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A nonhuman primate model of human non-suicidal self-injury: serotonin-transporter genotype-mediated typologies

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While non-suicidal self-injury (NSSI) occurs in the general population at a surprisingly high rate, with higher rates among certain clinical populations, its etiology is not well-understood. Consequently, the DSM-5 lists NSSI as requiring further research. This study utilizes a translational model of naturally-occurring NSSI to assess the role of early parental neglect and variation in the serotonin transporter genotype (5-HTT) in the etiology of NSSI. Subjects ($N = 161$) were rhesus macaques (*Macaca mulatta*) reared in one of three conditions (mother-reared (MR), peer-reared (PR), or surrogate peer-reared (SPR)), and classified as NSSI ($n = 18$) or non-NSSI ($n = 143$). Subjects were genotyped for 5-HTT and their behaviors were recorded during an ecologically-meaningful, stress-evoking, intruder paradigm. Two weeks prior to testing, blood samples were obtained and assayed for plasma cortisol and adrenocorticotropic hormone (ACTH) concentrations. NSSI subjects were more likely to be SPR, paralleling human studies showing that individuals that exhibit NSSI tend to have experienced abuse or neglect early in life. Results also indicated that variation in the 5-HTT genotype differentiated the NSSI subjects. NSSI subjects that were homozygous for the *L* allele exhibited high plasma ACTH and high rates of stress-induced stereotypies; whereas NSSI subjects with the *s* allele exhibited impulsive behaviors, including frequently approaching the potentially dangerous intruder, high rates of aggressive vocal threats, and more activity. These results suggest that there may be different 5-HTT genotype-mediated NSSI typologies and that both early experiences and variation in the 5-HTT genotype may be important factors in understanding the etiology of NSSI.

INTRODUCTION

Non-suicidal self-injury (NSSI) is a cluster of behaviors that involve the infliction of non-lethal harm to oneself [1]. NSSI tends to co-occur with certain clinical diagnoses [2, 3] and may escalate to suicidal ideation [4] and attempts [5]. The etiology of NSSI is not well understood and the DSM-5 lists it as requiring further study [6].

One of the most frequently cited risk factors for NSSI is aberrant early experiences, including abuse or neglect [7, 8]. The majority of those that experience early maltreatment, however, do not go on to exhibit NSSI [9]. Thus, elucidating why NSSI occurs in some individuals and not in others is an important step toward understanding its etiology.

At a more proximal level, some posit that NSSI is an aberrant means of reducing arousal [10], and studies suggest that self-injury brings temporary relief through tension and anxiety reduction [11]. Other studies show that individuals with NSSI tend to be more impulsive when compared to their non-NSSI peers [11, 12]. While impulsivity appears to be an important factor in humans with NSSI, researchers have generally not found a relationship between temperamental impulsivity and self-injury in nonhuman primates [13], although one study suggests that deficits in extinction learning among self-injuring monkeys evinces impulse-control deficits [14].

Given the role of anxiety and impulsivity in NSSI [15, 16], the present study investigates the role of the serotonin transporter genotype (5-HTT) in naturally-occurring NSSI. The 5-HTT genotype is a known predictor of a variety of stress- and impulse-control-mediated psychopathological outcomes in humans [17, 18] and nonhuman primates [19, 20]. Studies show that individuals possessing the short (*s*) allele often exhibit phenotypic susceptibility to stress and anxiety [21] and low levels of central serotonin are associated with anxious and impulsive behaviors [22, 23]. Studies in humans [24, 25] and nonhuman primates [26, 27] show that some self-injuring individuals respond to medications that modulate the serotonin system. Such findings indicate that inherent variation in the serotonin system may play a role in the etiology of NSSI for some. Others, however, do not respond to serotonin-related pharmacological strategies, suggesting a complex relationship between central serotonin and NSSI and that other systems are involved.

Anxiolytics reduce the rates of self-injury in some human populations, particularly those with intellectual disabilities [28]. In nonhuman primates, one study showed that benzodiazepines reduced rates of self-injury in some, but increased self-injury in others [29], with benzodiazepine-responders spending more time in a single housing and receiving more minor veterinary

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procedures. Another study in nonhuman primates shows that anxiogenic drugs increase the frequency of self-injury in some subjects, which the authors suggest is evidence of a dysregulated GABA system, perhaps as a consequence of GABA receptor genotype variation [30]. As anxiolytic drugs tend to reduce the activation of the hypo-thalamic-pituitary-adrenal (HPA) axis during stressful events [31], some suggest that NSSI is a self-soothing mechanism [32]. As further evidence of this, studies show that self-biting lowers the heart rate among self-injuring nonhuman primates [13] and that, for humans with a history of self-injury, imagining self-cutting leads to heart rate reductions [33]. Nevertheless, many do not respond to benzodiazepines, and in some individuals with NSSI, rates of self-injury may even increase following benzodiazepine administration [34].

