Rett syndrome: A preliminary analysis of stereotypy, stress, and negative affect

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ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 7 January 2014
Accepted 9 January 2014
Available online 25 February 2014

Keywords:
Rett syndrome
Stereotypy
Stress
Negative affect
Behavioral observation

ABSTRACT

Rett syndrome (RTT) is a neurodevelopmental disorder primarily affecting females. It is characterized by apparently normative development of motor and communicative abilities followed by deterioration in these domains. Stereotypic hand movements are one of the core diagnostic criteria for RTT. There is some anecdotal but limited scientific evidence that changes in hand stereotypy may be a sign of increased anxiety or arousal (i.e., a ‘stress response’) in RTT. Understanding stress responsivity is difficult in RTT because almost all individuals are nonverbal or otherwise severely communicatively impaired. This study used direct behavioral observation to quantify and compare the frequency of hand stereotypy and signs of negative affect during presumed periods of high and low stress associated with functional analysis conditions (negative reinforcement ['escape'] and control ['free play'], respectively) for 5 females with RTT (mean age = 17.8; range 4–47). Negative affect was more likely to occur during negative reinforcement ('stress') conditions for each participant whereas hand stereotypies did not differ across conditions for any of the participants. Although preliminary, the results suggest that hand stereotypy may not be a valid behavioral ‘stress-response’ indicator in females with RTT. Alternatively, the approach we used may have been limited and not sufficient to evoke a stress response. Either way, more work with direct relevance to improving our understanding of hand stereotypy and anxiety in RTT in relation to social context appears warranted.

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1. Introduction

Rett syndrome (RTT) is a neurodevelopmental disorder primarily affecting females. The syndrome is typically characterized by a mutation in the methyl-CpG binding protein 2 (MECP2) gene with a prevalence rate of 1:10–15,000 female live births. From birth to 6–18 months, girls develop apparently normally, reaching age-appropriate milestones, such as the use of motor skills and some language. With the onset of the disorder, however, there is a loss of much of their adaptive functioning, such as purposeful hand movement, communicative abilities, and in many cases, locomotion. Subsequently, the behavioral characteristics upon which the diagnosis is based emerge including stereotypic hand movements, growth retardation, breathing abnormalities, seizures, gait abnormalities, scoliosis, and thermal dysregulation (Lotan & Ben-Zeev, 2006; Neul et al., 2011).

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http://dx.doi.org/10.1016/j.ridd.2014.01.011
There is reason to believe that anxiety and related issues are also common among individuals with RTT. MECP2 deficient mice, which have been developed to recapitulate the core features of RTT, show significantly higher levels of anxiety-like behaviors (Adachi, Autry, Covington III, & Monteggia, 2009; Fyffe et al., 2008; Gemelli et al., 2006; McGill et al., 2006), and provide evidence of pathologically altered stress–response systems. It appears that MeCP2 modulates or otherwise regulates the expression of the corticotrohin-releasing hormone (CRH) gene, which is a part of a well-established pathway underlying anxiety (Fyffe et al., 2008; LeDoux, 2000; McGill et al., 2006). Although there has not been comparable work specific to anxiety among individuals with RTT, parents and other caregivers commonly report anxiety and stress-related issues. For example, in one study, 74% of caregivers reported that their daughters experienced fear or anxiety in unfamiliar situations (Mount, Charman, Hastings, Reilly, & Cass, 2002). In another, it was reported that 76% of individuals with RTT exhibited brief episodes of anxiety-like behavior, and these behaviors were reported to be precipitated by external events (Sansom, Krishnan, Corbett, & Kerr, 1993).

Although it is widely assumed that individuals with RTT have problems with anxiety (Lotan & Ben-Zeev, 2006), identifying if and when anxiety is being experienced is difficult, because of severe communicative impairments and therefore limited access to an emotional state (i.e., self-report is compromised; Didden et al., 2010). Most of the available information regarding emotional states among individuals with RTT is based on proxy reports such as rating scales or questionnaires, which is similar to, but not identical to, measuring symptoms. Another approach to examining anxiety among individual with RTT may be to use direct observation – which would be similar to measuring signs. To our knowledge, only two studies have utilized direct observation to assess behavioral or emotional states among individuals with RTT (Bergstrom-Isacsson, Lagerkvist, Holck, & Gold, 2013; Woodyatt, Marinac, Darnell, Sigafoos, & Halle, 2004). The results of these studies indicate that, although it is possible for trained observers to identify facial expressions and other indications of emotional/behavioral regulation in this population, agreement regarding the meaning or intention of these behaviors is generally poor, and individualized operational definitions of behavioral indications are necessary.

