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INVITED VIEWPOINT

α -Amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet!

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Summary Recent years have seen a growing interest in salivary α -amylase (sAA) as a non-invasive marker for sympathetic nervous system (SNS) activity. Saliva offers many advantages as a biomarker fluid and sAA is one of its most plentiful components. sAA is a digestive enzyme that breaks down starch, which provides a simple means of quantification by measuring its enzymatic activity. This commentary will address a number of common misconceptions and methodological issues that surround the use of sAA as a marker of SNS activity and limit its utility in biobehavioral research.

The usefulness of sAA as an SNS marker is undermined by the fact that the parasympathetic nerves also play a significant role in sAA release. Local parasympathetic nerves regulate sAA activity via: (1) α -amylase release from glands that are solely or mainly parasympathetically innervated; (2) via synergistic sympathetic–parasympathetic effects on protein secretion (known as 'augmented secretion'); and (3) via effects on salivary flow rate. Regarding methodology, we discuss why it is problematic: (1) to ignore the contribution of salivary flow rate; (2) to use absorbent materials for saliva collection, and; (3) to stimulate saliva secretion by chewing. While these methodological problems can be addressed by using standardized and timed collection of unstimulated saliva, the physiological regulation of sAA secretion presents less resolvable issues. We conclude that at present there is insufficient support for the use and interpretation of sAA activity as a valid and reliable measure of SNS activity.

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Recent years have shown a burgeoning interest in salivary α -amylase (sAA) as a non-invasive marker for sympathetic nervous system (SNS) activity. sAA is a digestive enzyme that breaks down insoluble starch into soluble maltose and dextrin. sAA can be measured quickly and reproducibly with commercially available kits based on substrates that utilize its enzymatic activity. This activity, expressed as units per

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milliliter (U/ml), is often taken as a proxy for sAA concentration although the two only modestly correlate ($r = .60$) (Mandel et al., 2010).

In early studies sAA activity emerged as a measure of parasympathetic activity, whereby sAA levels were found to increase during relaxation (Morse et al., 1983). The mid-nineties, however, saw the first studies showing that sAA activity is also increased during stress and correlates with norepinephrine release during exercise (Bosch et al., 1996; Chatterton et al., 1996). Although stress studies failed to replicate the latter observation (Nater and Rohleder, 2009), sAA activity was quickly adopted as a measure of SNS activity. If true, then collecting saliva would allow simultaneous assessment of the two major stress systems, the HPA-axis and the SNS, and it is therefore difficult to overstate its potential.

At a first glance the supporting data looks compelling. Numerous studies in humans and animals have revealed that sympathetic activation induced by stress, by exercise, by pharmacological means, or via local nerve stimulation, uniformly increases sAA release or its activity in whole mouth saliva (Bosch et al., 2002; Nater and Rohleder, 2009). Moreover, administration of the beta-adrenergic antagonist propranolol reduces amylase activity in unstimulated whole mouth saliva (Nederfors and Dahlöf, 1992) and abrogates stress induced increases in sAA activity (Nater and Rohleder, 2009). The hypothesis also seems consistent with the extensively characterized glandular biology in man and in animal models, whereby the parasympathetic nerves mainly (but not exclusively) control fluid secretion, and the sympathetic nerves mainly (but not exclusively) regulate salivary protein secretion, including the secretion of sAA (Proctor and Carpenter, 2007).

Notwithstanding, we will argue here that there is currently no strong scientific basis for the use of sAA activity as a reliable measure of SNS activity. This commentary will address a number of common misconceptions and methodological issues that, we hope, will clarify the limitations of sAA in biobehavioral research.

1. Is sAA activity determined by sympathetic activity?

Most psychophysicologists are aware that measuring sympathetic activity is a knotty issue: activation of the SNS does not occur in the generalized manner that is sometimes assumed and the various measures of SNS activation do not correlate well (Grassi and Esler, 1999; Folkow, 2000). The latter also applies to sAA: stress studies show that changes in sAA activity do not, or only modestly, correlate with changes in other SNS markers, such as cardiac pre-ejection period, skin conductance, and plasma norepinephrine. Even within the salivary glands the SNS does not act in a concerted fashion: the secreto-motor sympathetic nerve fibers, responsible for the glandular secretion of sAA, are activated independently of the vasoactive sympathetic nerve fibers that regulate vasoconstriction in glandular tissue (Proctor and Carpenter, 2007).

