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Disregulation of proopiomelanocortin and contagious maladaptive behavior

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Abstract

Self-injurious behavior (SIB) is an untreatable and often life-threatening problem among individuals with developmental disorders, especially those diagnosed with autism. Functioning, relationships and processing of the proopiomelanocortin (POMC) system are “uncoupled” in subgroups of self-injuring individuals resulting in different ratios of ACTH and opioids in the bloodstream, particularly under conditions of stress. In this study, relations between SIB and POMC were evaluated in a multi-year study of the largest prospective sample studied to date. Observations were collected on palmtop computers for 45 treatment-resistant patients who exhibited chronic SIB. Behavior of each subject was observed in natural settings without disruption or intrusion, for continuous, 2.5-h periods, two times a day (morning and afternoon), 4 days a week for two consecutive weeks, for a total of 40 h/subject. Blood was collected in the morning, late afternoon and immediately after an SIB episode on two separate occasions separated by at least 6 months. Levels of β -endorphin (β E) and ACTH were assayed by RIA. We discovered that the SIB was the best predictor of subsequent SIB. Moreover, the majority of subjects exhibited this contagious pattern of SIB. Levels of POMC fragments were reliable over a 6- to 9-month period. Subjects exhibiting POMC disregulation characterized by high morning levels of β E had the highest transitional probabilities of SIB (i.e. contagious patterns; $F=8.17$, $P<0.01$). These findings suggest that subjects with “contagious” SIB may represent a behavioral phenotype associated with disregulated expression of the POMC gene.

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Keywords: β -Endorphin; ACTH; Self-injury; Autism; Mental retardation

1. Introduction

1.1. Proopiomelanocortin (POMC)

Proopiomelanocortin (POMC) was the first mammalian endocrine or neuronal precursor to be cloned and the first prohormone shown to be differentially processed in a tissue-specific manner to yield multiple, active neuropeptides. The highly conserved POMC gene, assigned to human chromosome band 2p23.3 [1], has three exons and two introns. The large exon 3 contains nucleotide coding for all of the biologically active peptides of POMC. POMC CRF-responsive element (PCRF-REB-1) binding protein binds to a specific region [–171/–160] of the POMC promoter

and increases transcription of that gene sevenfold. POMC expression is controlled by the cellular response to corticotrophic-releasing factor (CRF).

POMC, as is true for most neuropeptide precursors, initially is synthesized as an inactive molecule that requires post-translational modifications to generate bioactive products [2]. Processing of the POMC molecule involves cleavage of the prohormone by peptidases at sites of dibasic amino acids, primarily arginine and lysine. In the secretory granules of neurons and endocrine cells, prohormone convertase 1 (PC1), prohormone convertase 2 (PC2) and carboxy peptidase E (CPE) are the predominant peptidases that cleave POMC [3]. Two other endoproteases, furin and Pace 4, also play a role in the cleavage of POMC [4,5]. In humans, most POMC is expressed in, and released from, the pars distalis of the anterior pituitary. POMC also is produced by other neurons in the hypothalamus, amygdala and pituitary stalk, and it is produced by a variety of cell types in the gut and lymphocytes. POMC gives rise to a variety of

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neuropeptides (or POMC fragments) including ACTH, MSH, LPH and endorphin [6,7]. These POMC fragments are involved in the stress response as part of the hypothalamic–pituitary–adrenal axis (ACTH) and are involved in the modulation of pain and pleasure because of their affinity for the opiate receptors (β -endorphin).

Because all elements of the POMC–PC peptide system unfold early in fetal life, uncoupling or abnormal levels of POMC products in mature organisms could be evidence of genetic expression during the prenatal period. For instance, both PC1 and PC2 are present in the fetus by midgestation but they are distributed differently in the brain. As organisms approach adulthood, the differences in distribution begin to disappear [8]. Because of this developmental pattern, the correlation among POMC fragments under normal circumstances, particularly ACTH and β E in adults, is very high [9,10]. Thus, evidence of chronic dysregulation may indicate that POMC expression was influenced during early development when enzyme distribution was heterogeneous. Studies from our group have demonstrated that (i) early exposure of rats to POMC peptides produces behavioral effects that persist over the life span [11–13] and (ii) in a subset of mature patients with developmental disorders (specifically those engaging in self-injury), there is “chronic” disturbance of the POMC system [14,15].