Considering the current state of knowledge, it is possible that caregivers of individuals with RTT may be over- or under-identifying possible sign of anxiety. Additional research is clearly needed to identify the specific behavioral indications of stress or anxiety among individuals with RTT. One possible indicator is stereotyped hand movements. Although this type of movement is one of the defining characteristics of RTT, it has been suggested that hand stereotypes may increase or intensify in response to stress or arousal (Temudo et al., 2008). In fact, one study reported differences in the percent of time that individuals with RTT engaged in hand stereotypes across social and non-social activities, although the specific patterns differed across participants (Sigafoos, Woodyatt, Tucker, Roberts-Pennell, & Pittendreigh, 2000). Similarly, Hetzroni and Rubin (2006) reported that females with RTT exhibited increased hand stereotypy in response to interruptions of familiar activities like watching television or listening to music, and Stasolla and Caffo (2013) reported decreases in hand stereotypy, along with increases in indices of happiness, associated with structured activities and environmental stimulation. In all of these cases, the observed differences may have been due to changes in anxiety or arousal. Another possibility, however, is that hand stereotypes decrease when the individual is engaged with or oriented to an ongoing activity (Fabio, Giannatiempo, Antonietti, & Budden, 2009). Despite the clinical implications of the assumption that hand stereotypes are indicative of a state of anxiety among individuals with RTT, the relationship between stereotyped hand movements and stress has not been systematically studied in this population.

The purpose of this exploratory study was to build on the existing literature by testing the hypothesis that individualized behavioral indicators of negative affect (such as whining, and negative facial expressions) and the percent of time engaged in hand stereotypy would differ across controlled environmental conditions that were expected to be relatively stressful or non-stressful in a small consecutively enrolled clinical sample of females with classic RTT.

2. Methods

2.1. Participants

Five females (mean age = 17.8; range 4–47) with a clinical diagnosis of classical Rett Syndrome who met the core criteria defined by Neul et al. (2011), participated in the study. Participants were recruited through the Minnesota Rett Syndrome Research Association. The University of Minnesota Institutional Review Board approved the study and parents or legal guardians provided informed consent. Participants of all ages and levels of physical functioning were included in the study except those with a recent onset of seizure disorder. All of the participants were nonverbal. SG was the only participant to use an augmentative communication device (Tobii C12 eye tracker). SG’s parents reported her using the device appropriately during activities such as mealtimes, but no formal assessments of language abilities were conducted. None of the other participants had any formal means of communication. The participant’s motor skills varied greatly: Three participants (ZC, SG, and JS) were ambulatory, whereas two (ML and KP) could not walk independently. Three (ML, KP, and JS) had little to no functional hand use, and two (ZC and SG) retained some skills, including the ability to pick up toys and feed themselves. Three of the participants (ML, KP, and JS), had histories of seizure disorders. One participant (ZC) lived in a group home, while the other four participants lived at home with their parents or legal guardians.
2.2. Materials and procedure

For the study, archived video was used from previously conducted functional analysis sessions. The original video recordings of the functional analyses included test conditions for positive reinforcement (social attention) and negative reinforcement (escape from a task) and a control condition (free play) according to the conditions described by Iwata, Dorsey, Slifer, Bauman, and Richman (1994). For the study, video from the control (free play) and negative reinforcement (escape) conditions (5 min sessions with each session conducted at least twice) were selected to analyze negative affect and hand stereotypy. All functional analyses were conducted over one or two days.

We reasoned that there may be different levels of arousal experienced and affect expressed during the different environmental manipulations associated with the control (free play) and negative reinforcement (escape) conditions. Free play was a baseline condition in which the participants had been given free access to attention and preferred activities. Due to their limited use of hands and communicative ability, free play consisted of preferred activities such as listening to music, watching television, playing with simple cause and effect toys. Positive social attention was provided every 10–15 s throughout the free play sessions. Therefore, it was expected that the participants would have experienced low levels of stress or arousal during the free play condition (low stress). In contrast, during the escape condition (high stress), participants were physically prompted to complete the activities of daily living such as folding laundry, brushing hair, and vacuuming. These activities were selected based on parental/caregiver reports that they were difficult or impossible for the participants to complete without assistance. It was therefore expected that these activities would produce more anxiety or arousal than the play activities of the control sessions. Video recordings of all of the free play and escape sessions for each participant were coded for the occurrence of hand stereotypy and signs of negative affect.