While such issues complicate the interpretation of sAA as a measure of SNS activity, more problematic is the evidence that parasympathetic activity likewise plays an important role in sAA and protein secretion, thereby invalidating the use of sAA as an exclusive read-out of sympathetic activity.

There are three main pathways whereby parasympathetic activity can influence sAA concentrations: (1) via α -amylase release from glands that are solely or mainly parasympathetically innervated, e.g., the palate and sublingual glands; (2) via synergistic sympathetic–parasympathetic interactions whereby parasympathetic activity amplifies sympathetic effects; and (3) via the effects of (parasympathetically-mediated) salivary flow rate. These aspects of salivary gland biology and physiology are further discussed below. Since collection of saliva is the key to reliable and interpretable results (c.f., Rohleder and Nater, 2009), we will discuss also the methodological pitfalls which are frequently encountered in saliva-based psychobiological research.

2. Basic concepts

2.1. Not all salivary glands respond the same

Most sAA literature refers to “saliva” without acknowledgement that this fluid is a complex mixture derived from many different glands and different cell types within glands. In short, saliva is produced by three pairs of major glands; the parotid glands, the submandibular glands and the sublingual glands. In addition there are numerous minor glands in the submucosa underlying the lip, cheeks and palate with a substantial contribution to salivary protein content (Humphrey and Williamson, 2001). Individually these glands differ greatly in the amount of sAA they produce (Veerman et al., 1996), in the autonomic innervations they receive, and the type of transmitter and neuropeptide receptor they express (Proctor and Carpenter, 2007). For example, the parotid and minor palatine glands contain the highest amounts of amylase (Veerman et al., 1996), and the parenchyma of these minor glands is mainly, if not entirely, innervated by parasympathetic nerves (Proctor and Carpenter, 2007). Thus, amylase release is most likely also elicited by parasympathetic stimulation of sAA-rich glands.

The latter point is relevant to psychophysiology: Bosch et al. (2003) showed that a passive-coping stressor that evoked parasympathetic activation (viewing a surgical video), as measured by increases in salivary flow and cardiac vagal tone, also strongly (2.5-fold) increased the release of sulfated-MUC5B; a protein that is almost exclusively secreted by the parasympathetically innervated palatine and sublingual glands. Significantly, this stressor also evoked an sAA release that was much larger than the release during a stressor that elicited a dominant (cardiac-) sympathetic activation in conjunction with a vagal withdrawal and a reduced flow rate (i.e., a time-pace memory task) (Bosch et al., 2003).

2.2. Sympathetic effects on sAA secretion are augmented by concurrent parasympathetic activity

Although heart rate has been successfully used as an index of psychological stress for more than one hundred years, and likewise has been erroneously labelled as a measure of sympathetic activity, it is now clear that heart rate responses reflect a mixture of additive and interactive changes in local parasympathetic and sympathetic activity (Berntson et al.,

2008). This principle also applies to the salivary glands, and in particular to the submandibular and the parotid glands where the two autonomic branches collaboratively evoke salivary secretion of both fluid and protein (there is no autonomic antagonism in the salivary glands) (Proctor and Carpenter, 2007). For example, an extensive experimental literature shows that the sympathetic effects on secretion of sAA, and other salivary proteins, are amplified by concurrent parasympathetic activity (Asking, 1985; Carpenter et al., 1998). This is termed 'augmented secretion' and refers to the fact that sAA secretion during concomitant sympathetic and parasympathetic nerve stimulation is much greater than the sum of the sAA secreted during individual nerve stimulation (Proctor and Carpenter, 2007).

3. Faulty methods

3.1. Ignoring the contribution of salivary flow rate

Probably one of the major causes of confusion in sAA research is that researchers do not consider secretion rate. Indeed, of the sAA studies published in Psychoneuroendocrinology only one attempted to quantify the possible confounding effects of flow rate. This omission is most likely inherited from cortisol research, in which, due to the nature of this analyte, flow rate does not play a significant role. In contrast to cortisol, sAA is synthesized in the acinar cells (i.e., the main secretory cells) of the salivary glands, where it is stored in granules before secretion. Upon neuronal activation, the content of these granules (containing amylase and other proteins) is secreted into saliva: i.e., the amount of amylase that is secreted per unit of time is directly related to the extent of sympathetic activity (Proctor and Carpenter, 2007). Therefore, the amylase output per unit of time, rather than its concentration, would appear the better proxy for neuronal activity. That is, sAA concentration reflects the combined effect of salivary flow rate (which is largely parasympathetic) and protein secretion (which, in sympathetically innervated glands, is largely sympathetic). If sAA is to be regarded as a valid measure of sympathetic activity, then the parasympathetic effect on salivary flow rate is a confounding factor that needs adjustment.

