Published in final edited form as:

J Oral Maxillofac Surg. 2012 October ; 70(10): 2256–2263. doi:10.1016/j.joms.2012.03.005.

UTILITY OF A SALIVARY BIOSENSOR FOR OBJECTIVE ASSESSMENT OF SURGERY-RELATED STRESS

Theodore F. Robles^B, Rassilee Sharma^A, Kwan-Soo Park^{A,C}, Lauren Harrell^A, Masaki Yamaguchi^D, and Vivek Shetty^A

^aOral & Maxillofacial Surgery, School of Dentistry, University of California, Los Angeles, 10833 Le Conte Avenue, Los Angeles, 90095-1668, USA

^bDepartment of Psychology, University of California, Los Angeles, Los Angeles, 90095-1563, USA

^cDepartment of Dentistry/Oral and Maxillofacial Surgery, Inje University Sanggye-Paik Hospital Sanggye 7 dong, Nowon-gu, Seoul, 139-707, Republic of Korea

^dGraduate School of Engineering, Iwate University, 4-3-5 Ueda, Morioka, 020-8551, Japan

Abstract

PURPOSE—To evaluate the clinical utility of a salivary alpha-amylase (sAA) biosensor for assessing oral surgery-related stress responses and the differential effect of the personality trait of pain catastrophizing.

PATIENTS AND METHODS—We conducted a prospective, cohort study of 76 healthy subjects who underwent elective removal of their third molars. Along with subjects' self-reports of anxiety and pain, biosensor-facilitated measurements of sAA levels were obtained at multiple time points during the preoperative consult, surgery, and post-surgical follow-up visits. Additionally, subjects completed the Pain Catastrophizing Scale at baseline. Mixed-effect regression models examined change in sAA and self-report ratings within and across visits, and the contribution of pain catastrophizing.

RESULTS—sAA levels were lower during surgery and post-surgical follow-up compared to the consult visit ($p < .01$). sAA decreased during the surgery visit ($p < .05$), and did not change during the consult or follow-up visits. Individuals who reported greater helplessness responses to pain manifested higher sAA levels during the surgery visit ($p < .05$). Self-reported anxiety ratings were highest during the surgery visit, and pain ratings were highest during the follow-up visit.

CONCLUSION—sAA did not show predicted elevations during the surgery visit compared to the consult and post-surgical follow-up visits, or increases during the surgery visit. However, individuals who reported responding to pain with helplessness had larger sAA levels in

Corresponding Author: Professor Vivek Shetty, Oral & Maxillofacial Surgery, University of California, Los Angeles, 10833 Le Conte Avenue, Los Angeles, CA 90095-1668. USA. vshetty@ucla.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

anticipation of surgery, providing proof of concept for the value of point-of-care assessments of surgery-induced stresses and the differential effect of personality traits.

Keywords

Biosensor; Salivary Alpha Amylase; Surgical Stress

INTRODUCTION

Preoperative stress and anxiety are commonly experienced by individuals scheduled for removal of their impacted third molars; one of the most frequent surgical procedures performed in the United States (1). Moderate levels of anxiety can help prepare patients for the distress of surgery; however, low levels of anxiety can leave the patient unprepared for postoperative pain and discomfort, whereas higher anxiety levels might sensitize the patient to noxious stimuli (2; 3). Importantly, individuals vary in their psychological and physiological responses to stress, in part due to underlying personality characteristics. In particular, *pain catastrophizing*, defined as the degree to which individuals magnify the pain experience and have difficulties coping effectively with pain, predicts increased pain ratings in the context of dental procedures (4; 5). Because the patient's orientation towards the painful stimuli and stressful experiences can significantly influence postoperative pain and discomfort, the ability to systematically recognize and assess psychological distress would allow the surgeon a more proactive and differentiated approach to pain management and improving the surgical experience.

