**Early life exposure to unpredictable maternal sensory signals influences cognitive development: A cross-species approach**

*Supporting Information – Measuring predictability: a cross-species approach*

## S.1 Overview

Maternal behavior in both rodents and humans was modeled as a stochastic process (specifically a time-homogeneous, stationary Markov chain) and predictability (as a continuous measure) was quantified through the entropy rate of the Markov process. The following sections describe how observed behaviors were coded, describe how Markov chains were constructed from the observed data, display visualizations illustrating sample data, and provide an overview of the estimation procedure and examples of different realizations of the entropy rate observed within the data.

### S.1.1 Constructing Sequences of Behavior

*Rodent Data*

Maternal behavior was recorded by directly observing the dams during 50-minute windows twice per day for eight days. The set of actions considered as characterizing maternal behavior were, licking/grooming the pups (LG), carrying pups (C), eating (E), nursing (N), nest building (NB), off pups and no other activities (O), or self-grooming (SG). Each dam could only be performing a single behavior at any given time point. This provides a continuous time-series of the dam's behavior. Figure S-1A is a visualization of this time-series. We posit that predictability of maternal sequences of sensory signals is critical to the developing brain. As a result, we focus on the transitions between behaviors. We combine data from the different observation periods; thereby assuming the sequence is a stationary process in time. This creates a discrete-time sequence of distinct behaviors, illustrated in Figure S-1B.

The sequence of behaviors can be summarized by considering how often specific *transitions* occur; e.g., how many times self-grooming behavior is followed by nursing. A matrix can be used to organize the transition frequencies with rows indicating the initial behavior in each pair of actions and columns the behaviors being transitioned into. Normalizing each row by the row total provides the proportion of time each "column" event follows each "row" event. These are known as transition probabilities. We visualize this in Figure S-2. Note that because we have

concatenated results from different observation periods there is a small probability of transitioning from a behavior to the same behavior.