There are limitations to studying NSSI in human populations. For example, research in humans tends to rely on retrospective memories and self-reports of the frequency of NSSI (for example, see [35]), which may be subject to underreporting and recall biases, and in humans, early life histories cannot be controlled. Rhesus monkey (*Macaca mulatta*) models provide an alternative because the frequency and severity of self-injury can be directly observed, and early rearing conditions can be closely-controlled. Furthermore, they possess marked genetic similarities to humans [36], including an orthologous serotonin transporter genotype [37]. They also show a high degree of similarity in the central nervous system (CNS) and the HPA axis [38]. Like humans, NSSI occurs naturally in the rhesus monkey. Rhesus monkeys engage in self-injury (~14–25% in captive settings; often termed SIB), typically in the form of self-biting [39], and rates are comparable to the percentages of humans that engage in NSSI [40]. While suicide has not been identified in rhesus monkeys, because this paper is intended to model human NSSI, the term NSSI is used to refer to self-injurious behavior in rhesus monkeys. As in humans, studies attribute NSSI in nonhuman primates to early parental neglect and limited social interactions [39, 41, 42]. Studies show that early maternal absence and limited social interactions not only increases the risk for NSSI but leads to CNS aberrations, including a dysregulated serotonin system [43] and HPA axis functioning [44, 45]. These factors make a rhesus monkey model of NSSI ideally suited for studying the etiology of NSSI.

The purpose of the present study is twofold: (1) to investigate the relationship between early parental neglect and NSSI status and (2) to investigate the role of 5-HTT genotype variation in the biobehavioral response to an ecologically meaningful stimulus among subjects with NSSI.

MATERIALS AND METHODS
Subjects
Subjects ($N = 161$) were rhesus monkeys (100 females, 61 males; $M_{age} = 4.67 \pm 0.19$ years) tested at the National Institute of Health Animal Center between 2002 and 2003. Subjects were part of a larger research program designed to study variables associated with variation in alcohol intake (cf. [46, 47]). Of the 161 subjects in this study, $n = 18$ subjects (10 females, 8 males) were identified by direct observation as engaging in NSSI in the form of self-biting on one or more occasions, while the remaining $n = 143$ subjects were not observed to self-bite.

As infants, subjects were reared in one of three different rearing conditions ($n = 75$) mother-reared (MR), $n = 37$ peer-reared (PR), $n = 49$ surrogate-peer-reared (SPR) (see [45]). Briefly, the MR animals were reared by their mothers in social settings (with 7–10 adult females, 1–2 adult males, and other similarly-aged infants). The PR animals were separated from their mothers following birth and hand-reared in the nursery for the first 30 days of life and were thereafter housed with 2–3 same-aged peers. The SPR animals were reared in a nursery for the first months of life with an inanimate surrogate and access to 2–3 same-aged, similarly-reared

peers for 2 h/day, 5 days/week. When subjects from each condition were ~7 months old, each cohort was permanently relocated into a larger social group of peers, receiving identical treatment until they were 3 years old, when the males and females were separated into separate groups of 8–12 monkeys so that breeding could be controlled. They remained in their respective isosexual groups for the present study. Rhesus monkeys live in troops that are governed by strict social dominance hierarchies. At the time of the study, dominance data was available for $n = 135$ of the subjects. Of these, $n = 35$ were high-ranking non-NSSI, $n = 45$ were middle-ranking non-NSSI, and $n = 40$ were low-ranking non-NSSI subjects. There were $n = 7$ high-ranking NSSI, $n = 3$ middle-ranking NSSI, and $n = 5$ low-ranking NSSI subjects. Protocols for the use of experimental animals were approved by the Institutional Animal Care and Use Committee of the National Institute of Alcohol Abuse and Alcoholism.