1.2. POMC and self-injuring behavior (SIB)

Self-injurious behavior (SIB) is largely untreatable, expensive to manage and a prevalent, often life-threatening problem among individuals with developmental disorders, especially those diagnosed as autistic. SIB can have many manifestations but most often involves using hands and/or fists to hit self (usually the head), banging one’s head against objects including sharp corners of tables and biting (usually hands and fingers). Most attempts to characterize SIB, including our own [14,24,30], have relied on determining the frequency or calculating the rate of behavior. There are no accepted causes for this bizarre behavior but most attempts to ameliorate SIB assume that the behavior reflects an attempt to obtain attention or to escape from aversive situations. This assumption fosters primarily behavioral strategies involving alternative methods/procedures for securing attention or techniques to improve adaptive abilities (e.g. communication) for avoiding unpleasant circumstances [16].

Evidence from studies conducted in several countries indicates that functioning, relationships and processing of the proopiomelanocortin (POMC) system are “uncoupled” in subgroups of self-injuring individuals resulting in different ratios of ACTH and opioids in the bloodstream, particularly under conditions of stress [14,15,17–26]. LeBoyer et al. [20] report a massive difference between resting levels of N-terminal β E_{1–23} and C-terminal β E_{20–29} POMC fragments in autistic patients some of whom exhibited SIB. They found that the β E_{20–29} fragment is elevated in plasma

resting levels of the patients but that the β E_{1–23} fragment is depressed or not different compared with controls. Moreover, they report that other fragments of POMC (e.g. ACTH) were not abnormal in their patients (further evidence of uncoupling of this system). Recently, this group [21] replicated their finding of elevated plasma C-terminal β E among autistic probands. Moreover, they report that mothers, but not fathers, of the probands express significantly elevated C-terminal β E. These findings are the first to suggest a maternal influence for POMC fragment variations. The authors conclude that these differences may be evidence of abnormal processing of the POMC gene among individuals with developmental disabilities including autism.

1.3. Opiate blockers and SIB

Medications that block aspects of POMC (i.e. opiate) activity can reduce SIB and improve other symptoms of autism [24,28–32]. These studies extend an older collection of studies conducted during the past 10 years that estimated between 30% and 70% of patients respond positively to opiate blocking drugs [26,33–38].

Observations of apparent insensitivity to pain among individuals who self-injure generated a *hypothesis* that SIB is a symptom of general sensory depression, including hypoalgesia [35,36,38–40]. Perhaps opiate blockers are effective in reducing SIB because they lower the sensory/pain threshold. Other observations indicate that some individuals who self-injure may enjoy the experience and seek means and methods to do serious harm to tissue. These observations led to speculations that one consequence of SIB is the pleasure associated with the release of opioids [26,34]. In this case, opiate blocking agents are successful in attenuating SIB because the (endogenous) opiate “high” (pleasure) is diminished.

There is preliminary evidence that opiate blockers are most effective in subjects with elevated levels of endorphin. We [14] determined that patients who exhibit the largest increase in plasma levels of β E after SIB have the most positive response to the opiate blocker naltrexone (NTX). These results are consistent with several other reports. Ernst et al. [18] report that baseline levels of β E are positively related to changes in behavior and Bouvard et al. [17] find that C-terminal β E decreased after NTX only in good responders. Positive responses to NTX are related to high levels of endogenous opiates [41] and Cazzullo et al. [28] report that patients responding with decreased β E levels after treatment with NTX have the best and most pervasive behavioral improvement.

