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Peripheral Innervation in Children with Global Developmental Delay: Biomarker for Risk for Self-Injurious Behavior?

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Abstract

The relation between somatosensory mechanisms and self-injury among children with neurological impairments associated with developmental delay is not well understood. We evaluated the feasibility of procuring skin biopsies to examine epidermal nerve fiber density and reported self-injury. Following informed parental consent, epidermal skin biopsies were obtained from a distal leg site with no pre-existing skin damage from 11 children with global developmental delay (55% male; mean age = 36.8 months, 17–63 mo.). Visual microscopic examination and quantitative analyses showed extremely high epidermal nerve fiber density values for some children. Children with reported self-injury (5/11) had significantly ($p < 0.02$) greater density values (138.8, $sd = 45.5$) than children without self-injury (80.5, $sd = 17.5$). Results from this novel immunohistological analysis of skin in very young children with neurodevelopmental delays suggests it may be a useful tool to study peripheral innervation as a possible sensory risk factor for self-injury.

Keywords

self-injury; developmental delay; risk; peripheral innervation; sensory

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

FS conceived of and designed the study, assisted with statistical analysis, and wrote the draft manuscript; RT was instrumental in participant referral/recruitment/evaluation, data interpretation, and manuscript draft development; CB & JD coordinated specimen procurement, initial processing, and manuscript draft revisions; BM, MS, & SF completed specimen processing and carried out histology and microscopy analysis; GWC directed immunohistochemistry work, statistical analysis, and mast-cell granulation quantification and manuscript draft preparation; WK directed skin biopsy preparation and interpretation and helped to draft the manuscript.

Self-injurious behavior (SIB) is among the most severe behavior disorders for individuals living with intellectual disability (ID) associated with neurodevelopmental disorders. It is estimated that 5–20% of the population with ID self-injure at some point in their lifetime. Topographies include but are not limited to head-hitting, head-banging, self-biting, eye gouging, and self-scratching.¹ Self-injury can be highly treatment-resistant and the cost of care is considerable. Over two decades ago, a 1991 NIH Consensus panel estimated expenditures associated with SIB and associated destructive behavior to be \$3 billion nationally.² There is little reason to think this estimate has changed.³ Quality of life is significantly reduced for individuals who self-injure and their families.⁴ The myriad issues associated with SIB collectively considered attest to its public health significance. Although there have been remarkable advances in the behavioral assessment and treatment of SIB,^{5,6} there is little evidence from an epidemiological perspective that the burden of the disorder has been reduced (i.e., no reduction in incidence).

The available evidence indicates that once manifest, SIB is likely to continue over the lifespan and can be highly resistant to even long-term treatment.⁷ A meta-analytic study⁸ found that individuals with severe/profound ID, a diagnosis of autism, and deficits in receptive and expressive communication were more likely to show SIB. Similar findings have been reported by others.⁸ It is also well appreciated that SIB is associated with several genetic developmental disorders, including Lesch-Nyhan, Prader-Willi, fragile X, and Smith-Magenis syndromes.⁹ Descriptive studies of SIB in children and adolescents come to comparable conclusions. Findings suggest that children with severe/profound intellectual disability are most likely to exhibit SIB.^{10,11} Among those children, younger developmental age,^{12,13} lower daily living skills,¹² impaired ambulation,¹³ and specific genetic syndromes⁹ have been associated with SIB in children and adolescents.

Prevalence estimates notwithstanding, the state of the scientific knowledge about biological risk factors for self-injury in ID is still quite limited. The majority of the findings have been cross-sectional, descriptive, and specific to adults (although some comparable data exist for children as noted above). Correlates of SIB have been identified, but there is little knowledge concerning causal mechanisms and the physiological pathways to developing the disorder. The term ‘risk’ and ‘risk factor’ have been used descriptively in SIB research with respect to correlational patterns, but rarely applied in an epidemiological sense of understanding mechanisms conferring relative risk for the disorder.

