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Safety, pharmacokinetics, and preliminary assessment of
efficacy of mecasermin (recombinant human IGF-1) for the
treatment of Rett syndrome

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Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder mainly affecting females and is associated with mutations in MECP2, the gene encoding methyl CpG-binding protein 2. Mouse models suggest that recombinant human insulin-like growth factor 1 (IGF-1) (rhIGF1) (mecasermin) may improve many clinical features. We evaluated the safety, tolerability, and pharmacokinetic profiles of IGF-1 in 12 girls with MECP2 mutations (9 with RTT). In addition, we performed a preliminary assessment of efficacy using automated cardiorespiratory measures, EEG, a set of RTT-oriented clinical assessments, and two standardized behavioral questionnaires. This phase 1 trial included a 4-week multiple ascending dose (MAD) (40–120 μg/kg twice daily) period and a 20-wk open-label extension (OLE) at the maximum dose. Twelve subjects completed the MAD and 10 the entire study, without evidence of hypoglycemia or serious adverse events. Mecasermin reached the CNS compartment as evidenced by the increase in cerebrospinal fluid IGF-1 levels at the end of the MAD. The drug followed nonlinear kinetics, with greater distribution in the peripheral compartment. Cardiorespiratory measures showed that apnea improved during the OLE. Some neurobehavioral parameters, specifically measures of anxiety and mood also improved during the OLE. These improvements in mood and anxiety scores were supported by reversal of right frontal alpha band asymmetry on EEG, an index of anxiety and depression. Our data indicate that IGF-1 is safe and well tolerated in girls with RTT and, as demonstrated in preclinical studies, ameliorates certain breathing and behavioral abnormalities.

Significance

This paper provides unique insights into mechanism-based
therapeutics for Rett syndrome (RTT), a devastating
neurodevelopmental disorder. This clinical trial was based on pioneer preclinical work from the laboratory of M.S. Outcome measures include clinical instruments, standardized behavioral measures, and biomarkers, the latter being not only objective but also applicable to experimental studies. We believe this work will have a major impact on the understanding and treatment of RTT, as well as other neurodevelopmental disorders.


The authors declare no conflict of interest.

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2Deceased March 10, 2009.

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(IGFBP3), a major IGF-1-binding factor that is increased in brains of RTT patients and Mecep2-null mice (11). Furthermore, administration of IGF-1 restores dendritic spine dynamics in Mecep2-deficient mice (12). The most compelling data supporting IGF-1 as a treatment for RTT come from two studies demonstrating that systemic administration of either full length IGF-1 or its active peptide fragment reverses, at least partially, many RTT-relevant features in Mecep2-deficient mice (13, 14). Among the latter are locomotor function impairment, breathing abnormalities, and heart rate irregularities. These improvements seem to reflect IGF-1’s effect on defective synaptic maturation and maintenance secondary to Mecep2 deficit (14).

Mecasermin, recombinant human IGF-1, is already Food and Drug Administration-approved for the long-term treatment of growth failure in children with severe primary IGF-1 deficiency (Laron syndrome) (15). We carried out a multiple ascending dose (MAD) study followed by an open-label extension (OLE) period with mecasermin in a group of 12 girls with MECP2 mutations, 9 of whom had RTT. Here, we report our findings on safety, tolerability, pharmacokinetics (PK), and preliminary assessments of efficacy. The latter include evaluations of neurobehavioral measures, exploratory biomarkers, and their corresponding pharmacodynamics (PD) data.

Results

Twelve girls with MECP2 mutations participated in the 4-wk MAD; 10 of them continued and completed the subsequent 20-wk OLE. Fig. S1 illustrates the timeline of this phase 1 trial. Participants’ demographic and baseline characteristics are shown in Table 1. Nine subjects met full diagnostic criteria for RTT and all continued in the OLE. The 4-wk MAD focused on obtaining PK data, determining cerebrospinal fluid (CSF) penetration, initial evaluations of safety and tolerability, and estimating feasibility of automated cardiorespiratory measures as biomarkers for treatment response. The OLE was designed to obtain additional information on safety, tolerability, and the aforementioned cardiorespiratory measures after chronic dosing, as well as preliminary data on neurologic and behavioral parameters of clinical relevance to RTT. These neurobehavioral evaluations were based on questionnaires and assessments used in an ongoing multisite longitudinal study [the Rett Natural History study (U54 HD061222)] and on two standardized measures of problem behaviors. Data on safety, tolerability, and PK is reported for all 12 MECP2 mutation-positive subjects, whereas preliminary efficacy and PD data only for the 9 subjects with RTT.

