



## Degranulated mast cells in the skin of adults with self-injurious behavior and neurodevelopmental disorders

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### ABSTRACT

The role of nociceptive processes in relation to chronic, tissue-damaging self-injury among individuals with neurodevelopmental disorders is poorly understood. Scientific investigation has been limited, in part, by the clinical reality that the majority of individuals with severe intellectual impairments have co-morbid communicative impairments making it difficult to ascertain information regarding pain. Recently, we found abnormal patterns of peripheral epidermal nerve fiber (ENF) innervation and increased neuropeptide (substance P; SP) content among a subset of individuals with chronic self-injury. Here, we provide initial evidence for peripheral neuro-immune activity specific to self-injury. Skin samples from non-injury body-matched sites were compared between non-verbal adults with and without self-injury matched on gender and disability level. Relative to disability-matched controls, individuals with chronic self-injury had significantly more degranulated mast cells and were more responsive to tactile stimulation during a sensory testing procedure. Thus, nociceptive mechanisms and peripheral afferent sensitization may play a part in mediating and maintaining chronic self-injury.

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### 1. Introduction

Self-injury is one of the most profoundly disturbing and difficult to treat behavior disorders among individuals with severe intellectual disabilities (i.e., mental retardation) and related developmental disorders (e.g., autism). Some individuals injure themselves with sufficient severity to produce permanent tissue damage and disfigurement with extreme instances resulting in brain damage and death. Little is known about self-injury's developmental course or its underlying pathophysiology. For many individuals behavioral learning mechanisms play a role in maintaining the disorder but for a significant minority no obvious environmental mediation appears to exist (Schroeder et al., 2001).

Chronic self-injurious behavior (SIB) typically involves repeated, forceful stimulation to specific, localized body sites (e.g., hitting

specific spot on head, picking an area on the leg, biting a specific part of the hand), rather than random patterns of SIB distributed to various points on the body (Symons and Thompson, 1997). A subset of individuals with chronic SIB and intellectual impairments may have dysregulated pain or nociceptive capacities (Sandman, 1988) manifest in the form of altered pain thresholds, impaired pain expression, and/or disrupted pain signaling. Unfortunately, most individuals with severe or profound intellectual disabilities are non-verbal or otherwise communicatively impaired making it difficult if not impossible to reliably ascertain pain status through conventional means (i.e., self-report). Because of this, there is no direct evidence characterizing whether pain function is altered among non-verbal individuals with chronic SIB, but indirect evidence consistent with this notion comes from studies documenting differences in peripheral autonomic markers (Symons et al., 2001), nociceptive biochemistry (Sandman et al., 1999; Symons et al., 2003), and abnormal peripheral innervation (Symons et al., 2008) among a subset of individuals with chronic SIB and mental retardation.

In the periphery, the sensory nerves in human skin contain a variety of bioactive neuropeptides related to nociceptive (Kennedy et al., 2005), inflammatory (Drummond, 2004) and immune (Paus et al., 2006) responses. One proposed mechanism underlying peripheral inflammatory and immune response is mast cell degranulation. Initial evidence supporting mast cell involvement

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with sensory nerves came from structural observations that non-myelinated nerves exist in close morphological relationship with mast cells in both normal (Dines and Powell, 1997) and diseased (Naukkarinen et al., 1996) skin. There is a functional bi-directional relation (i.e., nerve-immune 'cross-talk') between mast cells and nerves in which the activation of small diameter afferent neurons results in the release of neuropeptides, like substance P, peripherally as well as centrally. This activates mast cells, which in turn release mediators increasing neuronal excitability (Ferry et al., 2002; Suzuki et al., 1999).

The concept of neuro-immune interaction suggests that pathological conditions thought to be exclusively neural or immunological may be a joint product of the interaction between the two systems (Dines and Powell, 1997). Substance P (SP) released from small diameter sensory afferents, for example, has been shown to degranulate mast cells under a variety of conditions associated with pain and stress (Kawana et al., 2006). Increases in SP positive (SP+) nerve fibers and contacts between SP+ nerve fibers and mast cells (Naukkarinen et al., 1996) have been found in the skin of patients with identified cutaneous disorders associated with scratch/itch and pain. Moreover, there is increasing evidence that stress-induced peripheral neurogenic inflammation is mediated by SP release from peripheral nerve endings resulting in mast cell degranulation (Arck et al., 2003; Singh et al., 1999). Given our recent preliminary observation of elevated SP+ fibers in the skin of individuals with chronic SIB (Symons et al., 2008), the aim of the present study was to determine whether there were differences in mast cells in individuals with SIB compared to individuals without SIB controlling for age, gender, and degree of intellectual impairment.