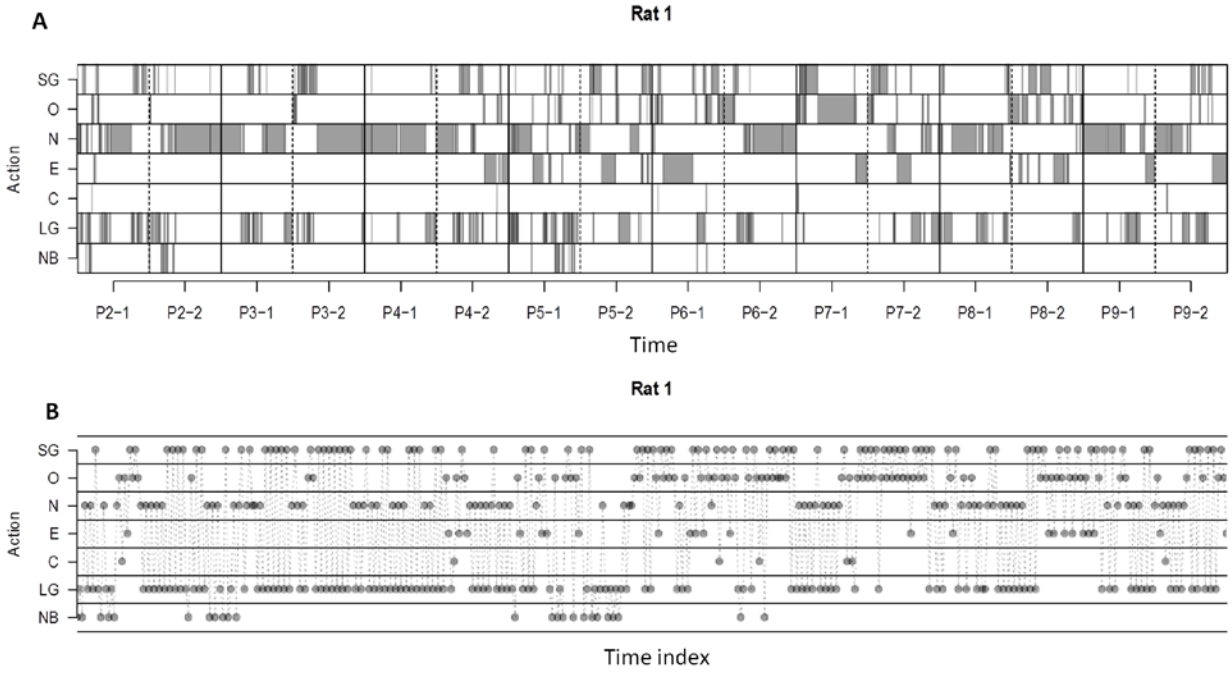


**Figure S-1.** S-1A displays a visualization of maternal behavior for two 50-minute periods in postnatal days P2-P9. Each row of the figure is a different action. The vertical lines crossing all rows in the figure correspond to beginning/ending of the 50-minute windows. Within each row, the solid blocks are durations during which each action was performed (these may appear as lines if the duration is short enough). S-1B is a Visualization of transitions in a dam's behavior. The data are the same as in Figure S-1 but each behavior has been reduced to a single dot so the duration is not pictured. Divisions between observation periods are not shown.

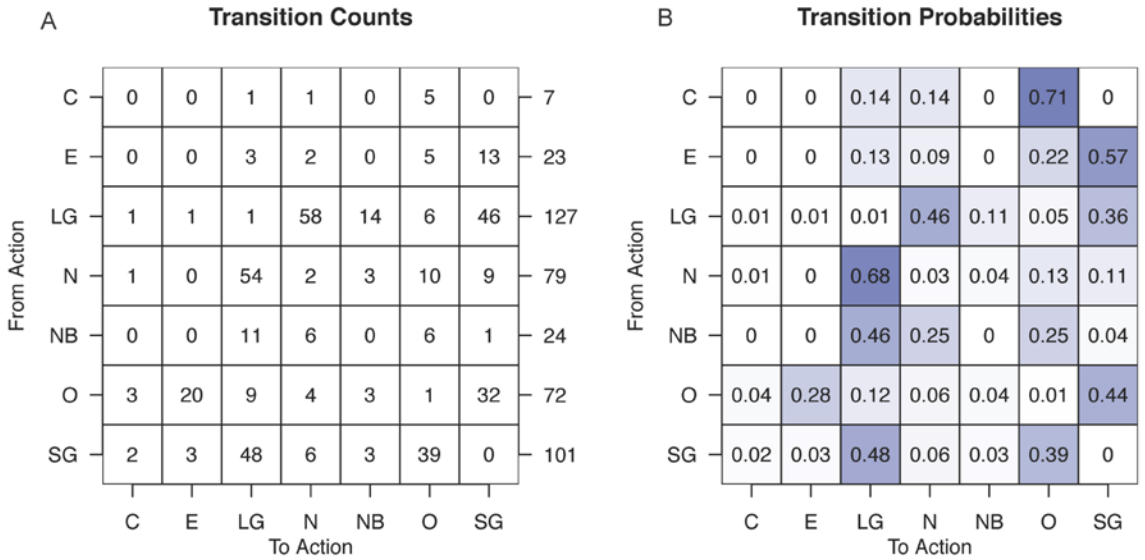


**Figure S-2**. This summary of the proportion of time an event follows another event can be considered as the empirical transition probability from the "row" event to the "column event" of a first-order Markov chain. S-2A displays the number of times an event follows another and S-2B displays the probability for each transition type.

*Human Data*

Maternal behavior was assessed during a semi-structured free play with her infant. Behaviors that were coded to characterize auditory, visual, or tactile sensory signals to the child are displayed in Table S-1. All behaviors were coded continuously in real time. The distribution of these sensory signals is shown in Table S-2. Figure S-3 is a visualization of a single mother's time sequence of events. As with the rodent data, it is our hypothesis that that predictability of maternal sequences of sensory signals is critical to the developing brain and thus, we focus on the transition between behaviors.

**Table S-1** – Description of behavioral codes used to characterize maternal sensory input to her infant.

| Sensory Signal | Action Type | Description |
|---|---|---|
| Auditory | Positive speech | Praising child or using an elevated or excited tone of voice |
| | Instructive speech | Content or instructive speech to the child |
| | Negative speech | Negative speech or scolding the child |
| | Laughing | Laughing |
| Tactile | Affectionate touch | Hugging, kissing, or patting the child. Affectionate instantaneous actions |
| | Functional touch | Fixing child's clothing, putting on child's shoes/socks, stopping child from falling over/getting them to sit, etc. |
| | Negative touch | Handling the child in a rough or negative manner such as roughly pulling baby's hand away to stop him/her from mouthing the toy |
| | Holding in lap | Holding the child while the child is in her lap |
| | Carrying/pick up child | Lifting the child up. Holding the child up in the air |
| | Restraining child | Touching child to stop him/her from doing something else for a sustained period such as stopping the baby from crawling or walking |
| | Supporting child | Providing support to baby when he/she is sitting on floor, or holding hands when he/she is standing up so that baby does not fall over |
| Visual | Manipulating object while child is observing | Child is attending to the object/toy that the mother is engaging in. (Ensures that the sensory signal is received by the child.) |