Intruder challenge test
When subjects were ~4 years old, they underwent a slightly-modified version of the standardized intruder challenge test, a stress-inducing experimental paradigm assessing the response to the territorial challenge of a same-age and same-sex unfamiliar monkey (the intruder), allowing researchers to measure stress and impulsivity in a standardized fashion while controlling for age and sex [48, 49]. Pilot testing indicated that it was difficult to maintain experimental control and accurately code the response of subjects to the intruder when the whole homecage group was tested at once. Thus, three same-age test subjects, living together in the same isosexual social group, were randomly selected and isolated from the larger group into the outdoor portion (264CM × 300CM × 244CM) of their homecage enclosure. None of the trios were comprised of more than one NSSI subject and the rearing condition of each the members of each trio was randomly distributed. Because the subjects from each homecage were all the same age and sex, the unfamiliar intruder could be matched for age, sex, and relative body size.

The intruder was separated from their social group and acclimated to a holding cage with an open mesh side (76CM × 63CM × 91CM) for a period of 30 min. Following segregation into the outdoor area of their living quarters, the test subjects were acclimated for a period of 10 min. The intruder holding cage was rolled directly in front of the outdoor portion of the test subjects' homecage enclosures, with the open mesh side facing into the enclosure. Test subjects could see, approach, and interact with the unfamiliar intruder through the mesh of the enclosure and transfer cage, limited touch was possible, but full contact was prevented to avoid injury (no animals were injured during the test). Each of the three test subjects' behaviors was recorded by separate observers for 30 min using handheld computers equipped with Observer™ software (Noldus, Leesburg, Virginia). All observers were trained by senior authors (JDH) and (SGL), each of whom had 10+ years of experience with the scoring system and achieved interobserver reliability ($r > 0.85$) using regression and an established criterion of deviating less than 10% on any behavior. Raters were blind to subjects' genotype and NSSI status at the time of data collection. The recorded behaviors were based on a behavioral ethogram developed and used extensively by the senior author's laboratory (see Table 1 for a detailed ethogram).

Hormone sampling and genotyping
Two weeks prior to the intruder challenge test, 3–4 ml of blood was obtained using EDTA coated vacutainers and a 22-g needle to assess levels of plasma cortisol and adrenocorticotropic hormone (ACTH). All subjects were captured and injected with ketamine anesthesia (15 mg/kg, intramuscular) within 8 min of entering their homecage, and samples were obtained within 5 min of injection. Samples were placed on wet ice and centrifuged at 4 °C

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Table 1. Intruder challenge behavioral ethogram.

Behavior	Description
Aggression	The focal subject gives/receives aggression to/from the intruder/cagemates. Contact aggression (e.g., bites, slaps) and non-contact aggression (e.g., threats, lunges) is recorded.
Latency to approach the intruder	Time from the start of the test until the focal subject first enters proximity (1 m) to the intruder.
Approach intruder	The number of times the focal subject enters proximity (1 m) to the intruder.
Activity	The focal subject moves across the substrate (e.g., walking, running, swinging across the cage, etc.).
Environmental exploration	The focal subject engages in the active manual, oral, or pedal examination, exploration, or manipulation of the environment.
Social contact	The focal subject is within arm's reach of the intruder/cagemates.
Behavioral withdrawal	The focal subject remains motionless and does not engage in social interaction, activity, or environmental exploration.
Stereotypy	The focal subject engages in repetitive, non-adaptive, rhythmic movement (e.g., pacing, flipping, saluting).
Threat vocalizations	The focal subject emits aggressive barks.

Behaviors were scored as duration (s), with the exception of aggression, approach intruder, and threat vocalizations, which were scored as frequencies.

Table 2. Frequency of genotypes by NSSI status.

	LL	LS/ss
NSSI	9	4
Non-NSSI	91	39

Table 3. Rearing condition of NSSI and non-NSSI subjects.

	Mother-reared (MR)	Peer-reared (PR)	Surrogate peer-reared (SPR)
NSSI	2	1	15
Non-NSSI	73	36	34

NSSI subjects were more likely to be surrogate peer-reared ($p = 0.00001$).

for 20 min, after which plasma was aliquoted and immediately frozen in liquid nitrogen. Samples were stored at $-70\,°C$ until they were assayed for concentrations of plasma cortisol and ACTH.