## 2. Methods

### 2.1. Participants

Twenty-five adults with severe to profound intellectual impairment (mean age = 41; 65% male; 80% profound mental retardation) were recruited from a residential population at a tertiary-care facility and two groups were formed following IRB approval from the University of Minnesota Committee for the Protection of Human Subjects and the J. Iverson Riddle Developmental Center IRB. The two groups consisted of a mental retardation plus SIB group (MR + SIB) ( $N = 16$ , mean age = 41 years, 65% male) and an MR-only contrast group ( $N = 9$ , mean age = 38 years, 67% male). Any individuals with known chronic illness or similar conditions considered to be painful or associated with discomfort (e.g., gastroesophageal reflux) were excluded as were individuals recently (within 1 week) having experienced acute illness (e.g., influenza) or injury severe enough to warrant medical attention (e.g., limb fracture). Individuals with specific syndromes associated with SIB (e.g., Lesch-Nyan syndrome, Cornelia de Lange syndrome) were also excluded. For the MR + SIB group, each individual had (1) a functional diagnosis of severe/profound mental retardation (based on DSM IV criteria and standard intellectual and adaptive behavioral test results as documented through existing chart review); (2) exhibited SIB either hourly or at least daily in bouts or discrete episodes (based on staff report, behavioral rating scales, and corroborated through direct observation); (3) chronic SIB (present for at least that last 12 months) (based on chart review); (4) received treatment for SIB that was currently stable (not planned on being changed in the next month); (5) parent or guardian informed consent. SIB body sites were associated with surface trauma marked by breaks in the skin, tissue rupture, and for some individuals, scarring. For the MR-only contrast group, each individual had (1) a functional diagnosis of severe/profound mental retardation (based on DSM

IV criteria and standard intellectual and adaptive behavioral test results as documented through existing chart review); (2) parent or guardian informed consent.

### 2.2. Design and procedures

A case-control cross sectional design was used. For all participants, (MR + SIB, MR-only) a single 3 mm skin biopsy from the upper middle back was obtained and compared. None of the individuals in the MR + SIB group had histories of self-injury at this body site. A non-SIB body site was selected to avoid potential confounds introduced by sampling damaged tissue produced by chronic self-injury. The specific body site (upper middle back) was selected because it proved to be a relatively straightforward procedure with this vulnerable participant population and it provides a basis for eventual normative comparisons (as more SIB cases accrue) through an existing dataset that includes the upper middle back. Biopsy sites were anesthetized by the application of a topical anesthetic and the biopsy was made with a 3 mm punch tool (Acupunch; Acuderm; Fort Lauderdale, FL). The primary dependent measure for the mast cell analysis was the quantification of mast cell granulation state (described below) for each subject.

### 2.3. Histological preparation of tissues

Biopsy specimens were processed for immunofluorescent localization of nerve and tissue antigens by methods developed for confocal analysis of nerve fibers (Kennedy et al., 2005). Biopsies were fixed in Zamboni's solution for 12–18 h at 4 °C, cryoprotected, and sectioned with a freezing sliding microtome (Leica, Nussloch, Germany). Diluent and washing solutions were 1% normal donkey serum (Jackson ImmunoResearch, West Grove, PA) in 0.1 M PBS with 0.3% Triton X-100 (Sigma, St. Louis, MO). Floating sections were blocked with 5% normal donkey serum in the diluent solution. Nerve and tissue antigens were localized by overnight incubations using a combination of two or three primary antibodies to protein gene product (PGP) 9.5 (1:800; Biogenesis, Kingston, NH), substance P (SP) (1:1000; Diasorin, Stillwater, MN), calcitonin gene-related peptide (CGRP) (1:1000; Diasorin, Stillwater, MN), and tryptase 1:4000; Chemicon, Temecula, CA), and type IV collagen (1/800; Chemicon, Temecula, CA), with each diluted in PBS-Triton X-100-NGS. Nonimmune serum was used with negative controls. Secondary antibodies labeled with cyanine dye fluorophores 2, 3 or 5 (Jackson ImmunoResearch), were used to locate two or three antigens in each section. Sections were washed 3 times over 3 h after each antibody incubation. After immunohistological processing, sections were adhered to coverslips with agar, dehydrated via an alcohol series, cleared with methyl salicylate, and mounted in DPX (Fluka BioChemika, Ronkonkoma, NY). Sections were evaluated visually with a Nikon Microphot-SA fluorescent microscope (Lake Success, NY) for assessment of granulation state (see below).

PGP 9.5/type IV collagen-stained sections were imaged with a CARV confocal system for quantification of ENFs. ENFs were traced from confocal image stacks following established guidelines (Kennedy et al., 2005). ENF density was expressed as the number of nerve fibers per mm within a 32  $\mu\text{m}$  section thickness (equivalent to 50  $\mu\text{m}$  wet section). SP and CGRP fibers within the subepidermal plexus were counted visually, using an epifluorescent microscope with a 20 $\times$  objective illuminated through narrow band fluorescence filters for Cy2 and Cy3.

### 2.4. Measurement of granulation state

The degree of mast cell granulation/degranulation has been used as a marker for immune activity (Taiwo et al., 2004,

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