recall how many sessions were involved for larger or older pieces. Therefore, we used only years since first tattoo, extent of body tattooed, and hours tattooed in tattoo experience calculations and analysis for the current study.

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 (pretest) 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 saliva sample (posttest), 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). We

selected this one-hour time increment for the second sample because we could not monitor each tattoo process individually and needed a time increment that would be sufficient to detect change but include anyone whose tattoo took longer to complete than one hour. Saliva samples were refrigerated overnight and shipped immediately after the festival to 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 min 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 < 0.007 µg/dL, 2.5 µg/mL, and 9.72 pg/mL, respectively. Correlation coefficients for each standard curve were better than 0.999. Intra-assay 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. Biomarker levels were standardized using Z-scores for statistical analysis to account for skewness typical of biological analytes.

Statistical Analysis

Statistical analysis and plotting of data were performed using SPSS Version 27 (IBM Corp., Armonk, NY) and statistical significance set as $p < 0.05$. We calculated means, standard deviations (SD), and range (min—max) or percent of sample for all 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, along with gender, age, and BMI. Other covariates were selected if they significantly correlated with the dependent variable (Table 2). Possible covariates included pretest and change (Δ) measures of other biomarkers, ethnicity, education, civil status, socioeconomic status, perceived stress, tattoo artist, tattoo delivery style (hand-tap or electric), handgrip strength, fat percentage, number of supporters, pain rating, medical complications from previous tattooing, recent sickness, alcohol use (past 24 h), tobacco use (cigarettes, vaping, or loose leaf; past week), marijuana use (past 24 h), medication use, and hours worked (past week).

Interactions among tattoo experience variables were explored. 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

Table 1 Sample characteristics

	Percent	Mean ± SD	Min—Max
Self-identified ethnicity			
Pacific Islander	46		
White	27		
Asian	15		
Black	6		
Native American	4		
Highest education completed			
Graduate	6		
Undergraduate	33		
Some college	29		
High school	29		
Some high school	2		
Civil status			
Married or engaged	44		
Committed relationship	19		
Casual relationship	2		
Single	31		
Other	2		
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		2.23 ± 2.070	0—7
Handgrip strength		41.33 ± 11.234	20—66
Tattoo experience			
Years since first tattoo		15.76 ± 10.973	0—52
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

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

	Pretest		Posttest		
	Mean ± SD	Min—Max	Mean ± SD	Min—Max	*P*
CORT	0.0031 ± 0.0038	0.0002—0.0148	0.0042 ± 0.0097	0.0003—0.0662	0.34
sIgA	0.6569 ± 0.6603	0.0737—3.4455	0.6554 ± 0.6934	0.0358—3.4939	0.99
CRP	9.9366 ± 27.7058	0.0000—141.2633	6.8177 ± 11.9910	0.0495—55.8908	0.34

variables were entered in the third block. We reexamined regression models using residual vs. leverage plots, removed influential variables, and reran models to determine the influence of such variables.

Results

Sample descriptives are outlined in Table 1.

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).

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 some biomarkers changes relative to these aspects of tattoo experience. Cortisol response appears relatively unchanged as a result of tattooing, though a slighter lower initial response and decrease between measures appears to take place among those with more of all three types of tattoo experience (A-C). By contrast, sIgA appears to increase between measures but only regarding extent of body (D) and hours tattooed (E) but not relative to years since first tattoo (F). CRP seems to remain unchanged from pre-posttest, with the elevation of the pretest slope (G and I) driven largely by a single outlier.

We investigated endocrine, immune, and inflammatory responses using posttest CORT, sIgA, and CRP as dependent variables, respectively, in separate hierarchical ANCOVA models. All variables were standardized. We included the pretest measure of each biomarker in the respective model. We included age, gender, body mass index (BMI), 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 $CORT_{posttest}$ ($r = 0.31$, $p = 0.04$), education with $sIgA_{posttest}$ ($r = -0.36$, $p = 0.01$), and recent sickness ($r = 0.39$, $p = 0.01$) 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. Interaction terms were created as cross-products of unstandardized variables and then standardized.

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 there was a significant extent-by-time interaction effect with a notable increase in adjusted r^2 (Table 4).

Because of the small sample size relative to the number of variables examined, we explored the distributions of the dependent variables in the regression models using Cook's Distance and Centered Leverage. We found one highly influential data point in each model, removed that data point, and reran the regression models. After removing the influential data point in the CORT model, hours tattooed became a significant negative predictor ($\beta = -0.15$, $p = 0.01$) and extent of body

Fig. 1 Pretest (Solid Circle and Line) and Posttest (Open Circle and Dashed Line) Cortisol (CORT [μg/dL]) (**A**-**C**), Secretory Immunoglobulin A (sIgA [μg/mL]) (**D**-**F**), and C-Reactive Protein (CRP [pg/mL]) (**G**-**I**) in Relation to Extent of Body Tattooed (**A**, **D**, **G**), Hours Tattooed (**B**, **E**, **H**), and Years Tattooed (**C**, **F**, **I**). Note. Variables in figure are untransformed ▶

tattooed approached significance ($\beta = -0.47$, $p = 0.06$). There were no significant changes to the sIgA or CRP models upon removal of the respective influential data point for each.