A subset of subjects ($n = 143$) was genotyped for 5-HTT. Genotyping procedures have been described in detail elsewhere (see [50]). Briefly, DNA was isolated from whole blood collected in infancy from the femoral vein after subjects were sedated (ketamine anesthesia, 15 mg/kg, intramuscular). The short (s) (398-bp) and long (L) (419-bp) alleles of the rh5-HTTLPR were identified by direct visualization following ethidium-bromide staining ($n = 100$ animals (47 MR, 23 PR, and 30 SPR) were homozygous for the L allele, $n = 40$ (26 MR, 4 PR, and 10 SPR) were heterozygous, $n = 3$ (2 MR and 1 SPR) were homozygous for the s allele). Preliminary analyses showed no difference between the heterozygotes and subjects homozygous for the s allele on the variables of interest. Therefore, subjects that were homozygous for the s allele were combined with the heterozygotes, as has been done in other studies [51]. Genotype frequency did not deviate from Hardy–Weinberg equilibrium ($x^2(2, N = 143) = 0.19$, $p = 0.66$). See Table 2 for the distribution of the genotypes.

Data analysis
Preliminary t-tests showed no difference between the time to capture or time to obtain blood samples for NSSI and non-NSSI subjects ($t(155) = -0.71$, $p = 0.48$) or for LL subjects or subjects with an s allele ($t(137) = -0.76$, $p = 0.45$). With the exception of cortisol, preliminary ANOVAs showed no effects of sex on variables of interest ($p > 0.05$), so sex was excluded from further analyses. A preliminary chi-square (x^2) test of independence showed no difference between NSSI and non-NSSI subjects in social dominance rank $x^2(N = 135$, df $= 2) = 2.46$, ($p = 0.29$).

To protect against inflating the Type I error rate, a two-way MANOVA was performed, with 5-HTT genotype and NSSI status as independent variables and plasma cortisol and ACTH and each of the nine behaviors as dependent variables. Then, follow-up ANOVAs were conducted, with 5-HTT genotype and NSSI status as independent variables and plasma cortisol or ACTH

concentrations or each of the observed behaviors as the dependent variables. All analyses were conducted in SPSS, version 26.

RESULTS
Rearing condition
Results from the x^2 test showed that there was a significant relationship between NSSI status and early rearing condition ($x^2(2, N = 161) = 26.79$, $p = 0.00001$), with SPR subjects more likely to exhibit NSSI than the MR or PR subjects (see Table 3).
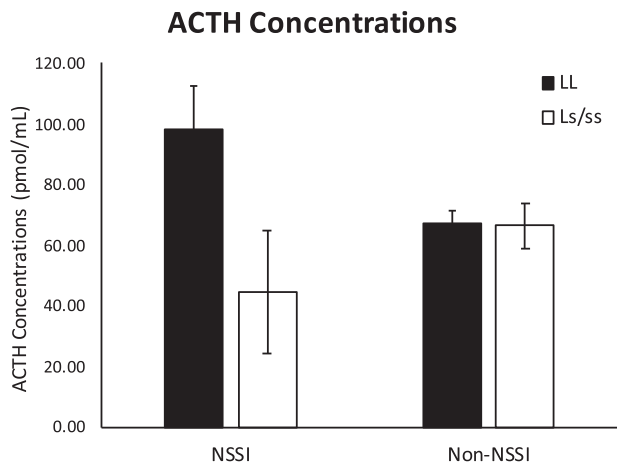
Two-way MANOVA
Results of the MANOVA showed a statistically significant effect of 5-HTT genotype ($F(11,94) = 3.07$, $p = 0.001$, Wilks' $\Lambda = 0.74$, $\eta_p^2 = 0.26$) and a statistically significant effect of NSSI status ($F(11,94) = 2.52$, $p = 0.008$, Wilks' $\Lambda = 0.77$, $\eta_p^2 = 0.123$). There was also a statistically significant 5-HTT-genotype-by-NSSI-status interaction ($F(11,94) = 2.93$, $p = 0.004$, Wilks' $\Lambda = 0.75$, $\eta_p^2 = 0.26$).

Follow-up ANOVAs
Plasma ACTH and cortisol concentrations. There was a significant main effect of 5-HTT genotype on ACTH concentrations ($F(1,121) = 4.25$, $p = 0.04$), with homozygous LL subjects exhibiting higher ACTH concentrations ($M = 69.70 \pm 43.26$ pmol/mL) when compared to subjects with an s allele ($M = 63.84 \pm 33.52$ pmol/mL).