The assessment of stress and anxiety is challenging because patients differ across personality characteristics, gender, culture, and prior surgical experience, which can modify the ways in which patients verbally express or report distress. As an alternative to subjective self-reports, stress researchers have increasingly focused on alternative methods that rely on the quantifying the biological changes associated with the stress response (6). One approach is measuring peripheral biomarkers of the neural response to stressful events. However, conventional methods for assessing the biological and physiological responses to stressful events rely on labor-intensive or expensive techniques, including psychophysiology recordings and catecholamine assays from blood samples. Blood is the most commonly used biofluid used of biomarker assessments; however, the discomfort and pain associated with procuring a blood sample distorts the stress biomarker profile (7). In contrast, saliva can be sampled noninvasively and easily and, as such, is an attractive alternate source of peripherally expressed stress biomarkers (e.g. cortisol, α -amylase, and chromogranin-A).

Of the various putative stress markers manifesting in saliva, salivary α -amylase (sAA) has emerged as a potential surrogate marker of autonomic nervous system activity, with some data suggesting sAA may be a specific marker of sympathetic nervous system activity generated by stress and anxiety (8–10). Furthermore, increases in sAA levels can be elicited by SNS activating drugs (yohimbine) (11) and diminished by beta-blockade (propanolol) (12). Capitalizing on the biometric potential of sAA, we developed a portable sAA biosensor to allow quick and easy point-of-care assessment of sAA levels (13). The salivary biosensor, comprising of a miniaturized handheld analyzer and disposable saliva collector strips, does

not need any elaborate preanalytical steps and provides rapid (< 1 minute) laboratory-quality measurements of sAA activity (14). Such a device that permits an objective, as well as rapid, point-of-care assessment of stress indicators could be valuable and relevant for management of perioperative stress and the shaping of the surgical experience.

In a previous study, we explored the utility of the sAA biosensor for evaluating stress experiences in dental students by contrasting academic exam stress with everyday activities. We found that greater biosensor-measured sAA was related to higher ratings of concurrent self-reported distress (15). The primary aim of the current study was to evaluate the clinical utility of the sAA biosensor for assessing the naturalistic stressful experiences surrounding third molar extractions. We hypothesized that biosensor-measured sAA levels would be elevated during the surgical visit compared to the consult and follow-up visits. In addition, we hypothesized that individuals with greater pain catastrophizing would show greater sAA levels at all visits, and during the surgery visit in particular. Additionally, we explored the relationship between sAA levels with self-reported anxiety and distress.

METHODS

Participants

Using a protocol approved by the UCLA Institutional Review Board, the study enrolled 76 healthy participants between the ages 18–40 who presented to the UCLA Oral and Maxillofacial Surgery clinic for surgical extraction of one or more impacted third molars. Potential subjects were presented with the opportunity of joining in the study at the time of clinic registration and if interested, they were screened and consented by a study personnel not affiliated with the participant's care. Eligibility criteria included absence of any acute illnesses or chronic health conditions that could impact endocrine system function, had never been diagnosed with a psychiatric disorder, and did not use psychotropic medications, steroids or illicit substances. All procedures were performed on an ambulatory basis, and patients were asked to refrain from eating and drinking for eight hours before the procedure.

Procedures

All study participants underwent three assessments conducted over three separate visits: preoperative consult, surgery, and post-surgical follow-up. During each visit, participants completed multiple psychological assessments, and sAA collection using the biosensor described below.

Preoperative consult—All eligible and consented participants completed brief questionnaires prior to actual consultation with the oral-maxillofacial surgeon, who was not involved in any data collection. Study personnel demonstrated how the biosensor measurement was performed, and provided instructions on how to self-report subjective ratings of anxiety, pain, and calmness. Four sAA readings and subjective ratings were obtained: 1) immediately after participants received a summary of the procedures, 2) when the participant arrived in the consult room, 3) after the surgeon completed clinical exam and made the treatment recommendation, and 4) following 5 minutes of resting after the surgical consultation was complete.

Surgery—During the surgical visit, four sAA readings and subjective ratings were obtained: 1) shortly after participants arrived at the clinic, 2) after research personnel reviewed the study procedures, 3) after arrival in the surgical suite for the surgical procedure and, 4) before local or general anesthetic was applied. No samples or measures were obtained from participants during or after application of anesthetic, or during or after the surgical procedure. Surgical techniques varied depending on the surgeon and complexity of impaction. Post-operative medications included a narcotic analgesic and an antibiotic.

