Experimental Combat-Stress Model in Rats: Histological Examination of Effects on Amelogenesis—A Possible Measure of Diminished Vagal Tone Episodes

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ABSTRACT: Developmental defects of enamel-stress histomarker rings (accentuated striae) may be a potential measure of diminished vagal tone in research on extreme stress such as exposure to combat. To develop an animal model of this measure, we examined the enamel of rat incisors which erupt continuously. We examined incisors from 15 stressed-colony rats and 7 control-group rats for these histomarkers using the Visible Burrow System (VBS). VBS was developed to study combat stress in rats. No stress rings were found in any of the rat incisors examined. In contrast to humans, rats have likely evolved to prioritize incisor strength during combat stress. Studies of amelogenesis during combat stress in other rodents with continuouly growing incisors are warranted. Laboratory animals such as rabbits or marmosets may be especially suitable, since they less frequently use their incisors for self-defense. *Dental Anthropology* 2004;17(3):79-82.

There has been a rapidly growing interest in developing animal models resembling human situations of extreme life threat (e.g. military combat). For example, heart-rate variability (HRV) is now extensively studied in animals since HRV has been one of the more consistent physiological markers for research on combat-related posttraumatic stress disorder (CR-PTSD) and post-deployment syndromes of unclear etiology (Gorman and Sloan, 2000; Malaspina *et al*., 1997; Shalev, 2002). Porges has recently called attention to the vagal motor neurons originating in the nucleus ambiguus and their link to HRV (Porges, 1995). To our knowledge, dental anthropological techniques have not been previously used in research on combat-stress biology (Bracha *et al*., 2003).

In the anthropological literature, there has been little attention given to the fact that in addition to their role in HRV, the vagal motor neurons originating in the nucleus ambiguus also control the moment to moment fluctuations in the parasympathetic regulation of blood flow to the enamel secreting ameloblasts (as well as to the adjacent salivary glands). In humans, several tissues (*e.g*., intestinal mucousae, other mucousae, skin, bone, teeth, hair, and nails) are of lower survival priority during life-threatening experiences such as war-zone exposure. These tissues grow predominantly during spans of high vagal tone such as rest and sleep (Appenzeller *et al*., 2002; Appenzeller, 1990; Bracha *et al*., 2003; Bracha *et al*., 2004; Bracha, 2004). Studying these anatomical structures of lowest survival priority may

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be a novel research approach to examine the negative effects of combat-related stress.

While little research has been done on the topic, amelogenesis of the still erupting teeth is one parasympathetic trophic "luxury" function likely to be among the lowest priorities during extreme stress and thus provides a sensitive stress indicator in humans (Yui *et al*., 2002; Bracha *et al*., 2002). Unlike nails, and most of the human bones, skin and mucousae, the parasympathetic innervation of the ameloblast layer and the nearby salivary gland and larynx originates not in the dorsal motor nucleus of the vagus, but in the more rostral and more limbic-connected nucleus ambiguus of the vagus. This neural circuit is known to be involved in the human fear response (Porges, 2001; Porges, 1995; Bracha *et al*., 2003).

The amelogenesis defects seen in human tooth enamel have been reproduced using laboratory-induced stress in large herbivores such as sheep, pigs, and deer (Guatelli-Steinberg, 2001; Guatelli-Steinberg and Lukacs, 1999; Suckling *et al*., 1986; Dean *et al*., 2001; Dirks *et al*., 2002). For our line of clinical research, which focuses on the effect of combat stress on mineralized tissues such as bones and teeth, rodent incisors are an especially attractive tissue in which to examine histological biomarkers of extreme stress. The constant gnawing motion of the rat's jaw rapidly wears the incisors. Therefore, new enamel is formed in the ameloblast layer to replace the worn incisor enamel throughout the lifetime of the rat. While enamel research in dental anthropology has focused on nutritional or chronic stress, this is to our knowledge, the first controlled study attempting to use dental anthropological techniques to understand the effects of combat stress.

To study the effects of combat-like stress on mineralized dental tissue, we used the Visible Burrow System (VBS) developed by Blanchard et al. (Blanchard *et al*., 1995). The VBS is an important novel system to study combat stress among rats (Monder *et al*., 1994). Using the VBS, acute episodes of combat stress can be experimentally induced at known intervals. Previous studies have shown that behavior highly reminiscent of human combat ensues among male rats in the VBS. For a review of the VBS, see Blanchard et al. (Blanchard et al., 1995) and Monder et al. (Monder et al., 1994).

METHODS

Using the VBS, we controlled the timing of experimental combat stress in male rats and subsequently studied its effects on mineralized dental tissue formation. We examined 22 male rats that were subjected alternately to stressed and unstressed periods over several months. During the three-week baseline (no-stress) period the male rats were kept in individual cages. During the second three-week (low stress) period, rats from the control group (n=7) were each placed in cages with a single female rat. During the same three-week (combat-stress) period, the test rats (n = 15) were placed in colonies of three male rats to one female rat. During this combat-stress period, a behavior highly reminiscent of human combat ensued among the male rats (Blanchard *et al*., 1995). After this period, the rats were returned to their individual cages for another three-week (no-stress) period. This cycle was repeated three times for all of the rats in the study.