There was also a significant 5-HTT-genotype-by-NSSI-status interaction on ACTH concentrations ($F(1,11) = 4.10$, $p = 0.04$), with homozygous LL/NSSI subjects exhibiting the highest ACTH concentrations ($M = 97.95 \pm 49.63$ pmol/mL), when compared to NSSI subjects with an s allele ($M = 44.38 \pm 48.30$ pmol/mL), or to LL/non-NSSI subjects ($M = 67.01 \pm 41.95$ pmol/mL) or to subjects with an s allele ($M = 66.52 \pm 31.18$ pmol/mL) (see Fig. 1).

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ACTH Concentrations

Fig. 1 Effect of 5-HTT Genotype by NSSI Interaction on ACTH Concentrations. There was a significant 5-HTT-genotype-by-NSSI-status interaction, with homozygous NSSI subjects exhibiting the highest ACTH concentrations ($F(1,11) = 4.10$, $p = 0.04$). Black bars indicate homozygous subjects; white bars indicate subjects with an s allele.

There was a significant sex-by-NSSI-status interaction for both plasma cortisol ($F(1,144) = 7.63$, $p = 0.006$) and ACTH ($F(1,124) = 4.09$, $p = 0.04$), with females that exhibited NSSI exhibiting higher cortisol and ACTH concentrations, when compared to NSSI males and non-NSSI subjects (cortisol: NSSI females: $M = 26.00 \pm 8.33$ pmol/mL, NSSI males: $M = 18.93 \pm 4.56$ pmol/mL; non-NSSI females: $M = 24.22 \pm 6.87$ pmol/mL, non-NSSI males: $M = 21.93 \pm 6.31$ pmol/mL; ACTH: NSSI females: $M = 82.02 \pm 59.23$ pmol/mL, NSSI males: $M = 50.52 \pm 25.96$ pmol/mL; non-NSSI females: $M = 66.79 \pm 40.86$ pmol/mL, non-NSSI males: $M = 54.40 \pm 16.75$ pmol/mL). There were no other cortisol effects.

Impulsivity
There was a significant main effect of 5-HTT genotype on the frequency of approaching the intruder ($F(1,139) = 13.17$, $p < 0.0001$), with subjects with an s allele approaching the intruder more often ($M = 21.65 \pm 16.80$ approaches) when compared to homozygous LL subjects ($M = 15.43 \pm 14.23$ approaches).

There was a statistically significant main effect of NSSI status on the frequency of approaching the intruder ($F(1,139) = 6.18$, $p = 0.01$), with NSSI subjects exhibiting a higher frequency of approaching the intruder ($M = 23.23 \pm 22.69$ approaches) when compared to non-NSSI subjects ($M = 16.71 \pm 14.30$ approaches).

There was also a significant 5-HTT-genotype-by-NSSI-status interaction on the frequency of approaching the intruder ($F(1,139) = 7.84$, $p = 0.006$), with NSSI subjects with an s allele approaching the intruder three times more often ($M = 43.75 \pm 25.97$ approaches), when compared to homozygous LL/NSSI subjects ($M = 14.11 \pm 14.67$ approaches), or the LL/non-NSSI subjects ($M = 15.56 \pm 14.26$ approaches) or to subjects with an s allele ($M = 19.38 \pm 14.22$ approaches) (see Fig. 2a).

Activity
There was a significant main effect of 5-HTT genotype on time spent active ($F(1,139) = 17.27$, $p < 0.0001$), with subjects with an s allele spending more time active ($M = 362.63 \pm 257.52$ s) when compared to homozygous LL subjects ($M = 263.94 \pm 200.17$ s).

There was also a significant main effect of NSSI status on time spent active ($F(1,139) = 8.33$, $p = 0.005$), with NSSI subjects spending more time active ($M = 403.30 \pm 311.63$ s) when compared to non-NSSI subjects ($M = 282.65 \pm 210.34$ s).

There was also a significant 5-HTT-genotype-by-NSSI-status interaction on the seconds spent active ($F(1,139) = 7.98$,

$p = 0.005$), with NSSI subjects with an s allele spending more time active ($M = 708.30 \pm 288.37$ s), when compared to homozygous LL/NSSI subjects ($M = 267.74 \pm 217.50$ s), or to LL/non-NSSI subjects ($M = 263.57 \pm 199.67$ s) or to subjects with an s allele ($M = 327.18 \pm 229.92$ s) (see Fig. 2b).