During the MAD, mecasermin dosing was escalated over a 4-wk period, beginning with twice daily (BID) injections of 40 μg/kg the first week, 80 μg/kg the second week, and 120 μg/kg during the third and fourth weeks, as depicted in Fig. S2. CSF samples were obtained before drug administration and after completing the fourth week (Fig. S2). Fig. 1 illustrates levels of IGF-1 and IGFBP3, the main IGF-1-binding protein (10, 11), in serum and CSF. There was a significant increase in IGF-1 but not IGFBP3 in both compartments at the end of the MAD. At the start of the OLE, subjects went through an identical dose escalation, staying on the maximum dose of 120 μg/kg for the remaining 17 wk of treatment.

Serum IGF-1 concentrations were first analyzed by a non-compartmental analysis (16) comparing different doses. A log-linear terminal phase was observed for 4-6 h postdosing (Fig. 24). The slopes of this decay allowed the estimation of terminal elimination half-lives ($t_{1/2}$) and mean residence times in the body (MRT$_{b}$) as shown in Table S1. Maximal concentrations ($C_{max}$) and the times to reach them ($t_{max}$) were also documented. The areas under the curve (AUC$_{t}$; time of last observation) up to the last observation lacked dose proportionality, suggesting nonlinear kinetics (Fig. 2B). The starting dose of 40 μg/kg elicited a mean AUC$_{t}$ = 2.050 ng·h/mL, while the area for twice that dose increased by just 75%. When the starting dose was tripled, the increment was nearly the same. Nonlinearity is also supported by the early parts of the concentrations profiles, with upward deviations after reaching maximum levels. We also carried out a compartmental analysis using a two-compartment model based on calculated Akaike and Bayesian information criteria, as well as on the residuals analysis (16). A Michaelis-Menten elimination kinetics (16) with first order absorption and a distribution clearance parameterization provided the best goodness of fit, compared with first order or mixed elimination alternatives. The volume of distribution for the central compartment ($V_{c}/F$) was estimated to be $7.71 \pm 0.78$ L (mean ± SE) and for the peripheral compartment it was $33.5 \pm 16$ L. The other parameters in the model were estimated for intercompartmental clearance as $0.58 \pm 0.048$ L/h, for the maximum elimination rate as $1.02 \pm 0.4 \mu$g·kg$^{-1}$·h$^{-1}$, and for the Michaelis-Menten constant as $4.62 \pm 3.7$ ng/mL. Individual subjects’ noncompartmental curves are depicted in Fig. S3 and Fig. S4 demonstrates the appropriateness of the proposed models (i.e., predicted vs. observed grouped data).

Based on direct compliance monitoring and serum levels, s.c. injections were well tolerated and no incidences of hypoglycemia or errors in dose administration were detected. During the MAD, one serious adverse event occurred (respiratory distress) and was determined to be unrelated to the study drug (10, 15). During the MAD, only two adverse events (nausea and vomiting) were considered as possibly related to the study drug and preceded withdrawal from the OLE. A similar profile of safety and