1.4. Naturalistic (in situ) observations of behavior

Clearly, the issue of how to measure human behavior is of central concern for the establishment of reliable and valid relations with biology [24,30,31,42]. Our previous studies [14,24,30,43] typically involved videotaping individuals in

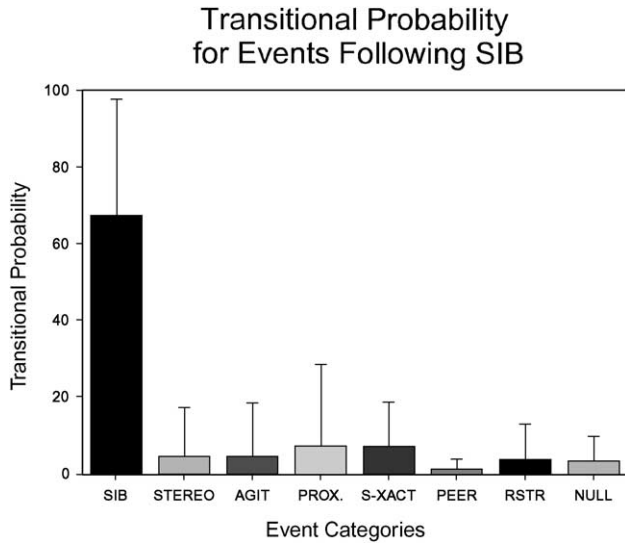


Fig. 1. The probability of events following SIB. SIB follows another SIB event almost 70% of the time but agitation follows SIB only about 5% of the time. Key: SIB, self-injuring behavior; STEREO, stereotypy; AGIT, agitation; PROX., subject proximity to others; S-XACT, staff activity related to subject; PEER, peer interactions; RSTR, restraining procedures; NULL, periods when nothing occurs within a time frame.

natural settings and using computer-assisted programs for scoring behaviors of interest. The primary measures collected with these methods are the frequency and rate of behavior. Advances in computer hardware and software provide new opportunities for direct observations, measurement and analysis of behavior [44]. We have developed a computer-assisted method that allows collection of extensive (40 h/subject) direct observations of maladaptive behavior and environmental conditions in a large group of subjects. We have adapted analytical methods to determine

the relationships (transitional probabilities) among the observed behaviors [24]. This method greatly extends the description from frequency of occurrence and rate of behavior to complex relations among behaviors.

2. Methods

2.1. Behavioral measures

Naturalistic observations were collected for 45 treatment-resistant patients who exhibited chronic SIB. A variety of traditional behavioral and pharmacological treatments had been attempted with all individuals in our sample but none were successful. Behavior of each subject was observed in natural settings without disruption or intrusion, for continuous, 2.5-h periods, two times a day (morning and afternoon), 4 days a week for two consecutive weeks, for a total of 40 h/subject. Real-time data (frequencies or durations) were entered on palmtop computers [24] by key presses which recorded the occurrence of an event (target behavior) and the clock time at which each behavior occurred. These data were downloaded to a host computer for analysis. Inter-observer agreement estimates of 0.75–0.85 were generated between two observers with independent recording systems observing the same subjects for extended periods of time.

The relationships among classes of behavior were determined with lag analyses to generate transitional (conditional) probabilities. A preferred behavioral pathway was constructed by analyzing relationships between a target behavior (SIB) and other recorded behaviors. The pathway was defined as the probability that an observed behavior would occur after an antecedent behavior. For our purposes, the relationship of interest was between SIB events, i.e. did

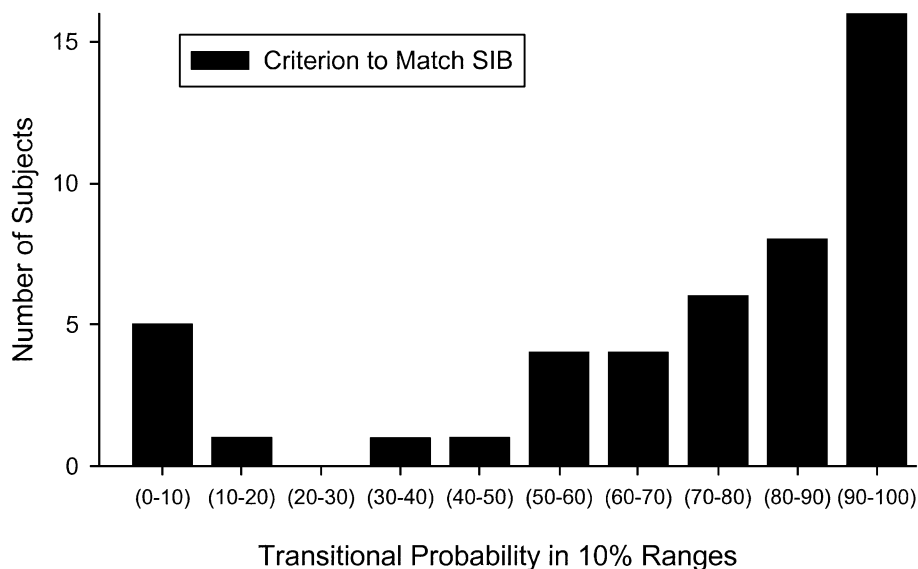


Fig. 2. Distribution of transitional probabilities of SIB following SIB. Figure indicates that 15 subjects have transitional probabilities for SIB between 90% and 100%. This pattern was interpreted in the text as supporting a contagious pattern for SIB.