Stereotypic behavior
There was a significant 5-HTT-genotype-by-NSSI-status interaction on seconds spent in stereotypic behavior ($F(1,139) = 5.01$, $p = 0.03$), with homozygous LL NSSI subjects exhibiting more time in of stereotypic behaviors ($M = 280.84 \pm 444.11$ s), when compared to NSSI subjects with an s allele ($M = 20.95 \pm 24.59$ s) or to LL/non-NSSI subjects ($M = 39.16 \pm 190.86$ s) or to subjects with an s allele ($M = 81.82 \pm 200.19$ s) (see Fig. 2c).

Threat vocalizations
There was a significant main effect of 5-HTT genotype on the frequency of threat vocalizations ($F(1,136) = 12.99$, $p < 0.0001$), with subjects with an s allele exhibiting more threats ($M = 20.12 \pm 34.81$ threats) when compared to homozygous LL subjects ($M = 15.03 \pm 36.08$ threats).

There was also a significant main effect of NSSI status on the frequency of threat vocalizations ($F(1,139) = 5.74$, $p = 0.02$), with NSSI subjects exhibiting more threats ($M = 23.77 \pm 41.83$ threats) when compared to non-NSSI subjects ($M = 15.84 \pm 35.09$ threats).
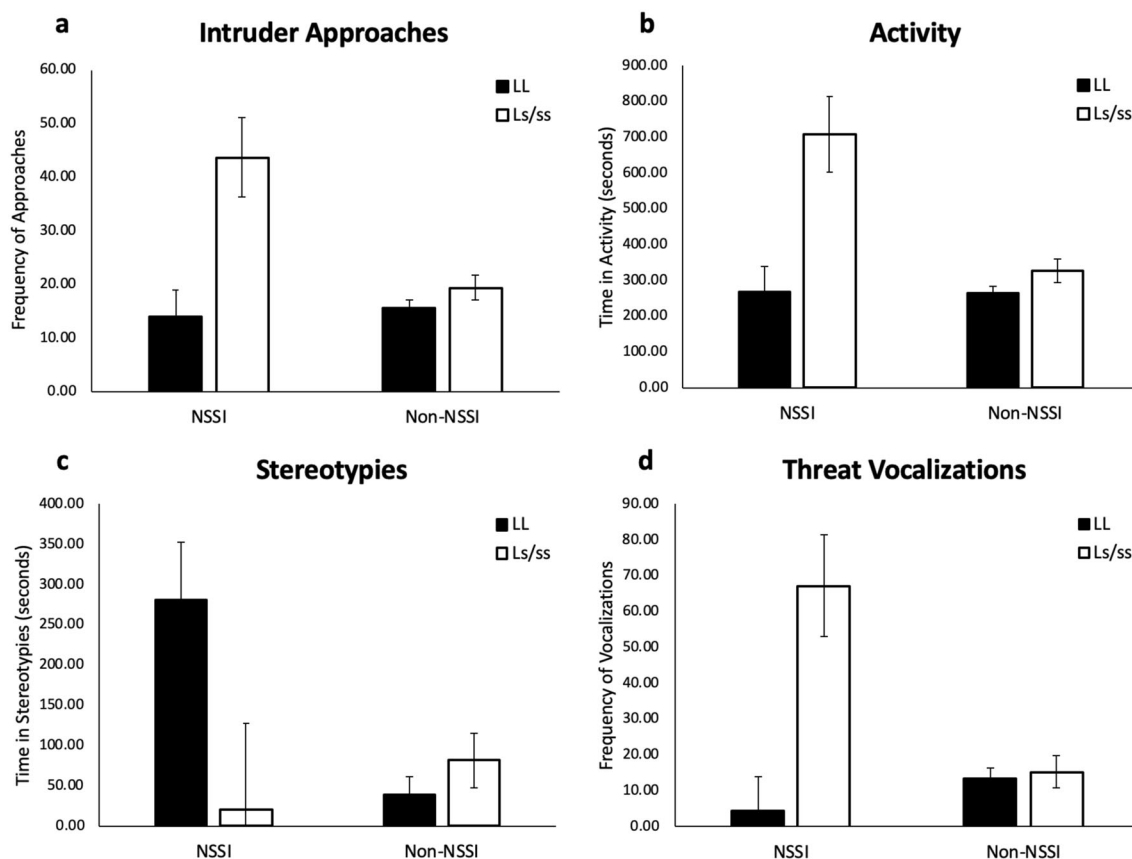
There was a significant 5-HTT-genotype-by-NSSI-status interaction on threat frequency ($F(1,139) = 11.55$, $p = 0.001$), with NSSI subjects with an s allele exhibiting the most threat vocalizations ($M = 67.25 \pm 57.57$ threats), when compared to homozygous LL/NSSI subjects ($M = 4.44 \pm 3.97$ threats) or to LL/non-NSSI subjects ($M = 16.08 \pm 37.66$ threats) or to subjects with an s allele ($M = 15.28 \pm 28.64$ threats) (see Fig. 2d).