**Table S-2**. Number of transitions and number of maternal sensory signals within auditory, tactile and visual domains.

| | **Number of transitions or events** ***M ± SD*** | **Proportion of total events** **M ± SD** |
|---|---|---|
| Number of Transitions | 335.02 ± 107.88 | - |
| Auditory Signals | 134.16 ± 50.62 | 77.6% ± 12.2 |
| Visual Signals | 19.61 ± 6.85 | 13.5% ± 8.9 |
| Tactile Signals | 14.36 ± 10.22 | 8.9% ± 6.5 |

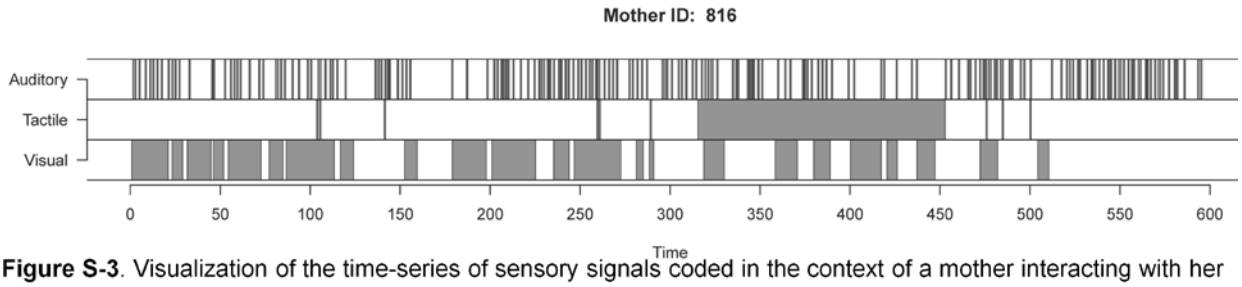


**Figure S-3**. Visualization of the time-series of sensory signals coded in the context of a mother interacting with her infant during the semi-structured play session.

Because our goal is to identify transitions between behaviors, as discussed above in the rodent section, we focused on the initiation of behaviors in analyzing the human behavioral data (e.g., initiation of a touch or a verbal utterance. It is necessary to standardize identification of discrete behaviors (e.g., to identify whether “Look.” “It is a truck.” is one event or two). Our process for doing so is as follows. We use verbal utterances to demonstrate. The start of each utterance was initially coded from video as a separate event. Thus, the example, “Look.” “It is a truck.” would initially be coded as 2 events. This instantaneous event time for each utterance was then extended to have a duration of 1-second. Thus, each event was right-padded with a one-second duration to standardize the lengths of the utterances and then instances that overlapped were merged into one event. This means that two maternal utterances separated by 1 second or more were characterized as two events and two maternal utterances for which the initiation was separated by less than 1 second were characterized as a single event. This can be seen in the Figure S-3 in the auditory category. The time interval of one second was chosen based upon an

empirical investigation of 10 randomly selected videos indicating that the average maternal utterance to her infant in this context was one second. An analysis was conducted with the entire sample to test the impact of selecting 1 second as the time interval on the calculation of entropy rate. Time intervals ranging from 0.5 to 2.0 seconds were tested. This analysis showed that the entropy rate estimates were not sensitive to the choice of interval within this range; the correlation of entropy rate estimates derived from different assumptions regarding the time interval (in the range from 0.5 to 2.0 seconds) were correlated with each other at the 0.90 or higher level. Although the issue of combining events most frequently affected auditory behaviors, a similar approach was applied to other brief events (e.g., affectionate touch).

In the human data, the codes for maternal sensory signals are not mutually exclusive (i.e., more than one event can happen at the same time). For example, a mother can be speaking while also touching the child. The eight possibilities listed in Table S-3 illustrate the possible categories. Changes between any of the 8 behaviors or combinations of behaviors were identified as transitions. We visualize the sequence of maternal behavior states for one mother in Figure S-4A (parallel to the rat data in figure S-1A). As with the rodent, the duration can be collapsed, as the "order" of the behaviors is the only thing that is important for assessing predictability of transitions between sensory signals. Transitions, therefore, are characterized as changes among any of the 8 possibilities in Table S-3. We visualize this in Figure S-4B (parallel to the rat data in Figure S-1B. Finally, based upon this sequence of events, the table of transition counts and the empirical transition matrix are constructed. They are provided in Figure S-5.