Post-surgery follow-up—At a follow-up visit conducted one week following surgery, study personnel repeated the sAA readings and subjective ratings at time points similar to the preoperative consult visit. Data were not collected on post-operative complications such as dry socket.

Measures

Pain catastrophizing—All participants completed the Pain Catastrophizing Scale (PCS; 16) during the initial preoperative consult. The 13-item PCS measures the degree to which individuals cope with painful experiences with cognitive activities that exacerbate the fearful aspects of their pain experience: rumination (excessive focus on the pain sensation), magnification (exaggeration of threat), and helplessness (feeling helpless to control the pain symptoms). Respondents report the degree to which they experience various thoughts and feelings with during painful experiences on a five-point Likert scale with scoring possibilities ranging from ‘not at all’ (0) to ‘always’ (4). The total score ranges from 0 to 52, and high scores indicate that more catastrophic thoughts or feelings are experienced. The PCS has been successfully used in a variety of experimental and clinical settings, and is one of the most widely used measures of this construct. It has excellent psychometric properties and is considered a reliable and valid measure (e.g., $\alpha = .87$) (16).

Salivary alpha-amylase (sAA)—Direct measurement of sAA was accomplished with a colorimetric salivary biosensor developed and validated previously (13; 14). The sAA biosensor comprises of disposable saliva collector strips and a hand-held reader with integrated analytic and reporting capabilities. The saliva collector strip is placed under the tongue, allowed to saturate with saliva for 15 seconds and then removed and inserted into the hand-held reader. Algorithms, inputted into the biosensor’s central processing unit normalize variations in ambient temperature and salivary pH and the measured sAA levels are shown on the digital display of the reader (14). To minimize variations, participants were instructed not to chew or otherwise manipulate their tongue or mouth shortly before and during the saliva sampling.

Subjective self-reports—During each visit, participants completed multiple single-item, self-reports of anxiety, pain, and calm during each saliva sample. Participants rated each item (“How anxious do you feel right now?”, “How much pain do you feel right now?”, and “How calm do you feel right now?”) on a scale from 1 (not at all) to 8 (extreme).

Statistical analyses

Unless otherwise specified, statistical tests involving sAA used $\log(x)$ transformed data, where x was the sAA level obtained from the biosensor. Data analyses were performed using SAS version 9.2 (SAS Institute, Cary, N.C.). Using mixed effects models with a random intercept and a first-order auto-regressive covariance structure for repeated measures, sAA levels were examined for any changes within and between the three visits. The changes in self-reported pain, anxiety, and calmness ratings, collected four times within each visit, were also investigated using mixed effects models with the same structure. The t-statistics reported are for the fixed effects parameters within these mixed effects models. Mixed models were built using the $\log(x)$ transformed sAA readings as the outcome, with PCS, self-reported anxiety, pain, and calmness, and time as covariates. Other mixed models examined to the relationship between PCS subscales and changes in self-reported anxiety, pain, and calm over time.

RESULTS

Participant characteristics

Most of the 76 subjects enrolled were young, with a mean age of 24.6 years, ± 5.2 (SD), and there were more women participants (64.5%) than men (35.5%). The majority of subjects (64.8%) had three or more their third molars removed surgically at the same appointment. The total pain catastrophizing scale scores obtained at the baseline visit ranged from 0 to 32, with a mean score of 14.39 ± 9.8 (SD), with scores on the three subscales of the PCS as follows: Rumination mean = 6.1, SD = 4.4, range 0 – 16; Magnification mean = 3.1, SD = 2.2, range 0 – 8; Helplessness mean = 5.2, SD = 4.6, range 0 – 21.

Subjective ratings of stress and pain

Figure 1 summarizes the changes in the participant self-ratings for pain, anxiety and calmness across the three visits. Focusing on changes across visits, pain ratings did not differ between the preoperative consult and surgery visits (note, the pain assessments were completed prior to the surgical procedure), but were significantly higher during the follow-up visit ($t = 5.78$, $p < .0001$). Compared to the consult visit, anxiety ratings were higher during the surgery visit ($t = 10.73$, $p < .0001$) and lower during the follow-up visit ($t = -3.47$, $p = .0005$), while calmness ratings were significantly lower during the surgery visit ($t = -8.17$, $p = .0001$) and higher during the follow-up visit ($t = 3.32$, $p = .0009$).