In one biologically-oriented line of research investigating peripheral physiology and self-injury, there have been a series of observations specific to nociceptive (i.e., pain) and sensory afferents (epidermal nerve fiber innervation) in the skin that may be relevant to the notion of individual difference variables or sub-clinical markers for biological vulnerability and SIB risk.¹⁴ A SIB subtype or subgroup associated with a specific pattern of peripheral biomarkers has been characterized in adults by differences in the density of small-diameter unmyelinated sensory nerve fibers and concentrations of substance P (SP), a neuropeptide relevant to nociceptive (i.e., ‘pain’) signaling.¹⁵ Initial observations in adult samples with intellectual disability and chronic SIB described intra-epidermal nerve density differences¹⁶ relative to non-disability controls which were subsequently replicated with a larger sample

of adult SIB cases relative to matched developmental disability controls.¹⁷ Increased SP-positive fiber counts in some, but not all, samples along with extensive mast cell degranulation (consistent with immune mediated inflammatory response) was documented. It was also found that individuals with SIB and altered peripheral markers were more (not less) responsive during a modified quantitative sensory testing protocol compared to matched controls without SIB.¹⁸

In the current study, a ‘peripheral biomarker’ approach was extended to a pediatric sample with global developmental delay at risk for ID. The specific purpose of this preliminary study was to replicate prior adult work by investigating whether there was any relation between early parent-reported concerns regarding self-injury and observed density differences in epidermal nerve fibers obtained through skin biopsy.

METHODS

Sample & Behavioral Characterization

Following IRB approval and informed parental consent, children (N=11) with global developmental delays (55% male; mean age = 36.8 months, 17–63) were consecutively recruited through a tertiary developmental pediatric clinic at a large children’s specialty rehabilitation hospital. Information regarding general developmental level and ratings of adaptive and maladaptive behavior were available through chart review. SIB cases were defined as endorsement on the ‘hurts self’ item from the Inventory for Client and Agency Planning scale (ICAP; described below: 5/11 parents reported SIB based on item endorsement on the ‘hurts self’ ICAP scale). For the sample, the mean overall general developmental score from the Child Development Inventory (CDI, described below) was used to characterize degree of developmental delay. To characterize language/communicative ability, the ‘Expressive’ and ‘Receptive’ CDI subscales were used. General adaptive/maladaptive behavior was derived from the ICAP (adaptive/maladaptive scores were not available for one of the No SIB participants). Overall, the SIB and no SIB groups were comparable on global developmental delay, language (expressive, receptive), and general adaptive and maladaptive behavior (See Table 1).

Child Development Inventory—The CDI¹⁹ is a parent report measure of development for children between the ages of 18 and 72 months. It contains 270 items that yield an overall General Development Scale and scores in eight specific domains (Social, Self-Help, Gross Motor, Fine Motor, Expressive Language, Language Comprehension, Letters, and Numbers). Ireton and Glascoe²⁰ reported acceptable levels of reliability and validity for the CDI in a sample that included young children with developmental delays. CDI scores are reported in terms of age equivalents in months.

Inventory for Client and Agency Planning—The ICAP²¹ is a standardized measure of adaptive functioning and maladaptive behavior designed to assess the service needs of people with developmental disabilities (infant through adult). The maladaptive portion of the ICAP includes eight problem behaviors (Hurtful to Self, Hurtful to Others, Destructive to Property, Disruptive Behavior, Unusual or Repetitive Habits, Socially Offensive Behavior, Withdrawal or Inattentive behavior and Uncooperative behavior). The frequency of problem

behaviors is rated on a 6-point scale as occurring ‘never’ to ‘hourly’ and severity of these behaviors is rated on a 5-point scale as ‘not serious’ to ‘extremely serious.’ Bruininks et al.²¹ reported test-retest and inter-rater reliabilities for each domain range from 0.80 to 0.90 as well as evidence of construct, concurrent, and content validity. The primary variable of interest in this study was whether the children exhibited self-injurious behavior within the last month. SIB was determined by the parent or caregiver endorsement of the ICAP item Hurtful to Self with a frequency of at least weekly (1 to 6 times per week).