### Table 1. Subject demographics and characteristics at baseline

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Diagnosis</th>
<th>Stage</th>
<th>MECP2 mutation</th>
<th>Concomitant medications</th>
<th>Breathing phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Classic</td>
<td>II</td>
<td>R168X</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>MRD*</td>
<td>n/a</td>
<td>C1135_1142 del</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>MRD*</td>
<td>n/a</td>
<td>C1135_1142 del</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Classic</td>
<td>II</td>
<td>C790_808 del</td>
<td>Levetiracetam</td>
<td>BH, HV</td>
</tr>
<tr>
<td>5</td>
<td>Classic</td>
<td>III</td>
<td>Large del exon 3 and 4</td>
<td>None</td>
<td>BH</td>
</tr>
<tr>
<td>4</td>
<td>Classic</td>
<td>III</td>
<td>C1159_1273 del</td>
<td>None</td>
<td>AE</td>
</tr>
<tr>
<td>8</td>
<td>Classic</td>
<td>III</td>
<td>R255X</td>
<td>Lamotrigine, lorazepam, melatonin</td>
<td>BH, AE</td>
</tr>
<tr>
<td>4</td>
<td>Classic</td>
<td>III</td>
<td>R255X</td>
<td>None</td>
<td>AE</td>
</tr>
<tr>
<td>8</td>
<td>Classic</td>
<td>III</td>
<td>T158M</td>
<td>None</td>
<td>BH, AE</td>
</tr>
<tr>
<td>3</td>
<td>MRD</td>
<td>n/a</td>
<td>R306C</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>Classic</td>
<td>III</td>
<td>Large del exon 1 and 2</td>
<td>Gabapentin, diastat</td>
<td>BH, AE, cyanosis</td>
</tr>
<tr>
<td>8</td>
<td>Classic</td>
<td>III</td>
<td>P322L</td>
<td>Levetiracetam</td>
<td>BH, AE, cyanosis</td>
</tr>
</tbody>
</table>

*AE, air expulsion; BH, breath holding; HV, hyperventilation.

*Subjects did not continue in OLE.

*Staging not applicable (n/a) to non-RTT.

*Subject with mild apnea (apneic episodes >10 s and <5 apneas per hour).

*Subjects with moderate-severe apnea (apneic episodes >10 s and >5 apneas per hour).
Levels of IGF-1 were not significantly different between pre- and post-MAD. The mean and SE of IGF-1 and IGFBP-3 in serum and CSF are shown (Table S1). Post-dosing, IGF-1 levels in serum and CSF were doubled, indicating IGF-1 reaches the CNS compartment. IGFBP3, the main IGF-1-binding protein, did not significantly increase in serum or CSF.

tolerability, with no unexpected, progressive, or related serious adverse events, was observed during the OLE. Although a high proportion of the subjects had abnormal cholesterol levels at baseline, these did not worsen during the trial. For details on adverse events, see Table S2.

Using cardiorespiratory data obtained with a BioTelemetry device (17), we calculated the apnea index (18) and hyperventilation index (19) and compared the start and end of the MAD (pre-MAD), start and end of the OLE (pre- to post-OLE), and beginning and end of the entire trial (pre-MAD to post-OLE). We applied paired t-tests, Wilcoxon signed rank tests, and a random intercept (RI) model, including time effects at each time point (post-MAD, pre-OLE, and post-OLE compared with pre-MAD). As illustrated in Table 2 (see “apnea index by time point” entries), based on the RI model which accounts for within-subjects correlation, the improvement in the apnea index was significant at the end of the OLE in comparison with start of the MAD. Improvements in the apnea index were comparable when only the five subjects with clinically significant apnea (apneic episodes >10 s), four of whom had moderate–severe apnea, were included in the analyses (Table S3). In the stress test, the effect of age as a covariate in the RI model for all subjects with RTT. The effect of age and its interaction with the respective time points was positive and significant, namely the improvements in the apnea index were more significant in older subjects. These patterns of improvement were not observed for the hyperventilation index (see “hyperventilation index by time point” entries in Table 2).

The specificity of the apnea index improvements is underscored when other respiratory parameters (20), typically not used in the clinical context, are examined. Table S4 shows that during the OLE, for instance, the percent epoch in slow respiratory rate and the mean total respiratory cycle times (Ttot) in slow respiration also decreased significantly but not the per cent epoch in rapid respiratory rate and the mean Ttot in rapid respiration. Similar results were found in the MAD. There were also changes in the cardiac parameters, namely a reduction in the percent epoch in normal heart rate with a concurrent increase in the percent epoch in rapid heart rate when the beginning and end of the OLE were compared. Variance in heart rate also decreased, although not significantly (Table S4). Similar to the breathing parameters, changes in cardiac variables demonstrated the same trend during the shorter MAD and the longer OLE. Preliminary PD analysis also suggested a positive response, namely a decrease in the apnea index, over the course of treatment. Just in a few cases this decrease leveled off, or, in one case, seemed to revert at the end of the MAD (Fig. S6 illustrates examples of different PD profiles).