We then examined relationships between PCS scores and subjective ratings during the various visits (Table 1). The PCS total score and three subscale scores were not significantly related to pain during the visits. The PCS total score, rumination, and helpless subscales were related to higher anxiety ratings during the consult and surgery visit, and lower calmness ratings during the consult visit. Higher magnification scores were also related to lower calmness ratings during the consult visit. In the surgery visit, the PCS total score and helplessness subscale were both associated with lower calmness ratings.

Temporal changes in salivary alpha-amylase levels

Boxplots showing average sAA levels at each visit are shown in Figure 2. Our mixed models showed that mean sAA levels during the surgery visit were an average of $-0.12 \log(1 + \text{U/ml})$ lower compared to the consult visit ($t = -2.58, p = .01$) and mean sAA levels during the follow-up visit were an average of $-0.16 \log(1 + \text{U/ml})$ units lower compared to the consult visit ($t = -3.28, p < .001$). We also examined change in sAA levels within each visit. The levels of sAA did not significantly change during the consult or follow-up visits. During the surgical visit, the sAA levels did not change significantly from arrival at the clinic to the informed consent process, but showed a significant decrease once participants arrived in the surgical suite and just prior to application of anesthetic ($\gamma = -0.095$, standard error = 0.04, $t = -2.23, p = .03$).

Relationships between PCS and sAA levels

We first examined whether the different facets of pain catastrophizing predicted sAA measurements within each visit. The magnification and rumination subscales did not predict sAA levels (data not shown), and the total score showed a trend for greater catastrophizing related to higher sAA levels during the surgery visit ($\gamma = 0.008, t = 1.77, p = .08$). The helplessness subscale emerged as a significant predictor of sAA. As shown in Table 2, greater helplessness ratings predicted elevated sAA levels during the surgery visit. Figure 3 shows that a 1 point increase in helplessness ratings was related to a $0.026 \log(\text{U/mL})$ unit greater average sAA during the surgery visit.

Relationships between subjective ratings and sAA levels

Based on previous work suggesting that greater subjective distress is concurrently related to elevated sAA levels (8; 17), we explored the relationship between self-reports of anxiety, calm, and pain ratings with sAA levels. Anxiety and calmness ratings were not related to sAA levels. Greater pain ratings were related to lower sAA levels ($\gamma = -0.04, t = -2.53, p = .01$). When we examined relationships between pain ratings and sAA within each visit, greater pain ratings during the consult visit were related to lower sAA ($\gamma = -0.08, t = -3.36, p = .0008$). Pain ratings during the surgery visit were not related to sAA levels, and greater pain ratings during the follow-up visit were related to greater sAA levels ($\gamma = 0.099, t = 3.33, p = .0009$).

DISCUSSION

Recently developed portable biosensors, that allow rapid, point of use measurement of putative stress biomarkers, have the potential to help clinicians objectively ascertain stress and anxiety levels preoperatively and anticipate pain control issues. To expand our studies of the clinical utility of such stress biosensors in naturalistic settings (14; 15), we evaluated the prototype sAA biosensor in the context of surgical extraction of impacted third molars. Contrary to our initial premise, sAA levels did not show the predicted increase during the surgery visit compared to the consult or follow-up. Subjective ratings of anxiety, pain, and calmness changed in expected directions at each visit, however, sAA levels were actually lower during the surgery visit compared to the initial consult visit, and similar to levels during the follow-up visit. Notably, sAA did not show significant changes during the course

of the consult or follow-up visits, and decreased during the surgery visit prior to administration of anesthetic. Finally, in contrast with our earlier investigations of sAA levels in dental students (15), where we found that sAA levels were related to concurrent self-reported anxiety in the context of academic stress, sAA levels in the context of a surgical procedure, were not related to self-reported anxiety. At the same time, greater pain ratings at the follow-up visit were related to elevated sAA levels.