one SIB predict a subsequent SIB? Conditional probabilities generated from the event and the 30-s temporal lag will be discussed in this report.

2.2. Biological measures

Venous blood was drawn in the morning (8 AM), late afternoon (4 PM) and after (i.e. within 10 min of) SIB episodes [14]. This entire procedure was conducted on two separate occasions for each subject to determine the stability of the measures. Blood samples (10 ml/draw) were withdrawn by antecubital venipuncture into EDTA (purple top)

vacutainers and chilled on ice immediately. Samples were centrifuged at $2000 \times g$ (15 min) and the plasma decanted into polypropylene tubes containing 500 KIU/ml aprotinin (Sigma; St. Louis, MO). The samples were stored at -70°C until assayed.

2.3. β -endorphin

Plasma levels of βE were determined by a commercially available direct solid phase two-site immunoradiometric assay (IRMA; Nichols Institute Diagnostics; San Juan Capistrano, CA). The βE assay incorporates two antibodies,

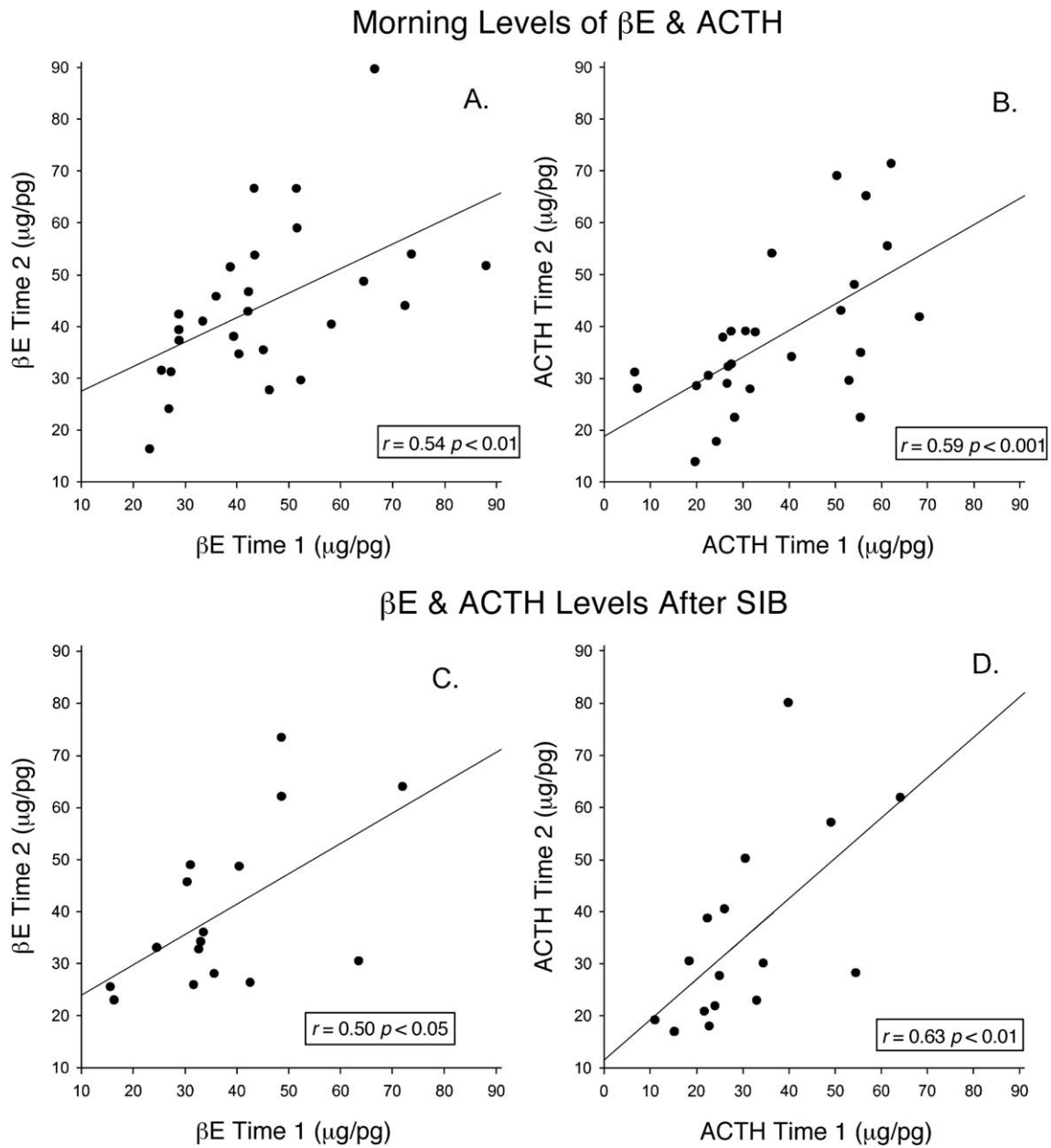


Fig. 3. Scatterplots of the relations between POMC fragments measured at 6–9 months: (A) relations between βE measured in the morning at two intervals; (B) relations between ACTH measured in the morning at two intervals; (C) relations between βE measured after SIB at two intervals; (D) relations between ACTH measured after SIB at two intervals.

both with high affinity and specificity for both N-terminal and C-terminal defined amino acid regions of the βE_{1-31} molecule. Both antibodies bind βE without competition or steric interference from each other and form a sandwich complex between the immobilized βE antibody on the plastic bead and ^{125}I -labeled βE antibody. The antiserum has 16% cross-reactivity with BLH at 500 pg/ml and has <0.01% cross-reactivity with related opiates at 5 μ g/ml. Samples were assayed in duplicate (200 μ l per assay tube). ^{125}I -anti- βE (rabbit) solution (100 μ l) was added to each tube and vortexed. The reaction was initiated by adding one anti- βE (rabbit) coated polystyrene bead to the assay tube followed by a stationary