Discussion

Through this study, we explored changes in stress (salivary cortisol), immune function (secretory immunoglobulin A), and inflammation (salivary CRP) among people receiving new tattoos. We expected to see increases in cortisol related to the pain and stress of tattooing, reduction in sIgA among those with less tattoo experience, and elevation of sIgA among those with more tattoo experience. We were unsure what to expect for CRP, as salivary CRP is strongly influenced by oral health (Pay & Shaw, 2019), which we did not screen.

There were no significant changes or relationships for cortisol until an influential data point was removed, whereupon we found that hours tattooed to be a significant negative predictor and hours tattooed negatively associated and approaching significance in predicting cortisol response to a new tattoo. The expected immunosuppression was detected in sIgA but only regarding extent of the body tattooed and not hours tattooed or years since the first tattoo. This may be because extent of body covered is a more accurate measure of tattoo experience than hours tattooed or years since first tattoo. Since artists vary in their tattooing speed for a variety of reasons and because retrospective estimates of time are likely very imprecise, hours spent being tattooed may be a relatively spurious variable. For CRP, there was a significant interaction effect, suggesting an increase in inflammation among those with higher tattoo experience.

In our study, cortisol production decreased slightly during tattooing for those with more tattoo experience, whereas there was no change for other participants in contrast to our prediction. It may be that tattooing for one hour does not meet the threshold load associated with stress-induced immunosuppression, as suggested by research in exercise science (Hill et al., 2008), though cortisol typically responds within 15–30 min of stress. The limited effect tattooing appears to have on cortisol may be due to the reduced fear of being tattooed among those with previous tattoo experience, especially those attending a tattoo festival. People seeking out a tattoo in festival spaces have no expectation of the more controlled experience (e.g., privacy, minimal onlookers, sound) of a tattoo shop. It is possible that people who choose to receive their tattoo in a festival environment seek this more exposed experience with the greater number of potential stressors it entails. Alternatively, it could mean that tattooing hurts less for those people with more tattoo experience, which is reported anecdotally by some but was not supported by the

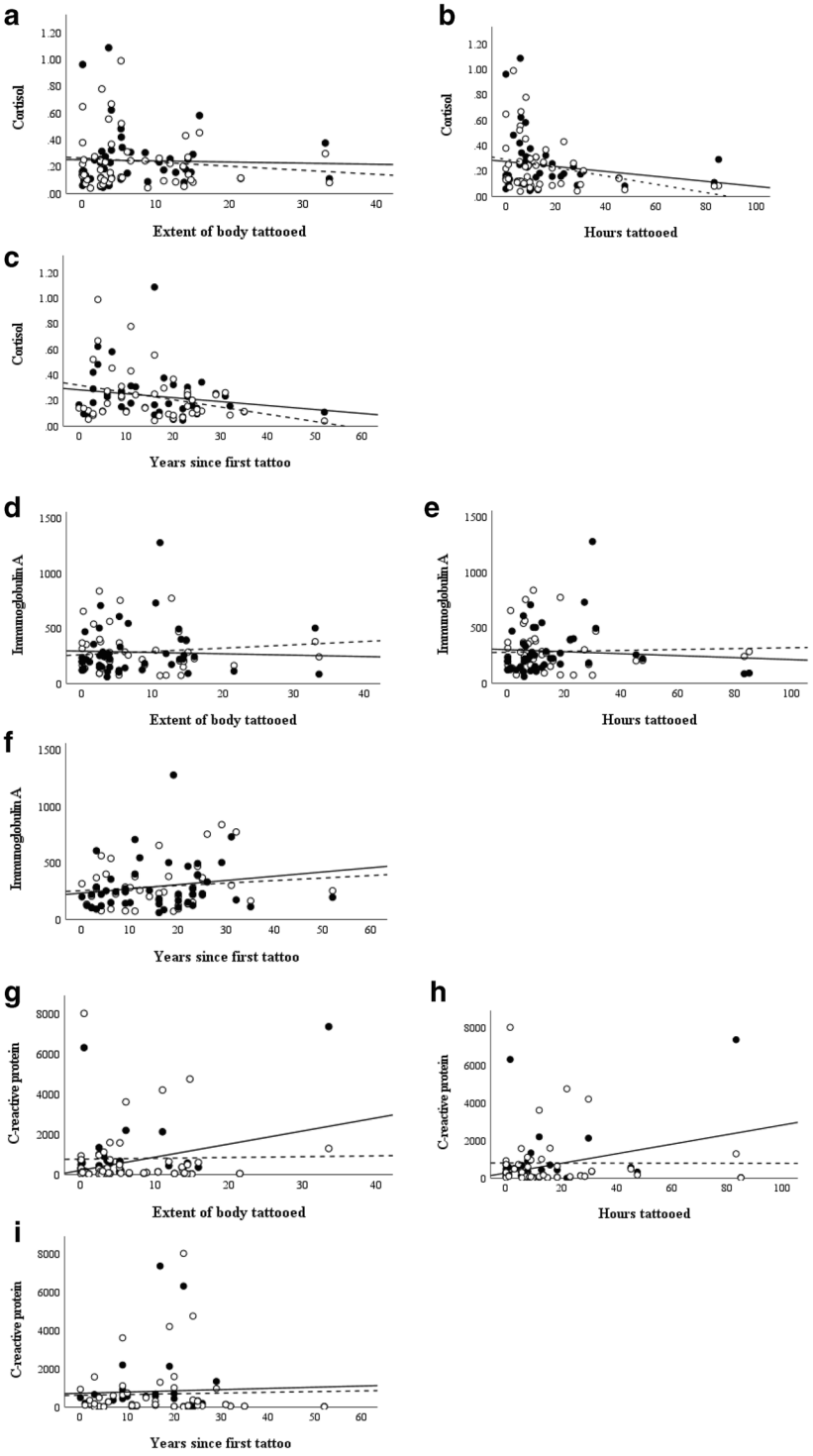
a
Cortisol
Extent of body tattooed
b
Cortisol
Hours tattooed
c
Cortisol
Years since first tattoo
d
Immunoglobulin A
Extent of body tattooed
e
Immunoglobulin A
Hours tattooed
f
Immunoglobulin A
Years since first tattoo
g
C-reactive protein
Extent of body tattooed
h
C-reactive protein
Hours tattooed
i
C-reactive protein
Years since first tattoo