One distinctive finding was the influence of pain catastrophizing on sAA levels. In particular, individuals who reported that they react with helplessness during pain experiences, such as feeling like pain was unending, overwhelming, and uncontrollable, had larger sAA levels in anticipation of the third molar surgery. The most plausible mechanism connecting pain catastrophizing with sAA responses involves the neural regions that regulate pain-related information processing. Several regions of the brain are critically involved in a circuit that processes the affective, attention, and sensorimotor aspects of pain, including the anterior cingulate and insula. Indeed, during mild acute pain, greater activity in those regions was related to greater self-reported pain catastrophizing scores (18). Many of the same regions are implicated in autonomic nervous system regulation, with the strongest evidence for cardiovascular measures of autonomic activity (19; 20). Thus, elevated activity in the circuit related to magnifying the pain experience may also lead to stimulation of the central autonomic network, leading to enhanced sympathetic activity. To our knowledge, this is the first demonstration that catastrophizing influences physiological responses in the context of a naturalistic, pain-salient setting. Taken together with the results from our primary analyses, our data suggest that sAA responses to a naturalistic stressor may not occur normatively for most individuals. Instead, for certain types of stressors, sAA responses and other sympathetic nervous system responses to surgery may only occur in individuals with particular vulnerabilities, such as pain catastrophizing.

We also found that the mean sAA levels were lower during the surgical visit compared to the consult and follow-up visits, due in part to a decrease in sAA over the course of the surgery visit. One possible explanation for our observed findings is that for many participants, the adrenergic response to the pending surgery may have peaked during or even before participants arrived at the clinic. Simply stated, perhaps the anticipation of the surgery visit and even the effort expended in getting to the clinic (e.g., rushing to be on time) could have led to an increase in autonomic activity, which then decreased over time as the patients arrived at the clinic and were able to acclimate to the setting. Unfortunately, few studies have directly addressed the dynamics of the autonomic responses to naturalistic events; that is, changes in autonomic responses before, during, and after a stressor in the naturalistic environment. Indeed, obtaining an adequate baseline is one of the challenges to studying physiological responses to naturalistic stressors. In contrast, in experimental stressor studies conducted under controlled laboratory conditions, participants are frequently allowed to acclimate to the environment for periods of 20 – 30 minutes in order for neuroendocrine and autonomic activity related to getting to the laboratory to return to baseline. For future work with similar types of stressors (i.e., clinic appointments), we suggest obtaining samples from participants prior to arrival to the clinic.

In addition to a significant decrease in sAA during the surgery visit, sAA did not show significant changes during the consult or follow-up visits. As we described above, one possible explanation is that sAA levels at arrival to the clinic were not true baseline levels, but were actually elevated above the individual's resting baseline and stayed elevated throughout the visit. However, the more likely explanation is that the consult and follow-up visits did not elicit a significant increase in autonomic activity. Indeed, the literature suggests that sympathetic activation during stress is conditioned on the level of mental effort exerted during the stressor. Given that in both the consult and follow-up visit, very little mental or physical effort is expended by patients, and other traditional dimensions of a stressor (e.g., controllability, novelty, social-evaluative threat) are at relatively low levels, it may not be surprising to see no change in sAA during the non-surgical visits. An alternate explanation may be that the sAA itself is not an exclusive read-out of sympathetic activity. Whole mouth sAA can be viewed as the sum of a large number of contributing factors including sympathetic-adrenergic stimulation during stress, as well as parasympathetic activity. In general, sympathetic activation of the parotid gland produces a degranulation of the preformed amylase stored as secretory granules in the acinar cells and the product is secreted by exocytosis into the saliva to raise the salivary amylase concentration. However, a considerable amount of sAA can also be produced through parasympathetic activation through a process that does not involve acinar cell degranulation (21; 22).