During the OLE, preliminary efficacy data were gathered by administering two RTT-oriented clinician assessments and two standardized behavioral measures to the nine RTT subjects. We focused on established instruments already reported in the literature (21–25), and did not include parent or clinician global impression assessments, to decrease data subjectivity and allow for future comparisons with other publications. Neurologic and behavioral parameters were measured by two evaluators (blind to the Rett Syndrome Behavior Questionnaire (RSBO) (22, 23) and the Anxiety Depression and Mood Scale (ADAMS) (24, 25). We performed exploratory comparisons between onset and end of the OLE using t-tests and the Wilcoxon signed rank test. Although not significantly different, total scores showed a trend toward improvement in all instruments. We then organized the subscales of these measures into neurobehavioral domains (e.g., motor, breathing/autonomic, problem behavior) and subjected them to exploratory t-tests comparing pre-MAD and post-OLE. We followed these hierarchical analyses by examining the items in the same subscales. These analyses revealed significant or trend-level changes in the breathing/autonomic and behavioral domains. However, the direction of change in breathing and peripheral autonomic subscales were inconsistent. For instance, breath-holding items in the RSBO showed improvement whereas those on the clinical assessment (CA) and motor–behavioral assessment (MBA) worsened. Similar inconsistencies were present for peripheral autonomic scales/items. Subscales and items representing alertness, activity, anxious behaviors, or abnormal mood demonstrated consistent improvements, whereas those recording irritability, aggressiveness, disruptive/hyperactive behavior, communication, and motor domains did not (Table 3 and Fig. S7).

Relative right-sided resting frontal (alpha band) EEG asymmetry has been used in multiple studies as an index of anxiety and depression (26), including pediatric populations (27). Left (L) greater than right (R) alpha power is typically interpreted as more positive vs. negative (less anxiety vs. more anxiety) behavior, whereas R > L is viewed as the reversed. As depicted in Fig. 3, six subjects evaluated during the OLE with EEG demonstrated R > L asymmetry (i.e., more anxiety). Although the degree of asymmetry was variable, five of the six showed a decrease in the asymmetry index and in three it was reversed. A paired-samples t-test revealed that this group trend toward L > R asymmetry (i.e., reduction in anxiety) was significant. Moreover,
the group reduction in the R > L asymmetry index correlated with improvements in measures of mood abnormalities and, to lesser extent in measures of breathing abnormalities and anxiety (Table S5). Analyses of cardiorespiratory and neurobehavioral parameters excluding the two individuals in Hagberg stage II (i.e., end of regression period) did not yield significantly different results from those including all nine RTT subjects.

Discussion
Our findings indicate that IGF-1 is safe for use in girls with MECP2 mutations, including those meeting diagnostic criteria for RTT. We found that mecasermin reaches the CNS and that its kinetics are complex, as expected from a protein that is cleaved and binds its receptor and interacting proteins (10, 11, 28, 29). Our preliminary efficacy analyses suggest that, when administered over several weeks, mecasermin improved certain aspects of the RTT phenotype, most notably, abnormal behaviors (i.e., anxiety) and breathing abnormalities (i.e., apnea). Changes in breathing abnormalities were better characterized using automated measurements of cardiorespiratory function. The evaluation of potential biomarkers also successfully delineated behavioral abnormalities with right-sided frontal alpha band EEG asymmetry, an index of anxiety and depression, showing a trend toward reversal in most RTT subjects exhibiting the phenomenon. Overall, the findings of this phase 1 trial are in agreement with preclinical data suggesting IGF-1 is a safe and beneficial treatment for RTT (13, 14).