The reciprocity between concentration and flow rate is also evident from stress studies. These show that when a condition has little or no effect on flow rate, then the confounding effects of flow rate are small (Bosch et al., 1996; Rohleder et al., 2006). However, this picture changes when there is a larger effect on flow rate. For example, we compared the effects of different laboratory stress tasks on saliva flow and sAA secretion and found that changes in sAA activity were for 25–40% due to changes in salivary fluid secretion (Bosch et al., 2003).

3.2. Use of cotton rolls for saliva collection

Researchers who use cotton sponges such as the Salivette will have noticed that saliva, a slimy and turbid fluid, comes out of the collection device watery and clear: what goes in evidently does not come out. Indeed, studies have shown that the salivette introduces measurement error, sometimes quite

substantial, to a number of salivary analytes, including sAA (Strazdins et al., 2005; DeCaro, 2008; Harmon et al., 2008; Beltzer et al., 2010). The sponge does not fully release its sAA, and this retention shows a strong inverse relation with the amount of fluid absorbed. Nearly complete sAA retention was observed when the cotton absorbed 0.25 ml of saliva, which approximates the normal amount of unstimulated saliva produced over 1 min (DeCaro, 2008). This implies that the amount of saliva, which is related to flow rate and/or duration of collection, will indirectly influence sAA values. The duration of saliva collection and the stimulus determining flow rate (see discussion below) are rarely standardized. A further limitation is that salivary flow rate is difficult to assess reliably using Salivettes, because the absorbent capacity decreases when more fluid is taken up and because values have a ceiling effect due to saturation of the material (see discussion by Beltzer et al., 2010). While recently newer absorbent materials have been marketed, there is little data to suggest that these will do a much better job with sAA.

Why use those absorbent materials in the first place? They may of course have an application in research where saliva collection is complicated, such as with newborns or during conditions like strenuous exercise. But otherwise it would seem sensible to adhere to what oral biologists have long established as the standard procedures for unstimulated 'whole saliva' collection (Navazesh, 1993). The suggestion that study participants are uneasy with spitting into a tube appears overstated and is contradicted by evidence (Strazdins et al., 2005). Admittedly, the "drooling method" may sound unappealing, but drooling is avoided with the equally reliable "spitting method" (Navazesh, 1993; Navazesh and Kumar, 2008). There is also no strong reason to assert that spitting in a tube is impractical: most laboratory suppliers provide a large variety of tubes and vials likely to fit every research condition. These collection vials come with another convenience: most cost only a fraction of absorbent products.

3.3. Stimulated and unstandardized collection of saliva

Stimulation of mechano-receptors in the mouth during chewing induces local autonomic reflex activity which is well known to enhance glandular secretion independent of central regulation, i.e., independent of the 'higher' neural effects of stress (Garrett, 1987). Indeed, sAA is principally a digestive enzyme and its secretion is heavily influenced by local reflex stimuli in order to deliver large amounts during eating. The fact that the majority of sAA studies use saliva collection methods which involve mechanical stimulation thus complicates interpretation of data: e.g., to what extent does activation of local reflexes modify or over-rule the central SNS effects on sAA release? This issue can be compared to the effects of movement and changing posture on heart rate, which similarly induce autonomic reflexes that will distort the heart rate effects of psychological stress (c.f., Bosch et al., 2009).

Complicating matters even further, in most studies the glandular stimulation is unstandardized. For example, participants are instructed "to gently move the Salivette around in the mouth" or "to chew on the Salivette". If not standardized, how can a researcher be certain that a relaxed

participant will chew with the same vigor as a distressed participant? Or, how will we know whether the instruction to 'gently move the Salivette around' will be acted upon with the same gentleness by female and male participants (or children vs. adults, etc.)? What we *do* know is that the strength of mechanical stimulation corresponds to the amount of saliva produced (Hector and Linden, 1987). This increased flow rate will likely affect sAA concentration since protein concentration is the combined result of protein output and flow rate, as discussed above. We may add that within the first several minutes of chewing-induced secretion there are decreases in sAA output and concentration from individual glands, even with standardization of the stimulus (Proctor and Carpenter, 2001). Regretfully, the duration of saliva collection is typically also not standardized.