Although our approach to examining a stressor in the naturalistic environment provides an ecological valid examination of sAA responses to stress, the approach has a number of limitations. As described above, we did not have an adequate measure of baseline sAA activity prior to patients' arrival at the clinic, such as a measure of sAA at the same time on a different day. Moreover, sympathetic activity may have peaked during the surgery itself, but because the nature of the surgery precluded obtaining saliva samples, we did not have a measure of post-surgical sympathetic activity. While there were restrictions on eating and drinking prior to the surgery, there were not similar restrictions prior to the consult or follow-up visit. Thus, the fact that our results were limited to surgery may have been due to confounding factors that influenced sAA levels during the consult and follow-up visit.

Clinical implications

Point-of-care biosensor systems such as the sAA biosensor may potentially improve patient care by allowing surgeons measure and analyze patterns of stress-related biomarkers. In combination with measures of underlying differences in cognitive and emotional processing of the surgical experience, such as pain catastrophizing, care providers will be able to anticipate pain control issues, thus allowing individualized antianxiety strategies and pain medications. Thus, portable stress measurement devices such as the sAA biosensor that use biological indicators to monitor underlying psychological differences and predict a patient's postoperative response may eventually result in better pain control in surgical patients, and thus improve patient care and satisfaction.

ACKNOWLEDGEMENTS

The project described was supported by Grant Number 1U01-DAO23815 from the NIH/National Institute on Drug Abuse (P.I. - V. Shetty). The authors would like to acknowledge the assistance of Dr. Payam Afzali with the logistical aspects of the study.

REFERENCES

1. Silvestri AR, Singh I. The unresolved problem of the third molar: Would people be better off without it? *The Journal of the American Dental Association*. 2003 Apr; 134(4):450–455. [PubMed: 12733778]
2. Vaughn F, Wichowski H, Bosworth G. Does Preoperative Anxiety Level Predict Postoperative Pain? *AORN*. 2007 Mar; 85(3):589–604.
3. Munafò MR, Stevenson J. Anxiety and surgical recovery: Reinterpreting the literature. *Journal of Psychosomatic Research*. 2001; 51(4):589–596. [PubMed: 11595247]
4. Sullivan MJ, Neish NR. Catastrophizing, anxiety and pain during dental hygiene treatment. *Community Dent Oral Epidemiol*. 1998 Oct; 26(5):344–349. [PubMed: 9792127]
5. Sullivan MJ, Neish N. Catastrophic thinking and the experience of pain during dental procedures. *J Indiana Dent Assoc*. 2000; 79(4):16–19.
6. Piazza JR, Almeida DM, Dmitrieva NO, Klein LC. Frontiers in the Use of Biomarkers of Health in Research on Stress and Aging. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*. 2010 Jul; 65B(5):513–525.
7. Landi MT, Caporaso N. Sample collection, processing and storage. *IARC Sci. Publ*. 1997; (142): 223–236. [PubMed: 9354922]
8. Nater UM, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C, Ehlert U. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology*. 2005 Mar; 55(3):333–342. [PubMed: 15708646]
9. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*. 2009 May; 34(4):486–496. [PubMed: 19249160]
10. Bosch JA, Veerman ECI, de Geus EJ, Proctor GB. [alpha]-Amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! *Psychoneuroendocrinology*. 2011 May; 36(4):449–453. [PubMed: 21295411]
11. Ehlert U, Erni K, Hebisch G, Nater U. Salivary alpha-amylase levels after yohimbine challenge in healthy men. *J. Clin. Endocrinol. Metab*. 2006 Dec; 91(12):5130–5133. [PubMed: 16968802]
12. van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: effect of beta blockade. *Psychoneuroendocrinology*. 2006 Jan; 31(1):137–141. [PubMed: 16046076]
13. Yamaguchi M, Deguchi M, Wakasugi J, Ono S, Takai N, Higashi T, Mizuno Y. Hand-held monitor of sympathetic nervous system using salivary amylase activity and its validation by driver fatigue assessment. *Biosensors and Bioelectronics*. 2006 Jan; 21(7):1007–1014. [PubMed: 15871919]
14. Shetty V, Zigler C, Robles TF, Elashoff D, Yamaguchi M. Developmental validation of a point-of-care, salivary alpha-amylase biosensor. *Psychoneuroendocrinology*. 2010 Feb; 36(2):193–199. [PubMed: 20696529]
15. Robles T, Shetty V, Zigler C, Glover D, Elashoff D, Yamaguchi M. Ambulatory measurement of salivary alpha amylase: Relationships with self-reported and naturalistic psychological stress. *Biol Psychol*. 2011 Jan; 86(1):50–56. [PubMed: 20955759]
16. Sullivan MJL, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and validation. *Psychological Assessment*. 1995; 7(4):524–532.
17. Chatterton RT, Vogelsong KM, Lu YC, Ellman AB, Hudgens GA. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol*. 1996 Jul; 16(4):433–448. [PubMed: 8842578]
18. Seminowicz DA, Davis KD. Cortical responses to pain in healthy individuals depends on pain catastrophizing. *Pain*. 2006 Feb; 120(3):297–306. [PubMed: 16427738]