As recently reported by Pini et al. (30), mecasermin administration is relatively safe and well tolerated. In our own phase 1 study, several expected adverse events, such as increased tonsil size and related snoring, were observed but were relatively mild and nonprogressive and did not lead to withdrawal from the trial. Most subjects had elevated cholesterol; however, this preceded IGF-1 administration and did not worsen with the drug. Therefore, concerns about metabolic syndrome raised by a recent animal study (31) were not supported by our trial. The most common adverse event, early signs of puberty, may be significant as some reports have shown accelerated puberty (i.e., early adrenarche) in RTT (32, 33). Nonetheless, because hormonal levels were within normal ranges throughout the study, this issue deserves further investigation. In summary, at the doses used in this (240 μg·kg⁻¹·d⁻¹) and the previously published (200 μg·kg⁻¹·d⁻¹) trial (30), mecasermin is a safe treatment.

Our data indicate that mecasermin administration increases IGF-1 levels in the CNS (10); therefore, our data on efficacy and some of the adverse events could be attributed to the presence of IGF-1 in the brain. The IGF-1 increase in CSF depicted in Fig. 1 is consistent with the increase in peripheral IGF-1 levels found in healthy volunteers and children with primary IGF-1 deficiency (39). As we observed, chronic treatment PK studies have suggested a plateau effect for doses between 160–240 μg·kg⁻¹·d⁻¹, probably reflecting saturation of IGFBP3 (38). The nonlinear PK kinetics, greater volume distribution in the peripheral than the central compartment, and lack of change in IGFBP3 in serum and CSF suggest that serum levels of IGF-1 for may not be the best basis for dosing and that higher or chronic dosing in RTT may not necessarily result in higher exposures or a sustained exposure–response relationship. This leads to careful consideration of dosing for future studies where acute intermittent pulses of mecasermin may be more effective than chronic dosing. The mouse model may be useful in exploring optimum dosing regimens.

Our study confirmed the feasibility of automated cardiorespiratory measurements as biomarkers of treatment response (40). It also indicates that these breathing evaluations may be more reliable and valid than clinical instruments because parent questionnaire data were in disagreement with clinicians’ observations. Whether these discrepancies reflect different lengths of observations (i.e., days to weeks for parents vs. minutes for clinicians) is unclear. Regardless, the measurements obtained during both the MAD and OLE demonstrate a consistent trend toward improved breathing. Other parameters obtained during the automated assessments further emphasize IGF-1’s selective effect on slow breathing, initially shown in the RTT mouse model (14). Although the lack of improvement in hyperventilation may have been influenced by technical issues (e.g., movement artifact), the selective effect on apnea is still desirable as it is perceived as more concerning clinically (41). Our preliminary dose–response analyses suggest that reduction in the apnea index is the result of IGF-1 administration; nonetheless, the reverting trend observed in a few subjects toward the end of the MAD (Fig. S6) may reflect the aforementioned saturation kinetics of IGF-1. Additional PD analyses, focusing on exposure–response relationships, need to be conducted to clarify this issue. The effects of IGF-1 on cardiac function were challenging to interpret. Although decreased heart rate variability may be seen as positive, its association with a trend toward higher heart rate may be considered a potential side effect. However, heart rate values remained within the wide normal range (42). Although the possible effect of mecasermin on heart rate warrants further investigation, our findings are in line with the partial correction of bradycardia in the Meep2-null mouse (14).

Our preliminary efficacy evaluations on neurobehavioral parameters provided a mixed picture. Whereas some measures indicated improvements, others worsened. This was particularly the case for abnormalities in breathing and peripheral autonomic function. A similar inconsistent pattern was found for externalizing problem behaviors, such as disruptive and irritable behaviors. Two other important domains—communication and motor function, including abnormal movements—did not show a change.