After the three combat-stress cycles, the two upper and two lower incisors from each rat were removed. The incisors from a total of 15 stressed-colony rats and 7 control-group rats were examined for "Developmental Defects of Enamel-Stress Histomarker Rings" (DDE-SH Rings; also known in dental anthropology as "accentuated striae"). The rat teeth were examined at 10X, 100X, and 400X by a trained dental anthropologist (JLLJ) who was blind to group assignment.

RESULTS

At least 3 teeth were available from each of the 22 rats. Because of the curvature of the rat incisors in the sagittal plane, one of the two lower incisors from each of four rats were not suitable for sectioning leaving us with 84 incisors out of a possible 88.

Unlike human teeth, the rat teeth showed markedly more decussation of the enamel rods giving them a twisted rope-like appearance. No DDE-SH Rings (accentuated striae) were found in any of the 84 incisors examined regardless of group assignment.

DISCUSSION

These negative results replicate and extend earlier research demonstrating the unusually high stress-resilience of rat amelogenesis. Fejerskov, using earlier stress-inducing methods reported similar negative results (Fejerskov, 1979). We propose that the explanation for this inter-species difference in the response to acute combat stress involves inter-species evolutionary differences in stress-response adaptation. It is likely that the rat genome has evolved to place high priority on incisor strength during life threatening experiences. Unlike humans and herbivores (such as sheep and deer), incisor strength is unlikely to be a luxury function for rats involved in combat. From an evolutionary point of view, short-term survival of the rat is more dependent on the stress-resilience of their incisors. Therefore, rat incisors may have evolved to achieve a greater degree of stress-resilience compared with sheep, deer, or human incisors.

Our finding that rat incisors show dramatically more enamel rod decussation is consistent with the above speculation. Enamel rod decussation is a histological feature known to increase the strength of

enamel (Fejerskov, 1979).

Preliminary data using enamel stress rings to chronicle episodes of diminished vagal tone in human teeth are promising (Bracha et al. unpublished). Therefore, it may be premature to abandon all laboratory animals as experimental models of acute episodes of extreme stress. For example, small herbivores, which in the wild only infrequently use their incisors for combat, may be a better choice than rats. Marmosets and rabbits, like rats, have constantly growing incisors and are as easy to study. However, marmosets and rabbits may resemble humans in stress prioritization with regard to the ameloblast tissue layer. Therefore their incisor enamel may be a promising model for research on combat stress.

Additionally, the newly developed animal research designs which induce extremely stressful but non-lethal exposure to larger predators (Cohen et al., 2003) may be especially useful for this line of research on the effects of acute combat stress on calcified tissue. The latest National Institute of Mental Health (NIMH) recommendations for future research directions on fear-circuitry disorders emphasize the "… need [for] research designed to develop better measures of the environment..." and the need to have "stress conceptualized broadly" (Davidson *et al*., 2002). Similar conclusions were drawn by Charney (Charney, 2004). Developing an experimental rodent model of dental biomarkers of acute stress is also consistent with the conclusions of the NIMH workshop on developing newer animal models of anxiety disorders (Shekhar *et al*., 2001). The line of research described here is well suited to address the above recommendations. A new technique for estimating vagal tone chronology may be a useful complement to the important research on HRV in laboratory animals and humans (Porges, 1995; Cohen *et al*., 2003).

In summary, laboratory animals that infrequently use their constantly growing incisors for combat may be a better choice than rats for this line of combat stress research. Research designs that provide extreme but non-lethal exposure to larger predators are especially recommended for this line of research.

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Editor's note:

This article is from the Honolulu VA Dental-Tissue Repository, and describes a new longitudinal study of predictors of psychosocial-stress resilience in young adults. The study includes a comparison of ameloblast distress episodes (*i.e.*, accentuated striae) that developed in the teeth between about 7 and 11 years (the period of third molar amelogenesis) along with the subject's self-reported and pediatrician-reported allostatic load between ages 7-11 and 11-18 years. Extensive psychosocial-allostasis measures are available from this unique American multi-ethnic group of 307 living, healthy, young middle-class men and women in Honolulu, Hawaii (in whom purely physiological, and nutritional allostasis is extremely low.) Open Access to some of the already published psychosocial-allostatic data is at:

http://www.annals-general-psychiatry.com/content/pdf/1475-2832-3-8.pdf

Two or more third molars are available on each of these 307 research participants. 100 of the participants already have enamel and dentin histological sections analyzed in collaboration with Donald J. Reed, PhD. Researchers interested in collaborations using this large database, or conducting further histological examination of the sections of the 307 teeth can contact the Principal Investigator at this address:

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