**Table S-3** – Descriptions of behavioral categories used to characterize transitions

| Grouping of Categories | New Category Descriptions |
|---|---|
| No Behavior | No Auditory, Tactile, or Visual Stimulation |
| Single Behavior | Only Auditory Stimulation |
| | Only Tactile Stimulation |
| | Only Visual Stimulation |
| Combinations of Two Behaviors | Both Auditory & Tactile Stimulation |
| | Both Auditory & Visual Stimulation |
| | Both Visual & Tactile Stimulation |
| Combination of All Behaviors | All States: Auditory, Tactile, & Visual Stimulation |

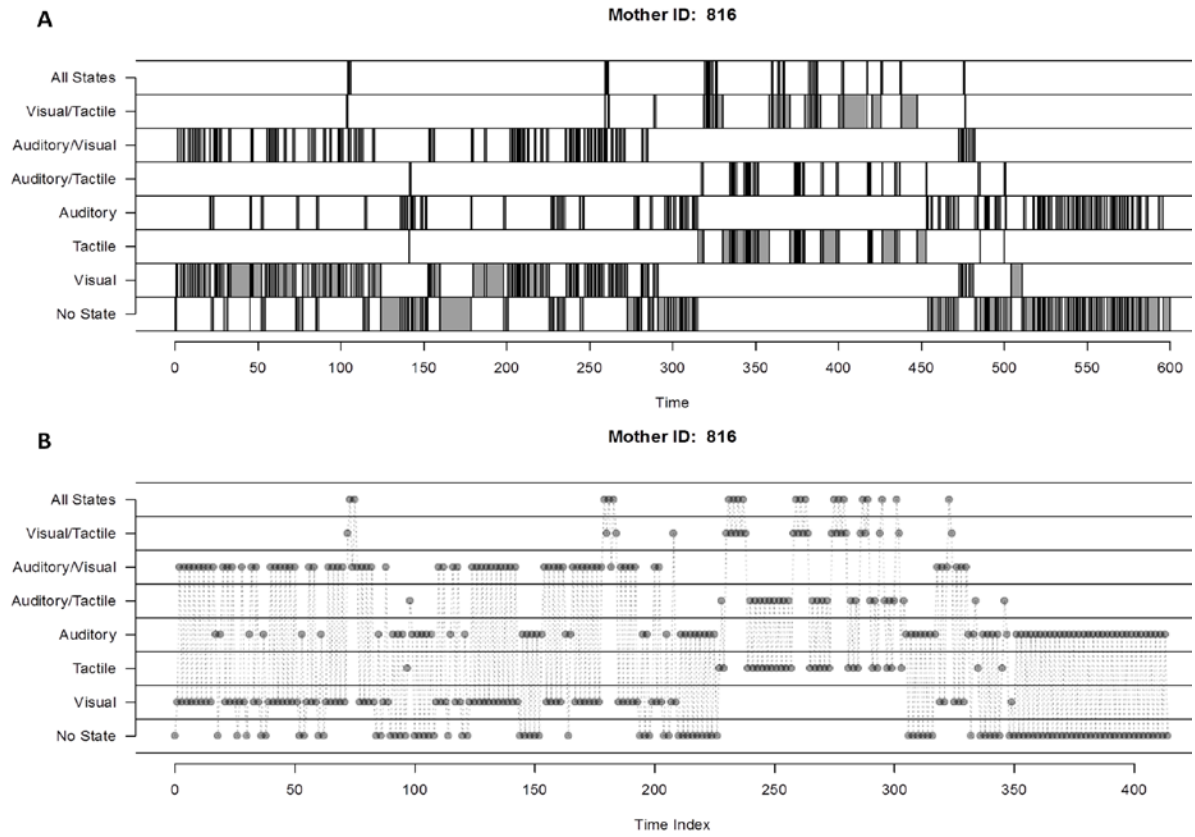


**Figure S-4**. S-4A displays the continuous time-series of maternal sensory signals in the context of interacting with her child and using the 8 categories shown in table S-3. Using the 8 possible combinations, a time series of mutually exclusive behaviors, similar to the rodent data, is created. At each instant in time the mother can only be in one of the eight states listed in Table S-3. S-4B displays the discrete time-series of human maternal sensory signals where each sensory signal has been reduced to a single dot so duration is not pictured. This is similar to the rat data shown in Figure S-1B.