2.3. Operational definitions

After viewing several video clips of the participants engaging in a variety of activities, two raters developed individualized operational definitions of hand stereotypy and negative affect. Based, in part, on Mahone, Bridges, Prahme, and Singer (2004) stereotyped hand behavior included hand clasping, hand clenching, hand clapping, hand clapping, hand wringing, and finger rubbing. *Hand Clasping* (KP, ML, ZC) was defined as any instance in which the participant clasped her hands together without moving her hands and then released them. *Hand Clenching* (KP, ML) was defined as any instance in which the participant opened and closed her fingers to make a fist with either one or both of her hands more than once within 3–s or less in between clenches. *Hand Flapping* (SG) was defined as any instance where the participant moved her arms and/or wrists with her hands loose so that her hands would repeatedly move in a back and forth motion without making contact with any surface. *Hand Mouthing* (ML, ZC) was defined as any part of the participant’s hands except for her thumb being inside of her mouth (thumb sucking was coded separate and did not occur during sessions within the two conditions). *Hand tapping* was observed for two participants, but in slightly different forms. For KP, *hand tapping* was defined as any instance in which one or all of her fingers or her palm repeatedly made contact with a non-toy surface (carpet or her legs) more than once with either one or both of her hands within three-seconds or less in between taps. For SG, *hand tapping* was any combination of her clapping her hands together and tapping her chest with one or both of her hands. JS was the only participant to engage in *hand wringing*, defined as any instance in which she brought her hands together at her torso and moved her hands in a clockwise or counterclockwise rotation together until they ceased to make contact. Finally, *finger rubbing* (ZC) was defined as any instance where one or more of the participant’s fingers in one hand made repeated contact with the fingers in the same hand by moving back and forth together.

Negative affect was based on three domains adapted from the anxiety subscale of the Diagnostic Assessment for the Severely Handicapped scales (DASH-II) including distressed vocalizations, facial grimacing, and trembling (Matson, Gardner, Coe, & Sovner, 1991). *Facial grimacing* was defined as any scrunching of the eyes, brows, nose, and/or mouth in a downward motion for any period of time with a subsequent release back to a neutral expression but does not occur while crying. *Trembling* was defined any instance in which the participant’s head, torso, arms or legs moved in a quick, short, shaky fashion for more than 1 s (trembling was not coded during body rocking). *Distressed vocalization* was defined as any audible instance of a gasping, sobbing, screeching, or yelling utterance in the absence of a visible smile for any period of time.

2.4. Measurement system and inter-observer agreement

A 10-s partial interval coding system was used (Gast, 2010). There were a total of 30 intervals per session. For each interval, target behaviors (stereotypy and negative affect) occurrence (1) and nonoccurrence (0) was scored. All data were collected by watching digital video and hand scored (i.e., paper and pencil) then entered into an Excel spreadsheet for analysis. Following training and calibration sessions, for inter-observer agreement (IOA) purposes, two coders independently coded 37.5% of the sessions for each individual participant. Agreement was defined as the number of agreements between observers divided by the sum of agreements and disagreements multiplied by 100 (to yield a percentage agreement). Individual IOA percentages as follows: KP = 99.9%, ML = 86%, JS = 100%, ZC = 96%, and SG = 90.5%.
2.5. Data analysis

Because the number of sessions of each condition varied across participants, and was insufficient to compare within-sessions rates of behaviors across conditions, data from each condition (low stress and high stress) were pooled to create two-by-two contingency tables for each participant to compare the number of intervals with and without target behaviors (hand stereotypy, negative affect) between the two conditions. The proportion of intervals with and without hand stereotypy, and negative affect across high- and low-stress conditions was then analyzed using Pearson’s Chi-square coefficients.