A third, and perhaps the most significant concern associated with mechanically stimulating flow rate, is that it drastically changes salivary protein composition, owing to: (1) the different responsiveness of the parotid and submandibular glands to stimulation; and (2) the differing amounts of sAA and other proteins that these glandular salivas contain. Under passive conditions (i.e., without mechanical or gustatory stimulation), most saliva is secreted by the submandibular glands and only about 20% derives from the parotid gland, which happens to be very rich in sAA (Schenkels et al., 1995; Humphrey and Williamson, 2001). However, in response to chewing the contribution of individual glands changes, whereby now about half of all saliva is from the parotid glands. This is highly significant as parotid saliva contains a 4–10-fold higher AA concentration than submandibular saliva (Veerman et al., 1996).

In sum, the commonly used method of unstandardized mechanical stimulation invokes local autonomic reflex activity, shifting the balance from submandibular secretion (modest sAA concentration) to parotid secretion (high flow rate, high α -amylase content) independent of central SNS regulation. Overall therefore, it is extremely difficult to obtain a value from stimulated 'whole mouth' sAA that can confidently be attributed to central effects on local SNS activity.

4. Conclusion

While biopsychology boasts a strong tradition of endocrine, immune, and cardiovascular research, scholars of this field rarely had training in oral biology. It is likely that with such training many would have made different choices with regard to methods and the interpretation of sAA data. Currently most researchers adhere to a methodology that was validated for cortisol research. For example, among the sAA studies published in Psychoneuroendocrinology to date, virtually none controlled for the potentially confounding effects of salivary flow rate, most do not standardize saliva collection in terms of stimulation or collection duration, and the majority used absorbent materials that are known to distort sAA values. We found that a significant number of studies did not provide any detail on how saliva was collected or how participants were instructed (apart from the simple statement that 'Salivettes were used'). Also, many authors confuse the distinctions between sAA activity, sAA concentration and sAA secretion, in one example presenting data in implausible concentration units while citing the use of an

assay that does not exist. We hope to have clarified how such methodology makes the bulk of sAA findings difficult to interpret.

But even with more rigorous methodology one should perhaps anticipate disappointment. Experimental evidence from oral biology research indicates that the idea of sAA activity as a valid and reliable measure of SNS activity is too simplistic. The salivary glands are a sophisticated and heterogeneous group of organs, capable of responding with a high level of specificity to stimuli relevant to digestion, speech, and immune function. Whole mouth sAA can be viewed as the sum of a large number of contributing factors; glandular sympathetic–adrenergic stimulation during stress is only one of such factors.

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Conflict of interest

The authors have no conflicts of interests to declare.

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References

- Asking, B., 1985. Sympathetic stimulation of amylase secretion during a parasympathetic background activity in the rat parotid gland. *Acta Physiol. Scand.* 124 (4), 535–542.
- Beltzer, E.K., Fortunato, C.K., Guaderrama, M.M., Peckins, M.K., Garramone, B.M., Granger, D.A., 2010. Salivary flow and alpha-amylase: collection technique, duration, and oral fluid type. *Physiol. Behav.* 101 (2), 289–296.
- Berntson, G.G., Norman, G.J., Hawkey, L.C., Cacioppo, J.T., 2008. Cardiac autonomic balance versus cardiac regulatory capacity. *Psychophysiology* 45 (4), 643–652.
- Bosch, J.A., Brand, H.S., Ligtenberg, T.J.M., Bermond, B., Hoogstraten, J., Nieuw Amerongen, A.V., 1996. Psychological stress as a determinant of protein levels and salivary-induced aggregation of *Streptococcus gordonii* in human whole saliva. *Psychosom. Med.* 58 (4), 374–382.
- Bosch, J.A., de Geus, E.J., Carroll, D., Goedhart, A.D., Anane, L.A., van Zanten, J.J., Helmerhorst, E.J., Edwards, K.M., 2009. A general enhancement of autonomic and cortisol responses during social evaluative threat. *Psychosom. Med.* 71 (8), 877–885.
- Bosch, J.A., de Geus, E.J.C., Veerman, E.C.I., Hoogstraten, J., Nieuw Amerongen, A.V., 2003. Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. *Psychosom. Med.* 65 (2), 245–258.
- Bosch, J.A., Ring, C., de Geus, E.J., Veerman, E.C., Amerongen, A.V., 2002. Stress and secretory immunity. *Int. Rev. Neurobiol.* 52, 213–253.
- Carpenter, G.H., Garrett, J.R., Hartley, R.H., Proctor, G.B., 1998. The influence of nerves on the secretion of immunoglobulin A into submandibular saliva in rats. *J. Physiol.* 512 (Pt 2), 567–573.
- Chatterton Jr., R.T., Vogelsson, K.M., Lu, Y.C., Ellman, A.B., Hudgens, G.A., 1996. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin. Physiol.* 16 (4), 433–448.