There were no significant relationships found between NSSI status or 5-HTT genotype and aggression, latency to approach the intruder, environmental exploration, social contact with conspecifics, or behavioral withdrawal ($p > 0.05$).

DISCUSSION
Results showed that the majority of NSSI subjects experienced limited social experience (83% of NSSI subjects were SPR). To the extent that these limited social experiences are comparabl to early parental neglect in humans, these findings are consistent with studies showing that aberrant early experiences, including child abuse and neglect, are risk factors for NSSI in human [52] and nonhuman primates [13, 39, 41]. The results also showed evidence for two 5-HTT genotype-mediated typological variations in the stress-response of individuals that engage in NSSI. Homozygous LL subjects showed tendencies for overactive HPA axes and hyper-responsiveness to stress, as evidenced by high ACTH concentrations and stereotypies; whereas subjects with an s allele showed evidence of impulse control deficits, exhibiting longer overall activity and high rates of aggressive threat vocalizations and more instances of approaching the unfamiliar, potentially dangerous, intruder.

Studies show SPR subjects tend to have a variety of behavioral pathologies, such as rocking, stereotypies, and self-biting [13, 42]. One interpretation of these results is that SPR subjects may engage in NSSI as a maladaptive self-soothing mechanism. This interpretation may not be complete, however, as 34 of the 49 SPR subjects did not engage in NSSI, indicating that factors other than early neglect likely contribute to the etiology of NSSI. It is of note, that the PR subjects were no more likely to exhibit NSSI than the MR subjects, replicating an earlier finding [42]. While both PR and SPR subjects were deprived of maternal care, PR subjects had continual social interactions with their agemates, whereas SPR subjects had only limited social interactions. Studies show that such limited interactions reduce the ability of SPR subjects to

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Fig. 2 Effects of 5-HTT Genotype by NSSI Interaction on Stress-Induced Behaviors. a There was a significant 5-HTT-genotype-by-NSSI-status interaction, with NSSI subjects with an *s* allele exhibiting the highest rates of approaching the intruder ($F(1,139) = 13.17$, $p < 0.0001$). **b** There was a significant 5-HTT-genotype-by-NSSI-status interaction, with NSSI subjects with an *s* allele spending the most time active ($F(1,139) = 7.98$, $p = 0.005$). **c** There was a significant 5-HTT-genotype-by-NSSI-status interaction, with homozygous NSSI subjects exhibiting the most time in stereotypies ($F(1,139) = 5.01$, $p = 0.03$). **d** There was a significant 5-HTT-genotype-by-NSSI-status interaction, with NSSI subjects with an *s* allele exhibiting the most threat vocalizations ($F(1,139) = 11.55$, $p = 0.001$). Black bars indicate homozygous subjects; white bars indicate subjects with an *s* allele.

develop social competence when compared to PR and MR subjects [53]. Given these differences, NSSI may develop as a consequence of limited social interactions, although this explanation is speculative. What is clear, however, is that NSSI is not simply the result of parental absence; nor is it the result of early maltreatment, but rather is, at least in part, a consequence of limited social experiences, an interpretation that parallels work in humans showing that NSSI is more frequent in individuals who experience peer-rejection and loneliness [54, 55].