3. Results

3.1. Hand stereotypy

When the data were combined across all five participants (Fig. 1), the percent of intervals that hand stereotypy occurred did not differ statistically across the two conditions (low vs. high stress) ($\chi^2(1, N = 98) = 1.06, p = .30$). Individual results are presented in Table 1. ML’s hand stereotypy (hand clenching, hand clasping) was not significantly different across the two conditions ($\chi^2(1, N = 270) = .033, p = .85$) (Fig. 2a); KP’s hand stereotypy (hand tapping) was not significantly different across the two conditions ($\chi^2(1, N = 240) = .022, p = .88$) (Fig. 2b); ZC’s hand stereotypy (hand clapping, finger rubbing) was not significantly different across the two conditions ($\chi^2(1, N = 150) = .643, p = .42$) (Fig. 2c); SG’s hand stereotypy (hand flap, hand tap) did not differ significantly between conditions ($\chi^2(1, N = 150) = 1.08, p = .29$) (Fig. 2d); JS’s hand stereotypy (hand tapping) did not differ between conditions, with hand stereotypy occurring in 100% of intervals across both conditions (Fig. 2e).

3.2. Negative affect

When the data were combined across all five participants (Fig. 1), the percent of intervals in which negative affect was expressed was significantly greater in the high-stress (escape) versus low-stress (free play) condition ($\chi^2(1, N = 979) = 111.58, p < .001$). Individual comparisons were as follows: for ML, negative affect was more likely to be expressed in the high-stress condition ($\chi^2(1, N = 270) = 18.58, p < .001$), for KP, negative affect was more likely to be expressed in the high-stress condition ($\chi^2(1, N = 240) = 98.017, p < .001$) (Fig. 2b); ZC’s negative affect was more likely during the high-stress condition ($\chi^2(1, N = 150) = 5.19, p < .05$) (Fig. 2c); SG’s negative affect was more likely during the high-stress condition ($\chi^2(1, N = 150) = 14.95, p < .001$) ($\chi^2(1, N = 150) = 1.08, p = .29$) (Fig. 2d). For JS, there was a marginal significant difference for negative affect, such that it was more likely to be expressed in the high stress condition ($\chi^2(1, N = 180) = 3.37, p = .06$) (Fig. 2e).

In terms of individual level expression of negative affect, there were differences across participants for the three main coding categories (‘distressed vocalizations’, ‘trembling’, ‘facial grimacing’; see Table 1). ML and KP were both more likely to express distressed vocalizations, trembling, and facial grimacing in the high stress versus low stress conditions, whereas ZC was only more likely to express trembling and JS was only more likely to express distressed vocalizations in the high stress conditions.

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### Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>ML</th>
<th>KP</th>
<th>ZC</th>
<th>SG</th>
<th>JS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand Clenching</td>
<td>25%</td>
<td></td>
<td>20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand Clasping</td>
<td>20%</td>
<td></td>
<td>15%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand Flapping</td>
<td>15%</td>
<td></td>
<td>10%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 1.** Relationship between the percent of intervals negative affect and hand stereotypy occurred across the high stress and low stress conditions combined across all 5 participants. ***p < .001, **p < .01, *p < .05; error bars represent 95% confidence intervals with continuity correction as described by Newcombe (1998).**
versus low stress conditions. Similar to ML and KP, SG expressed more distressed vocalizations and facial grimacing in the high stress conditions, but did not exhibit any instances of trembling.

4. Discussion

The present study examined the hypothesis that behavioral indicators of negative affect and the occurrence of hand stereotypies would differ across putatively high- and low-stress environmental conditions in a small consecutively enrolled clinical sample of females with classic RTT. Despite the sense that hand stereotypy (a diagnostic feature of RTT) may be an indicator of increased anxiety or arousal associated with stress (Lotan & Ben-Zeev, 2006), there were no observed differences in hand stereotypy across the low and high stress conditions for any of the participants. There were, however, clear differences in the frequency of signs of negative affect across the two conditions (more frequent signs of negative affect were

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### Table 1

Percent of intervals different forms of negative affect observed across participants during high-stress condition and low-stress condition.