Specimen Collection

Epidermal skin biopsies were obtained painlessly while the child was under general sedation for an indicated procedure (MRI). Specimens were obtained from the postero-medial calf - an area that had no pre-existing skin damage and no history of SIB among the SIB cases. The biopsy was made with a 3 mm punch tool (Acupunch; Acuderm; Fort Lauderdale, FL). Biopsies were fixed in Zamboni’s solution, cryoprotected, and sectioned with a freezing sliding microtome (Leica, Nussloch, Germany). After immunohistological localization of nerve (PGP 9.5 and substance P) and tissue antigens (type IV collagen and tryptase), sections were adhered to coverslips with agar, dehydrated via an alcohol series, cleared with methyl salicylate, and mounted in DPX (Fluka BioChemika, Ronkonkoma, NY). All processing and reviews of microscopy images were ‘blind’ to child status with respect to whether or not they self-injured. The primary outcomes measured for this preliminary analysis were ENF density (ENF count/mm), frequency of substance P (SP) positive fibers in the ENF density count, and mast cell granulation state. Mast cell degranulation was scored: 0 = no degranulation, 1 = degranulation limited to deep dermis, 2 = degranulation limited to deep and mid dermis, 3 = degranulation throughout tissue, 4 = extensive degranulation throughout tissue.

RESULTS

SIB-Risk Comparison

Visual microscopic examination and qualitative analyses of the microscopy images suggested innervation differences characterized by extremely high epidermal nerve fiber innervation densities (ENFd > 100) for some but not all children (see Figure 1 for representative images). Three exploratory analyses were conducted to quantitatively investigate within group innervation differences for the children being evaluated for global developmental delay. Specifically tested was the relation between the peripheral innervation density observations and parent-reported self-injury. Also investigated was whether there were differences in substance P positive fiber counts and mast cell degranulation status between the groups. We also checked whether body surface area differed between groups. There was no significant difference between groups (SIB, No SIB) for body surface area (SIB mean = 0.65, no SIB mean = 0.59).

There was no statistically significant difference between the SIB and no SIB group for substance P positive fiber (SP+) counts (SIB mean SP+ = 12.6 [range = 7–15]; no SIB mean SP+ = 9.5 [range = 3–15], $t_{10} = 0.15$, ns), although the SIB group on average had higher counts with less variability (all of the values were 15 except one case which was 7). There

was no significant between-group difference in mast cell granulation state. For the majority of children, regardless of group membership, mast cells were intact (i.e., not degranulated, score = 0). One SIB case was scored a '1' as were two no SIB cases (1 = degranulation limited to deep dermis).

Using the self-injury subscale ("hurts self") of the ICAP, a chi-square analysis was conducted based on the presence/absence of SIB and ENF density values greater/less than 100. Children with ENF density values greater than 100 were more likely to be reported to SIB; χ^2 (df = 1) = 4.4, $p < 0.03$; Yate's Correction – $p < 0.07$.

Group means (SIB vs No SIB) were compared using a two sample t-test assuming unequal variances. Children with reported SIB had greater ENF density values as a group than children without reported SIB; mean ENF density for the SIB group = 138.8 (SD = 45.5), mean ENF density for the no SIB group = 80.5 (SD = 17.5), $t_5 = 2.7$, $p < 0.02$ (Figure 2).

A relative risk ratio analysis²² was performed based on the ratio of the proportion of SIB cases 'exposed' to the putative risk factor of high-density ENF values (>100) relative to the proportion of cases not exposed (ENF d values < 100). Children with high ENF densities were 4.8 times more likely to be reported to SIB; RR value = 4.8, $z = 1.67$, $p < 0.09$.

Relative risk was calculated based on the following formula. Relative Risk (RR) = $(a / (a + b)) / (c / (c + d))$ with a = # of 'positive' outcomes (SIB +) in the 'exposed' group (ENF d > 100), b = # of 'negative' outcomes (SIB -) in the 'exposed' group, c = # of 'positive' outcomes (SIB +) in the 'not exposed' group (ENF d < 100), d = # of 'negative' outcomes (SIB -).