There were two 5-HTT genotype-mediated responses to the intruder challenge test among NSSI subjects. In the first, homozygous *LL*/NSSI subjects exhibited high rates of stereotypies. Stereotypies are non-adaptive, repetitive behaviors [56], tending to occur among subjects that experienced early maternal deprivation [57]. It is of note that the homozygous *LL*/NSSI subjects spent 13 times more time in stereotypies than subjects with an *s* allele, suggesting a trait-like maladaptive response to stress. For homozygous *LL*/NSSI subjects, one cannot rule out the possibility that NSSI is an extension of such stereotypies. Homozygous *LL*/NSSI subjects, however, exhibited significantly higher ACTH concentrations, when compared to NSSI subjects with an *s* allele. While speculative, homozygous *LL*/NSSI subjects may attempt to reduce arousal by engaging in self-soothing techniques, like stereotypies and NSSI, an interpretation corroborated by other work suggesting that NSSI decreases arousal (i.e., decreases heart rate) in monkeys [13] and humans [33].

In contrast, NSSI subjects with an *s* allele exhibited more frequent approaches and more threat vocalizations directed toward the unfamiliar intruder and more time in general activity. Studies suggest that the *s* allele is associated with high rates of impulsivity [58, 59]. In line with this, on average, NSSI subjects with an *s* allele approached the intruder three times more often than homozygous *LL*/NSSI subjects. Approaching an unfamiliar conspecific in the context of the intruder challenge test is a high-risk behavior and may indicate impaired impulse control. While CNS serotonin activity is not reported in this manuscript, other studies in nonhuman primates show that, on average, NSSI subjects administered fluoxetine show reductions in rates of self-injury [60], suggesting that the serotonin system may be involved in NSSI. NSSI subjects with an *s* allele also exhibited lower ACTH concentrations and less time in stereotypies than homozygous *LL*/NSSI subjects, an indication that their NSSI was not driven by anxiety or arousal. While speculative, these impulse control deficits may lead to an inability to successfully inhibit NSSI tendencies, an interpretation consistent with human research suggesting that NSSI individuals tend to exhibit impulsivity [12].

CONCLUSIONS
Taken together, these 5-HTT genotype-mediated response typologies among the NSSI subjects suggest that some individuals with NSSI may exhibit NSSI due to an inability to inhibit impulsive behavior, while others may exhibit NSSI in an attempt to cope

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with high levels of stress and anxiety. While other studies show that a dysregulated serotonin system may play a role in the etiology of NSSI [61], this study parses out important genotype-mediated nuances that may better explain the differences between subjects.

It is clear that early experiences are, in part, foundational in the development of NSSI. The results of this study also suggest that the proposed 5-HTT genotype-mediated typologies may be useful in determining appropriate NSSI treatment modalities, and could explain why some individuals that self-injure respond to serotonin-specific treatments [24, 25], while others respond to anxiolytics [28]. While this study has sample sizes comparable to others investigating NSSI in nonhuman primates [14, 26, 29], one limitation to the interpretation of these results is the small number of NSSI subjects with the *s* allele, which tempers somewhat the generalization of the findings. As such, this study provides evidence for a promising area of future study. Future larger-scale studies of self-injury in nonhuman primates should attempt to replicate these results with a larger sample size of subjects possessing an *s* allele. Nevertheless, these results are compelling and suggest a novel way of dichotomizing human populations with NSSI. While this study focuses on the 5-HTT genotype, it is likely that other systems play a role in the etiology of NSSI. For example, NSSI leads to the release of endogenous opiates [62], and some studies show that blocking opioid release reduces NSSI [63]. It would be of interest to investigate the μ-opioid genotype in future studies.

An important step in providing better treatment for individuals with NSSI is understanding variables that are relevant to its etiology. These findings provide evidence for factors that are important in the development of NSSI. That SPR subjects were at greatest risk for developing NSSI suggests that early life events, in particular limited social experiences, are an important underlying risk factor in the development of NSSI. The results of this study also suggest that there may be different NSSI typologies, modulated by 5-HTT genotype variation. To the extent that these results generalize to humans, they suggest that 5-HTT genotype variation may play an important role in determining which NSSI treatment modalities may be most effective.

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AUTHOR CONTRIBUTIONS
EKW, RK, JPD, SMD, and JDH were responsible for the study concept and design. SGL, CSB, MLS, DG, SJS, and JDH contributed to the acquisition of the data. EKW, RK, and JDH conducted the analyses and interpreted the findings. EKW, RK, and JDH drafted the initial manuscript. JCH critically revised the manuscript for important intellectual content. EKW, RK, JPD, SMD, JNH, LN, SGL, CSB, MLS, DG, SJS, JCH, and JDH reviewed the manuscript and approved the final version for publication.

ADDITIONAL INFORMATION

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