Table 3 Bivariate correlations between potential covariates and dependent variables

	$CORT_{posttest}$	$sIgA_{posttest}$	$CRP_{posttest}$
$CORT_{pretest}$	-	-0.135	0.158
$CORT_{\Delta}$	-	0.222	-0.032
$sIgA_{pretest}$	-0.140	-	-0.103
$sIgA_{\Delta}$	-0.075	-	-0.075
$CRP_{pretest}$	-0.071	-0.149	-
CRP_{Δ}	-0.080	-0.133	-
Ethnicity	-0.155	-0.056	0.239
Education	-0.106	-0.358*	-0.020
Civil/marital status	-0.152	0.179	0.183
Socioeconomic status (1–10)	-0.162	-0.001	-0.062
Perceived stress	0.025	0.279	0.016
Tattoo artist	-0.033	0.098	-0.102
Tattooing style (hand-tap or electric)	0.028	-0.444**	0.007
Handgrip strength	0.306*	-0.272	-0.237
Fat percentage	-0.252	0.194	0.194
Number of supporters	0.167	0.174	-0.111
Pain rating (1–10)	0.171	-0.182	-0.164
Problems with previous tattoos (no/yes)	-0.029	-0.090	0.015
Recent sickness (no/yes)	-0.050	-0.027	0.378**
Alcohol consumption (past 24 h)	-0.089	-0.046	0.032
Tobacco use (past week)	-0.131	-0.104	0.269
Marijuana use (past 24 h)	-0.051	-0.108	-0.098
Medication use	-0.061	0.156	-0.082
Hours worked (past week)	-0.024	-0.107	0.024

* $p < 0.05$ (2-tailed), ** $p < 0.01$ (2-tailed)

pain ratings we collected after each tattoo. Or it could mean that people with more tattoo experience can psychologically compartmentalize the pain better than novices. We observed many people chatting with family or friends who had accompanied them or the tattoo artists, playing with their phones, or listening to earbuds while being tattooed, which may distract them from pain. We have observed tattoo novices faint at the beginning and others at the end of their tattoo because of the combination of psychological tension and physical pain, whereas those same people report no such experiences for subsequent tattoos.

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

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

		Standardized β	P	Adjusted r^2
CORT[a]				
Tattoo experience	Extent	-0.195	0.232	
	Hours	-0.062	0.707	0.469
	Years	0.004	0.983	
Extent-by-hours		0.168	0.388	0.465
sIgA[b]				
Tattoo experience	Extent	-0.463	0.004	
	Hours	0.111	0.463	0.559
	Years	-0.179	0.321	
Extent-by-hours		-0.050	0.799	0.545
CRP[c]				
Tattoo experience	Extent	-0.183	0.241	
	Hours	-0.081	0.635	0.604
	Years	-0.103	0.554	
Extent-by-hours		-0.578	0.002	0.698

[a] Model covariates include age, gender, BMI, $CORT_{pretest}$, handgrip strength

[b] Model covariates include age, gender, BMI, $sIgA_{pretest}$, tattoo delivery style

[c] Model covariates include age, gender, BMI, $CRP_{pretest}$, recent sickness

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 between tattoo extent and hours. 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 examine raw data in this and previous tattoo studies (Lynn et al., 2016, 2019), it appears that sIgA decreases in response to tattooing as well, but when controlling for tattoo experience, it is clear this effect is driven by those with lower experience. We noted increases in CRP and sIgA among participants with higher tattoo experience, as with moderate exercise doses.