DISCUSSION

We found preliminary evidence for the possibility of already altered peripheral innervation (i.e., increased ENF densities) in a high-risk clinical group of very young children (high risk for intellectual disability given global developmental delay and therefore presumably at risk for self-injury). It is important to underscore that the children were not recruited into the study based on the presence or absence of SIB, but because of developmental delay concerns. The results from this novel application of immunohistological analysis of skin and the apparent differences in innervation density do not definitively confirm or refute a SIB subgroup model, but do suggest that there may already be significant differences in peripheral innervation early in development that may somehow be related to the emergence of SIB.

The direction of peripheral density differences observed in the pediatric sample were consistent with prior observations in an adult sample comparing SIB vs no SIB (SIB ENFd > No-SIB ENFd), but the absolute values were different¹⁷. The ENFd mean of an adult SIB group (n=16) with intellectual and developmental disabilities was 79.8, sd = 22.3 compared with 138.8, sd = 45.5 for the current pediatric SIB group. The ENFd mean of the adult no-SIB group (n=9) was 65.2, sd = 17.8 compared with 80.5, sd = 17.5 for the current pediatric no-SIB group. There were 3 cases in the adult SIB sample with innervation density values greater than 100 and no cases with innervation values greater than 100 in the adult no-SIB

control sample. Considering these observations, there seem to be numerous heretofore unasked (and unanswered) neurodevelopmental questions concerning the increased ENFD values in the SIB samples (pediatric & adult), most likely related to trophic factors for afferent fiber growth and guidance, genetic/epigenetic regulation, and the clinical significance of these observations as possible markers for SIB risk.

The biological markers of increased ENF and substance P fiber density (in some cases) together with mast cell degranulation (observed in older SIB cases) suggests that a neuro-immune mechanism could be related to self-injury, but probably not directly. It should also be noted, though, that in this sample, unlike prior samples of older individuals (older children/adolescents²³ and adults¹⁷), the majority of mast cells were intact. It may be that the variability in peripheral innervation density is natural and is modulatory with respect to sensory input that, in turn, may relate to SIB under certain pathological circumstances (this notion is discussed in more detail below).

The significance of the findings in the present study is underscored by the fact that we recruited based on developmental delay (not presence/absence of SIB) and sampled at body sites with no history of tissue damage. But, a critical limitation is the inadequate (lack of) pediatric normative ENF data from skin biopsy. As far as we and others know (A. Oaklander, personal communication; S. Beggs, personal communication), there are no published referent values for typically developing children birth through five years of age. It is also worth pointing out that how a SIB case is defined varies across studies, and this study is no exception, further complicating comparisons. The measure of SIB (the subscale items from the ICAP) may not be the optimal SIB measure, but it was chosen for continuity with prior work with comparable populations.²⁴ In the current study, the sample size was small and although formed prospectively by consecutive clinical referral it was a sample of convenience and the results should be considered in relation to the specific sample and not representative of the population of children with global developmental delay. Mono-method bias (exclusive reliance on parental report for behavioral measures) may have been operating and we were unable to corroborate SIB using direct observation.

To the degree that there may be individual differences in peripheral innervation and epidermal nerve fiber density, there are a number of interesting but highly speculative issues that are not typically considered with regard to the problem of SIB among ID and related neurodevelopmental disorders. Initial risk may be related to variation in the underlying early tactile and nociceptive circuitry and the nature of early experience. Basic neuroscience work on peripheral nociception has found that prolonged neuroinflammation in the dorsal horn of the spinal cord can produce a switch between normally inhibitory to excitatory spinal pathways resulting in hyperalgesic phenotypes (exaggerated pain sensitivity/responsivity) such that non-noxious touch evokes pain (allodynia).²⁵ It appears that the circuitry dedicated to nociception is 'co-opted'; spinal nociceptive neurons are able to be activated by low-threshold mechanoreceptors (touch) because of inflammation-enhanced Na(+)-K(+)-Cl(-) cotransporter 1 (NKCC1) activity. Whether this is an issue for children with global developmental delay and increased ENF densities is completely unknown, but the finding is relevant in so far as it promotes inquiry into the nature of tactile and noxious experience and

the consequences of pathology in ‘pain pathways’ (in this case, neuroinflammation) as possible risk factors for SIB.