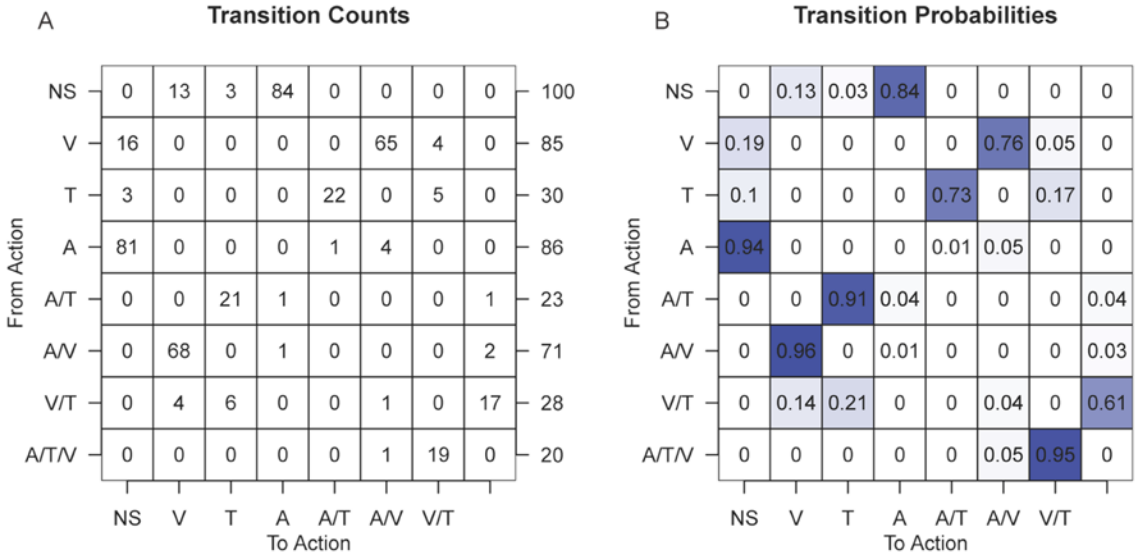


**Figure S-5.** Summary of the transitions from the "row" events to the "column" events. S-5A displays the observed counts of the transitions from the "row" events to the "column" events and S-5B displays the empirical transition matrix obtained by normalizing each row of the table of counts.

### S.1.2 Measuring Degree of Predictability – Entropy & Entropy Rate

The empirical transition matrix is a summary of the way in which a mother transitions among sensory signals and this matrix has significance when modeling behavior. In this study, the observed sequence of behaviors is modeled as a time-homogeneous, stationary Markov chain. A Markov chain is a specific type of stochastic process (i.e. a sequence of random variables) with a finite state space. In a first-order Markov chain the probability of the next observation is related only to what has most recently occurred. This is precisely the information contained in the matrix of transition probabilities. The assumption of time-homogeneity implies that this probability function does not change with time, while the stationary assumption implies that in the long term the distribution of behaviors will approach a stationary distribution $\pi$. The entropy rate of a Markov chain is a quantitative measure of the degree to which a future behavior can be predicted from the most current behavior. We provide a brief introduction to the concept and then describe how it is calculated for a Markov chain.

Entropy is a continuous measure that quantifies the predictability of a single random variable (33). As an example, consider rolling a single die and recording the number of spots observed. If the die were perfectly fair, each side of the die would have equal probability of one-sixth of occurring. This corresponds to maximum entropy and maximum unpredictability. If the die were such that the same side always landed on top, then the die would be perfectly predictable and have zero entropy. Mathematically, if $X$ is a discrete random variable capable of taking on $k$ values and $p_i$ is the probability of the $i^{th}$ value, then the entropy $H(X)$ is computed as (32)

$$H(X) = -\sum_{i=1}^{k} p_i \log_2 p_i$$

Entropy rate extends the concept of predictability from a single random variable to sequences of random variables. The entropy rate of a first-order Markov chain is calculated from the matrix of transition probabilities $P$ where $P_{ij}$ gives the probability of transitioning from state $i$ to state $j$ (as shown in Figures S-2 and S-5, where the row index represents the “from” state and the column index represents the “to” state). The entropy rate of a first-order stationary Markov chain is,

$$H(\mathcal{X}) = -\sum_{ij} \pi_i P_{ij} \log_2 P_{ij}$$

where $\pi_i, i = 1, \ldots, K$ is known as the stationary distribution and summarizes the long-run characteristics of the process. Entropy rate is a continuous measure bounded between zero and $\log_2 K$, where $K$ is the number of possible states. Therefore in the rat experiment the $H_{max,rat} = \log_2 7 = 2.807$ and for the human observational study $H_{max,human} = \log_2 8 = 3$.