19. Lane RD, Wager TD. The new field of brain-body medicine: What have we learned and where are we headed? *Neuroimage*. 2009 Sep; 47(3):1135–1140. [PubMed: 19524045]
20. Gianaros PJ, Derbyshire SWG, May JC, Siegle GJ, Gamalo MA, Jennings JR. Anterior cingulate activity correlates with blood pressure during stress. *Psychophysiology*. 2005 Nov; 42(6):627–635. [PubMed: 16364058]
21. Asking B, Proctor GB. Parasympathetic Activation of Amylase Secretion in the Intact and Sympathetically Denervated Rat Parotid Gland. *Experimental Physiology*. 1989 Jan; 74(1):45–52.
22. Garrett JR, Thulin A. Changes in parotid acinar cells accompanying salivary secretion in rats on sympathetic or parasympathetic nerve stimulation. *Cell and Tissue Research*. 1975 Jun.159(2)

Figure 1. Boxplots illustrating self-reported pain, anxiety, and calmness levels during each visit. Self-report ratings are averaged within each visit.

Figure 2.
Boxplots depicting sAA levels during each visit.

Figure 3. Scatterplot indicating the relationship between scores on the PCS helplessness subscale and sAA levels during the surgery visit.

Table 1

Relationships between PCS subscales and subjective ratings at each visit

PCS subscale	Consult			Surgery			Follow-up		
	Pain	Anxiety	Calm	Pain	Anxiety	Calm	Pain	Anxiety	Calm
Total score	-0.02	0.047**	-0.062***	-0.01	0.09***	-0.05*	-0.02	0.02	-0.01
Helplessness	-0.03	0.095**	-0.12***	-0.03	0.19**	-0.13**	-0.06	0.04	-0.04
Magnification	-0.07	0.13	-0.22**	0.04	0.23	-0.14	-0.07	0.05	-0.09
Rumination	-0.05	0.097**	-0.12**	-0.03	0.19**	-0.1	-0.02	0.04	-0.02

* $p < .05$,

**

 $p < .01$,

 $p < .001$

Note. Parameters are unstandardized coefficients showing the relationship between the PCS subscale of interest and the subjective rating of interest at each visit. Coefficients represent the change in subjective ratings associated with a 1 unit change in the PCS subscale of interest. Analyses were conducted separately for each PCS subscale.

Table 2

Parameter estimates from mixed regression models predicting sAA levels from visit and PCS helplessness subscale scores.

Parameter	Estimate	Standard error	<i>t</i>	<i>p</i>
Fixed parameters				
Consult (average sAA level)	3.78	0.10	38.06	<.0001
PCS helplessness × Consult	.004	0.01	0.23	.79
Surgery (difference from consult)	−0.23	0.07	−3.39	.0007
PCS helplessness × Surgery	0.02	0.01	2.22	.03
Follow-up (difference from consult)	−0.17	0.07	−2.31	.02
PCS helplessness × Follow-up	0.002	0.01	0.22	.82
Variance parameters				
Intercept variance	0.25			
AR(1) variance	0.23			
Residual variance	0.21			

Note. Parameters are unstandardized coefficients. The PCS helplessness × visit (Consult, Surgery, Follow-up) interactions indicate the relationship between the PCS helplessness scores and sAA levels averaged across the visit.