incubation at room temperature for 20 ± 4 h. The beads are then washed twice with phosphate-buffered saline and aspirated to dryness. The labeled antibody complex bound to the solid phase was measured using an ICN Biomedical (formerly Micromedic) Isoflex Gamma Counter. The amount of radioactivity is directly proportional to the amount of intact βE_{1-31} since the formation of the sandwich complex occurs in the presence of an intact βE molecule containing both N-terminal and C-terminal regions. The Allegro Beta-Endorphin Immunoassay system has a minimum detectable dose MDD=14 pg/ml (95% confidence limit) with a coefficient of variance CV=4.1% (intra-assay) and CV=9.0% (inter-assay) at the highest concentrations in the present study.

2.4. ACTH

ACTH ^{125}I -antibody solution (100 μ l) was added to the samples, vortexed and incubated at room temperature for 20 ± 2 h after the addition of an avidin-coated bead. The solid matrix was washed with buffered surfactant in phosphate-buffered saline to remove unbound components and the bound radiolabelled antibody complex quantified using a Micromedic Isoflex Gamma Counter. The ACTH assay has a MDD=1.0 pg/ml (95% confidence) with CV=3.0% (intra-assay) at 35 pg/ml and CV=7.8% (inter-assay) at 36 pg/ml.

Data reduction for the RIA and IRMA assays was done by a computer-assisted four-parameter logistics program by Rodbard et al. [45].

3. Results

A primary goal of the behavioral dimension of the study is to determine the antecedents and consequences of SIB. The results indicate that transitions between SIB incidents are significantly higher than SIB following any of several other behavioral/environmental events recorded (Fig. 1). On the abscissa are listed several behaviors and environmental events recorded for all subjects, and on the ordinate is the average transitional probability of SIB following any of the listed behaviors/events. It is clear that SIB is the best predictor of subsequent SIB. These data establish in a large

sample the contagious nature of SIB and as such provide reliable measures and methodology to test the biological hypothesis.