### S.1.3 Rat Data – Assignment of Environment Induces Unpredictability

In the rat study, the dams were randomized into two maternal care environments: one that provided optimal materials for bedding and nesting (CTRL) and an alternative where the dam had a limited supply of bedding and nesting materials (LBN). The LBN environment has been shown to produce fragmented and unpredictable maternal care (20). The entropy rate captures this phenomenon as shown in Figure S-6. As shown in Figure 1, rats reared by dams exhibiting less predictable behavior sequences performed poorly on a spatial memory task (reduced novel/familiar location ratio) during adolescence. Figure S-7 illustrates that exploration times during training (A) and during testing (B) did not differ between the two groups.

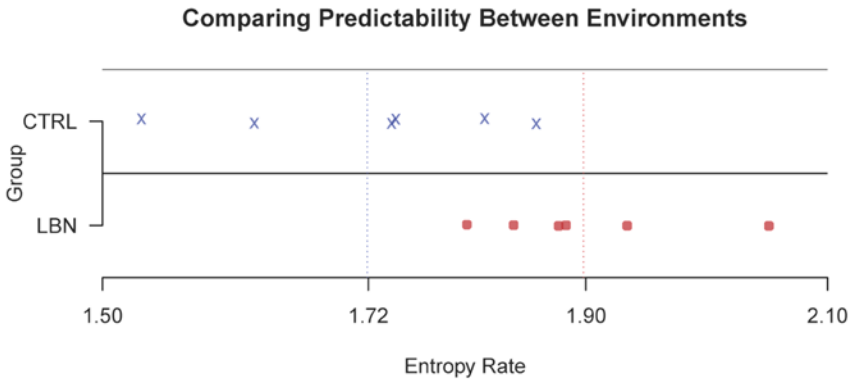


**Figure S-6**. Comparing the entropy rate between the two environmental care groups: LBN vs. CTRL. The LBN environment induced less predictable maternal behavior in the dams.

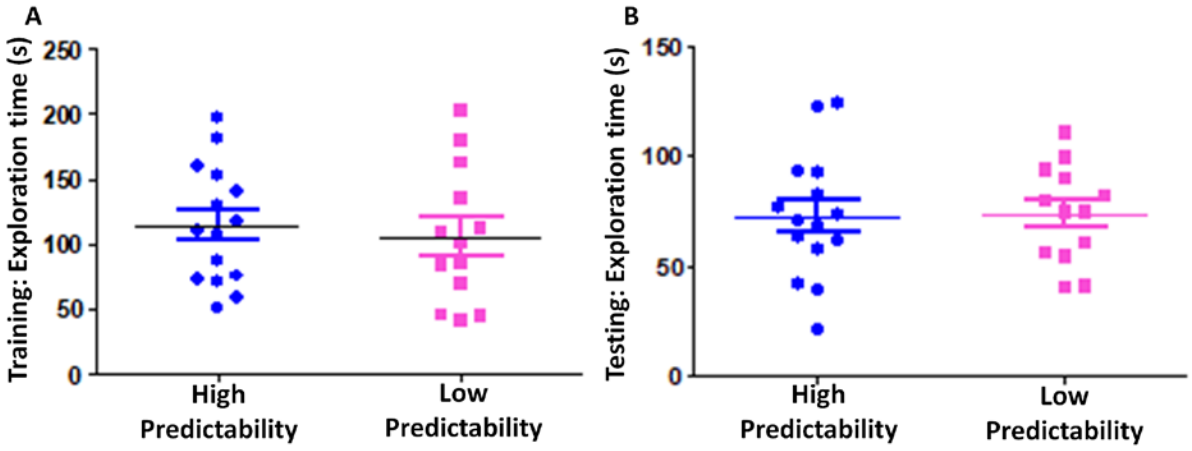


**Figure S-7**. Although rats reared by dams exhibiting low-predictability of their caring behavior sequences performed poorly on a spatial memory task (reduced novel/familiar location ratio) during adolescence, exploration times during training (A) and during testing (B) did not differ between the two groups.