Table 2. Summary of breathing indices for all RTT subjects by time point (n = 9)

<table>
<thead>
<tr>
<th>Breathing indices</th>
<th>Pre-MAD</th>
<th>Post-MAD</th>
<th>Pre-OLE</th>
<th>Post-OLE</th>
<th>Pre-MAD to Post-OLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apnea index (mean ± SE)</td>
<td>10.11 ± 19.34</td>
<td>5.11 ± 9.68</td>
<td>4.67 ± 6.81</td>
<td>3.00 ± 5.72</td>
<td>−7.12 ± 4.58</td>
</tr>
<tr>
<td>Student’s t P</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.159</td>
</tr>
<tr>
<td>Wilcoxon signed rank P</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.094</td>
</tr>
<tr>
<td>RI model P</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.018</td>
</tr>
<tr>
<td>Hyperventilation index (mean ± SE)</td>
<td>3.55 ± 6.71</td>
<td>3.00 ± 6.59</td>
<td>6.44 ± 16.86</td>
<td>3.66 ± 8.97</td>
<td>0.12 ± 0.93</td>
</tr>
<tr>
<td>Student’s t P</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.908</td>
</tr>
<tr>
<td>Wilcoxon signed rank P</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.675</td>
</tr>
<tr>
<td>RI model P</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.963</td>
</tr>
</tbody>
</table>
It is important to recognize the limitations of the present study. The first limitation is the relatively small sample and age range considering the dynamics of RTT. Nine of the subjects met RTT diagnostic criteria and only seven were at a stable period (Hagberg stage III) (2). Nevertheless, analyses excluding the two individuals in stage II did not yield different results. Although the inclusion of twins with MECP2-related disorder (MRD) allowed for the examination of safety and PK in individuals with other MRDs, it also decreased the variability of the sample. This study was designed to assess CNS penetration and PK profile of IGF-1, and to test the feasibility of automated cardiorespiratory measures; as such, RTT subjects were not selected on the basis of breathing abnormalities or specific profiles of neurobehavioral impairment. This increased the heterogeneity of the already small sample, leading to diminished statistical power. Analyses of the clinically oriented measures used discovery type statistics without correcting for multiple comparisons and emphasizing the consistency of the body of data rather than specific parameters. On the other hand, comparisons between onset and end of the OLE, without considering intermediate time points may have overlooked transient positive effects of IGF-1. Although measures from the Rett Natural History study (21) were selected because of their relevance, these instruments have not been validated as outcome measures, and discrepancies between the parent questionnaire and clinician assessment need to be further examined. Also, the ADAMS (24, 25), has not been validated in RTT. Increased care and placebo effect could have also influenced our neurobehavioral findings. Nonetheless, the use of automated measures such as the BioRadio for cardiorespiratory function (17) or EEG asymmetry profiles for anxiety and mood (26, 27) strengthened clinician- and parent-reported data and support future exploration of biomarkers. Additional biomarker data—namely the Q sensor (47) for recording motion and hand stereotypes and visual evoked potentials for examining cortical function (48)—was collected as part of this trial and needs to be analyzed and reported in future publications.

### Methods

**Sample.** Characteristics of our cohort are shown in Table 1 and SI Methods. The study was approved by the Institutional Review Board of Boston Children’s Hospital and informed consent was obtained from the parent of each participant. Further information is provided in SI Methods.

**Study Design and Safety Measures.** Unblinded phase 1 study designed to establish PK profile (4-wk MAD) and long-term safety and tolerability (20-wk OLE) of IGF-1 in girls with RTT (Fig. S1). Subjects received twice daily (BID) s.c. injections at 40 μg/kg (week 1), 80 μg/kg (week 2), and 120 μg/kg (weeks 3, 4, OLE) (Fig. S2). Safety was assessed by evaluations listed in Table S6. Detailed information is provided in SI Methods.

**PK and PD Analyses.** Sera were obtained at different daily time points during the MAD, and at each visit during the OLE, while CSF only at the beginning and end of the MAD (Fig. S2). Methodologies for IGF-1 and IGFBP3 measurements, and PK and pharmacodynamics analyses, are detailed in SI Methods.

**Automated Cardiorespiratory Measures.** Time synchronized chest respiratory inductive plethysmography, three lead electrocardiography, and video recordings are detailed in SI Methods.
Neurobehavioral Assessments. Table S4 lists the multiple measures of neurologic and other functions obtained during the OLE. Additional information is presented in SI Methods.

EEG Recordings. EEG recording, spectral power analysis, and frontal asymmetry scores were performed as reported (49–51) and detailed in SI Methods.

Statistical Analyses. Standard descriptive and comparative statistics were employed. Specific tests are specified in Results and SI Methods.