The utility of salivary CRP as a biomarker of inflammatory response to tattooing remains unclear. We found a significant interaction effect of extent of body and total hours tattooed associated with reduced CRP but no main effects, which indicates a crossover interaction. The reliability of salivary CRP as a proxy of inflammation

has been questioned (Pay & Shaw, 2019; Slavish et al., 2015), which makes CRP difficult to interpret across studies. Analyses of serum and salivary CRP in relation to exercise have indicated contradictory results, with reductions in CRP detected in aerobic but not resistance exercise paradigms (Donges et al., 2010). In this sense, the lack of change between CRP measures in response to tattooing is similar to those found for cycling, weightlifting, and swimming, whereas serum CRP reductions were observed in relation to aerobic dancing and jogging (Kasapis & Thompson, 2005). CRP production is stimulated by interleukin-6, but studies of inflammatory response to acute exercise find that interleukin-6 and CRP can have differing responses to the same stressor because of the interrelationships among endocrine and immune homeostats (Kasapis & Thompson, 2005; Slavish et al., 2015).

Finally, we expected to see differences in baseline biomarkers relative to previous tattoo experience as observed in another study (Lynn et al., 2019), but there were no pretest differences based on tattoo experience. Secretory IgA response to getting a new tattoo was influenced by extent 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).

Limitations

Our study is statistically underpowered for the number of variables collected. Future research could aggregate previous and current data to reassess relationships among tattoo variables and biomarkers, except for CRP, which was not examined in the 2016 study. CRP was included to assess baseline inflammation, but it may be more a reflection of oral health than circulating inflammatory markers (Pay & Shaw, 2019). One means to address this in future research would be to include assays of interleukin-6 and interleukin-1, which induce and amplify CRP gene expression, respectively (Weinhold et al., 1997).

This study was also short-sighted in the initial design because we conceived it as an addendum to research conducted in American Samoa to increase the sample size. However, the American Samoa data, though limited in quantity, elicited results comparable to the first study and has already been published as validation of those findings (Lynn et al., 2019). An a priori research design focused on psychological appraisal and physiological response should include more questions specifically about psychological states of mind before, during, and after the tattooing. Another limitation may have been the one-hour time increment before the second sample; since previous studies indicated changes in cortisol and immunoglobulin A from the beginning to the end of the tattoo, one hour may not have been sufficient time for detectable changes to occur (though this would be unusual for cortisol). However, the first study included samples from participants whose tattoos took less than one hour to complete—measurements that were even closer together in time (Lynn et al., 2016).

Conclusion

Our results partially support two previous studies of tattooing and health biomarkers that explored tattooing from an evolutionary perspective (Lynn et al., 2016, 2019). Of note is the clear influence previous tattoo experience has on endocrine, immune, and inflammatory biomarkers. It appears justified to view the allostatic accommodations made with respect to tattooing as rheostatic in nature, rather than as simply on or off. How these mechanisms interact in vivo is still unclear but can be addressed through a larger sample and the ability to assay more of the relevant interacting biomarkers.

Our data suggest but do not yet fully support that positive psychological appraisal may have adaptive influences on endocrine and immune responses to stressors repeatedly experienced 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. Future research should examine attitudes before tattooing begins, as well as sickness symptoms after tattoos, in combination with biomarkers, to clarify the dynamic psychophysiological benefits we may receive from this extraordinarily popular form of body modification.

Author Contributions Conceptualization: CDL, MEH, MPM.
Methodology: CDL, MEH.
Investigation: CDL, MEH, GEC, HW.
Visualization: CDL.
Funding acquisition: CDL, MEH.
Project administration: CDL.
Supervision: CDL, MEH, MPM.
Laboratory analyses: TJN, JG.
Writing – original draft: CDL.
Writing – review & editing: CDL, MEH, MPM, GEC, HW, JG.
Other:
Muiz Awan, Liana Donsbach, Rebecca Modisette, and Vy Nguyen assisted with laboratory assays. Teresa Gladen provided onsite support.
Blue Chen-Fruean and Whitey Chen invited our team and provided on-site accommodations to collect data at the tattoo festival.
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Declarations

Competing Interests Authors declare that they have no competing interests.

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