The distribution of transitional probabilities for the event analysis is illustrated in Fig. 2. On the abscissa is the probability (in 10% increments) that SIB will follow SIB. On the ordinate is the number of subjects at each probability range. For example, in the event lag, 14 subjects had a transitional probability between 90% and 100% and six subjects were between 0 and 10%. Clearly, for a majority of subjects, the conditional probability of one SIB incident preceding another SIB incident was greater than 50% with the plurality of subjects between 90% and 100%.

The stability of the biological measures over a 6- to 9-month period are illustrated in Fig. 3A–D. Measures of morning levels of βE collected at Time 1 and Time 2 (A) are significantly correlated ($r=0.53$, $p<0.01$). Similarly, measures of ACTH collected at two separate times (B) are significantly related ($r=0.51$, $p<0.01$). Levels of βE (C) and ACTH (D) after two incidents of SIB separated by 6–9 months also are significantly related ($r=0.57$, $p<0.05$). These findings indicate that biological measures of POMC are stable and reliable markers of individual differences in the patient population.

Morning levels of βE and ACTH are tightly coupled ($r=0.87$) in this patient group as we have reported previously [14]. We calculated a disregulation index (DI) [24] to examine the relation between SIB and deviations from the coupled association between βE and ACTH. According to median values of DI, subjects were divided into high (higher βE levels relative to ACTH levels) and low groups. Fig. 4, illustrates that subjects with high morning levels of βE had significantly higher conditional probability than subjects with DI levels below the median ($F=8.17$, $p<0.01$).

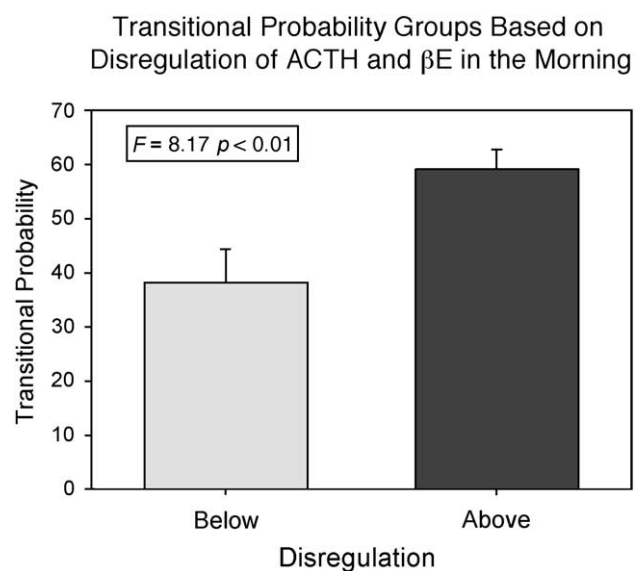


Fig. 4. Illustrates that subjects with a high disregulation index (higher βE levels relative to ACTH) have significantly higher levels of contagious SIB (higher transitional probability).

4. Discussion

We have argued [25] that a contagious distribution of SIB is compatible with a biological explanation for this behavior. In our current studies, we found that for most, but not all subjects, SIB is the best predictor of subsequent SIB. These preliminary findings suggest that subjects with “contagious” SIB may represent a phenotype whose behavior is maintained by biological rather than behavioral factors.

Our results also suggest that subjects with deviations from the tight coupling between ACTH and β E, specifically with relative elevations in β E, are most likely to exhibit “contagious” patterns of SIB. These findings suggest a prominent role for POMC generally, and the endogenous opioid system specifically, in the maintenance of SIB.

We have preliminary results collected in a small pilot sample (five adults with autism and SIB) that suggest that the fragment of the gene that codes for the opioid region of POMC is highly polymorphic [15]. This is the first evidence that we are aware of that the POMC gene may be involved in behavioral symptoms. These findings complement the report [27] of the first deficit related to a deletion in MSH region of the POMC gene defining a new monogenic disorder with early onset. The reasonable stability of POMC values over a 6- to 9-month interval suggests that the biological pattern is a stable trait in this population. These results support the conclusion that POMC patterns represent a neurochemical phenotype. We view these encouraging findings as a first step in characterizing a possible and plausible genetic anomaly associated with self-injuring individuals.

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