In a very general sense, there may be a constellation of factors at work that result in altering sensory thresholds and the nature of some forms of tactile or nociceptive sensory experience (‘input’) for children with global developmental delays. In clinical burn models²⁶ increased ENF density correlates with chronic pain from scar tissue but that there is also increased density at control (i.e., non-burn) sites within the same individuals (similar to our observations of increased ENF density at non-SIB sites). It is also known that substance P and glutamate, key molecules in amplifying the nociceptive signal at the level of spinal cord, are ‘at work’ in the periphery²⁷ suggesting that pathology in the periphery could be the first link in a signal amplifying chain to the spinal cord and up to brain (recall the increase in substance P positive fibers in the sample). Last, Jensen, Granmo, and Schouenborg²⁸ showed that c-fiber activity (c-fibers are the majority of the fibers making up epidermal nerve fibers) ‘gets’ to the brain and the nature of the signal is affected by the context of a hyperalgesic state. Whether or not the children in our sample or at risk children with ID and early SIB are in a hyperalgesic state is unknown, they may not be – certainly *not all* children with global developmental delay would be expected to be, but it would be important to know whether some might be. Observational findings from adults with chronic SIB (vs a no-SIB group) showed increased (not decreased) reactivity to a range of sensory (tactile) and nociceptive stimuli consistent with hyperalgesia.¹⁸

Although any one model is likely to be underspecified, the findings from basic work specific to the physiology of sensory nerves and nociception suggest some plausible new candidate variables to include in models of SIB in I/DD. Consistent with the conceptual and empirical work on individual differences in SIB vulnerability suggested by Devine and colleagues,²⁹ further work appears warranted to (a) conduct confirmatory tests of individual differences in the density of epidermal nerve fibers in relation to SIB, (b) determine the regulatory processes underlying early development of epidermal nerve fibers and later plasticity, and (c) understand the functional significance of density differences in ENFs among children with neurodevelopmental disorders.

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LIST OF ABBREVIATIONS

ID	intellectual disability
SIB	self-injurious behavior
ENF	epidermal nerve fiber
ENFd	epidermal nerve fiber density

PGP	pan neuronal protein gene product
SP	substance P
MC	mast cell
ICAP	Inventory for Client and Agency Planning
CDI	Child Development Inventory

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Figure 1.

Confocal images of epidermal nerve fibers (ENFs) in skin in children with global developmental delay with (a) and without (b) self-injurious behavior (SIB). **a.** Nerve, protein gene product 9.5 immunolocalization, (green) distribution in superficial skin of a representative pediatric SIB case. Basement membrane, type IV collagen immunolocalization (red), reveals capillaries (Cap) and the dermal-epidermal junction (DEJ) that separates the epidermis (Epid) from deeper dermis. ENFs were counted as they crossed the DEJ. Epidermal nerve fibers (ENFs) arising from the sub-epidermal neural plexus (SNP) are abundant. The density for this participant was 209.39 ENFs/mm. **b.** Developmental delay skin sample with no SIB and a more typical normal innervation pattern. ENF density for this participant was 77.3 ENFs/mm. Thickness of epidermis may vary from participant to participant regardless of disease state. Scale bar = 100 μ m for all images.

Figure 2.

ENFd quantification via dot-plot comparison of individual ENFd values between No SIB (lft, red) and SIB (right, blue) groups. Mean ENF density for the SIB group = 138.8 (SD = 45.5), mean ENF density for the no SIB group = 80.5 (SD = 17.5), $t_5 = 2.7$, $p < 0.02$.

Table 1

Select developmental and clinical characteristics of the sample.

	Overall M (range)	SIB group M(SD)	No SIB group M(SD)	p=
CDI				
General development	22.0 (0–45)	26.6 (14.3)	17.4 (20.4)	.44
Expressive language	17.5 (2–40)	17.6 (20.0)	17.4 (11.3)	.49
Receptive language	17.0 (4–38)	17.0 (8.6)	17.0 (13.4)	.50
ICAP				
Adaptive behavior	63.2 (40–94)	66.4 (19.6)	60.0 (23.7)	.43
Maladaptive behavior	–16.3 (–2 – –43)	–15.6 (10.9)	–19.8 (17.7)	.35