<table>
<thead>
<tr>
<th></th>
<th>Distressed vocalizations</th>
<th>Facial grimacing</th>
<th>Trembling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>ML</td>
<td>14% (0–16%)</td>
<td>3% (3–23%)</td>
<td>18% (10–23%)</td>
</tr>
<tr>
<td>KP</td>
<td>16% (0–33%)</td>
<td>3% (0–10%)</td>
<td>27% (16–37%)</td>
</tr>
<tr>
<td>ZC</td>
<td>0% (0–0%)</td>
<td>0% (0–0%)</td>
<td>2% (0–3%)</td>
</tr>
<tr>
<td>JS</td>
<td>47% (47–47%)</td>
<td>30% (14–48%)</td>
<td>10% (10–10%)</td>
</tr>
<tr>
<td>SG</td>
<td>52% (40–63%)</td>
<td>28% (7–47%)</td>
<td>63% (50–77%)</td>
</tr>
</tbody>
</table>

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Fig. 2. Relationship between the percent of intervals negative affect and hand stereotypy occurred across the high stress and low stress conditions for ML, KP, ZC, SG, and JS. ***p < .001, **p < .01, *p < .05; error bars represent 95% confidence intervals with continuity correction as described by Newcombe (1998).
associated with the high stress condition) for each of the participants. These preliminary findings suggest that the assessment conditions elicited behaviors that are considered related to stress and anxiety (Matson et al., 1991).

These results differ from previous studies that found changes in the rates of hand stereotypy across different environmental conditions (Hetzroni & Rubin, 2006; Sigafos et al., 2000; Stasolla & Caffo, 2013). The contexts examined in those studies, however, were not designed to evaluate the effects of stress or anxiety through the use of controlled, program contingencies created on the basis of a preference assessment. However, the previous studies may have observed differences reflecting changes in the level of engagement or attention, or other unknown cognitive or biological factors.

In addition to overall increased negative affect, there were differences in the three negative affect domains (face, vocalize, tremble) coded across the participants. Although a larger sample size would be needed to tease out whether the differences in expression modality related to specific genotype–phenotype relationships or to the severity of the stressor, it does indicate a range of response that providers and caregivers should be willing to consider with regard to mounting a stress response. This is consistent with the findings of Bergström-Isacsson et al. (2013), who demonstrated that trained observers could reliably identify emotional facial expressions associated with different types of music using the Facial Action Coding System (FACS; Ekman, Friesen, & Hager, 2002) among a sample of females with RTT.

Interestingly, Bergström-Isacsson et al. (2013) also found that certain facial expressions, such as false smiles, exhibited by the participants with RTT were associated with brainstem activation, which appears to be immature in RTT. It is therefore possible that some displays of facial affect by individuals with RTT are unrelated to their emotional states or the environmental context. Nevertheless, the rates of clearly identifiable positive and negative facial expressions that they observed, along with the consistent differences in signs of negative affect across the high- and low-stress conditions in the current study support the notion that individuals with RTT display their emotional states using facial expressions and other behavioral indicators. Determining with more certainty whether the observed changes reflected anxiety or stress, however, would require additional study.

Hand stereotypy, on the other hand, may not be a reliable sign of stress or anxiety in this population. This claim certainly needs to be qualified by the limits of the study design (small clinical sample of convenience, assumption that behavioral assessment sessions were reliably discriminated as ‘high’ or ‘low’ stress, and lack of intensity measure of motor stereotypy). Additional research is therefore needed to clarify the issue, and to identify reliable and objective indicators of stress and anxiety in this population. In the meantime, the automatic assumption that changes in hand stereotypy are related to stress or anxiety in this population may lead parents and other caregivers to over- or underestimate the frequency and intensity of anxiety that their children may be experiencing.

Despite the preliminary nature of the current study, the results clearly indicate that there were increases in the rates of behaviors consistent with negative affect during the ‘high stress’ conditions for all of the participants, suggesting that the participants discriminated between conditions and responded accordingly. For future work, one way to improve our clinical understanding of anxiety in RTT would be to incorporate a ‘biomarker’ approach paired with behavioral tests such that indicators of stress physiology (such as salivary cortisol or heart rate variability) would be included in the analysis model to more fully account for known endocrine and autonomic regulatory problems in RTT (McGill et al., 2006; Nuber et al., 2005). Combining such physiological measures with objectively defined behavioral indices, such as the FACS model, may lead us to a better understanding of the experience and expression of anxiety and stress in this population. Clinically, the ability to identify reliable and unbiased signs of anxiety in females with RTT would help practitioners, educators, and parents better understand the emotional status of their patients, students, and children to help them lead a more healthy and functional life.

Acknowledgments

We are grateful to the families and their daughters for their willingness to participate in research. We would like to recognize Dr. Celia Gershenson and the late Dr. Herb Pick for their contributions to the REU program. Support for this work was provided, in part, by NSF Grant # SMA-1063006 at the University of Minnesota and NICHD Grant No. 44763.

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