- DeCaro, J.A., 2008. Methodological considerations in the use of salivary alpha-amylase as a stress marker in field research. *Am. J. Hum. Biol.* 20 (5), 617–619.
- Folkow, B., 2000. Perspectives on the integrative functions of the 'sympatho-adrenomedullary system. *Auton. Neurosci.* 83 (3), 101–115.
- Garrett, J.R., 1987. The proper role of nerves in salivary secretion: a review. *J. Dent. Res.* 66 (2), 387–397.
- Grassi, G., Esler, M., 1999. How to assess sympathetic activity in humans. *J. Hypertens.* 17 (6), 719–734.
- Harmon, A.G., Towe-Goodman, N.R., Fortunato, C.K., Granger, D.A., 2008. Differences in saliva collection location and disparities in baseline and diurnal rhythms of alpha-amylase: a preliminary note of caution. *Horm. Behav.* 54 (5), 592–596.
- Hector, M.P., Linden, R.W., 1987. The possible role of periodontal mechanoreceptors in the control of parotid secretion in man. *Q. J. Exp. Physiol.* 72 (3), 285–301.
- Humphrey, S.P., Williamson, R.T., 2001. A review of saliva: normal composition, flow, and function. *J. Prosthet. Dent.* 85 (2), 162–169.
- Mandel, A.L., Peyrot des Gachons, C., Plank, K.L., Alarcon, S., Breslin, P.A., 2010. Individual differences in AMY1 gene copy number, salivary alpha-amylase levels, and the perception of oral starch. *PLoS One* 5 (10), e13352.
- Morse, D.R., Schacterle, G.R., Furst, M.L., Esposito, J.V., Zaydenburg, M., 1983. Stress, relaxation and saliva: relationship to dental caries and its prevention, with a literature review. *Ann. Dent.* 42 (2), 47–54.
- Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* 34 (4), 486–496.
- Navazesh, M., 1993. Methods for collecting saliva. *Ann. NY Acad. Sci.* 694, 72–77.
- Navazesh, M., Kumar, S.K., 2008. Measuring salivary flow: challenges and opportunities. *J. Am. Dent. Assoc.* 139 (Suppl.), 355–405.
- Nederfors, T., Dahlöf, C., 1992. Effects of the [beta]-adrenoceptor antagonists atenolol and propranolol on human whole saliva flow rate and composition. *Arch. Oral Biol.* 37 (7), 579–584.
- Proctor, G.B., Carpenter, G.H., 2001. Chewing stimulates secretion of human salivary secretory immunoglobulin A. *J. Dent. Res.* 80 (3), 909–913.
- Proctor, G.B., Carpenter, G.H., 2007. Regulation of salivary gland function by autonomic nerves. *Auton. Neurosci.* 133 (1), 3–18.
- Rohleder, N., Nater, U.M., 2009. Determinants of salivary alpha-amylase in humans and methodological considerations. *Psychoneuroendocrinology* 34 (4), 469–485.
- Rohleder, N., Wolf, J.M., Maldonado, E.F., Kirschbaum, C., 2006. The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology* 43 (6), 645–652.
- Schenkels, L.C.P.M., Veerman, E.C.I., Nieuw Amerongen, A.V., 1995. Biochemical composition of human saliva in relation to other mucosal fluids. *Crit. Rev. Oral Biol. Med.* 6 (2), 161–175.
- Strazdins, L., Meyerkort, S., Brent, V., D'Souza, R.M., Broom, D.H., Kyd, J.M., 2005. Impact of saliva collection methods on sIgA and cortisol assays and acceptability to participants. *J. Immunol. Methods* 307 (1–2), 167–171.
- Veerman, E.C., van den Keybus, P.A., Vissink, A., Nieuw Amerongen, A.V., 1996. Human glandular salivas: their separate collection and analysis. *Eur. J. Oral Sci.* 104 (4 (Pt 1)), 346–352.