### S.1.4 Human Data – Characterization of Entropy Rate in the Study Sample and Relation with Quality Measures

The human data are observational in nature. We observe a roughly normal distribution of entropy rates, as shown in Figure S-8. The entropy rate is derived from transition data so it is natural to explore the association of entropy with the total number of behavioral transitions. Figure S-9 shows that the correlation of the number of transitions and the entropy rate is weak (r=-0.15). Our hypothesis is that it is predictability of transitions (entropy rate) and not the count or number of transitions that influences brain development and thus predicts the cognitive outcomes. Thus, we focus in the manuscript on entropy rate as our cross-species predictor of cognitive outcomes. Consistent with our hypothesis, we note that number of transitions does not significantly predict either of the cognitive outcomes (t=1.67, β=0.12, p=0.10 for MDI and t=-1.11, β=-0.154 and p=0.31 for delayed recall).

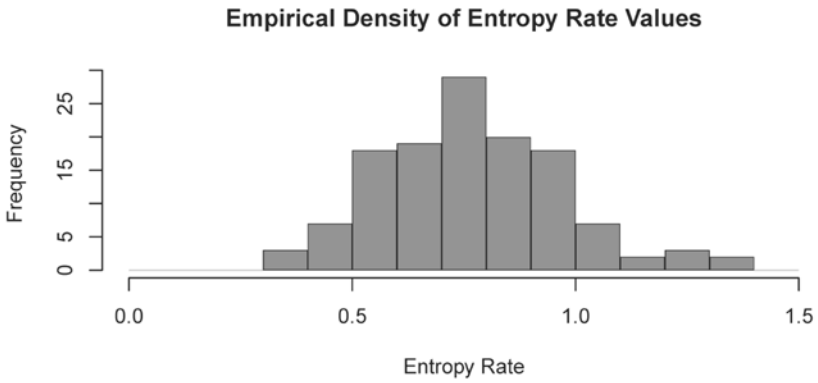


**Figure S-8.** Distribution of the entropy rate for human mothers is roughly normally distributed. This highlights that the study observed diversity in the degree of predictability of mother sensory signals to her infant in the context of the semi-structured play interactions.

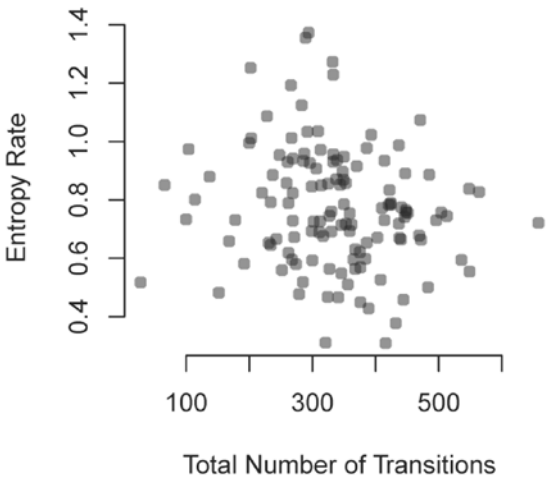


**Figure S-9.** Relation between entropy rate and number of transitions. Entropy rate is weakly related to the total number of transitions that occurred in the interaction (r=-0.15).

A large literature supports the importance of quality of care for child outcomes (1-6). We report in the article body that maternal sensitivity is a significant predictor of MDI, but not delayed recall. Table S-4 presents the interrelation between measures of kind of care employed in this study (sensitivity, and number of auditory, tactile and visual signals). Table S-5 displays the relation between these quality measures and child cognitive outcomes. Consistent with evidence from the literature, we find in our sample that the quality measures predict child outcomes. What is new in the present

manuscript is the evidence that that predictability of sensory signals is an additional important measure that is associated with cognitive outcomes cross-species. Further, entropy rate remains a better predictor of cognitive outcomes than either number of transitions or auditory, tactile and visual event counts. When tested in a regression model entropy rate remained a significant predictor of 2-year cognitive performance after considering each of the five quality of care measures (sensitivity, number of transitions and auditory, tactile and visual counts). Entropy rate remained a significant predictor of 6.5-year outcomes when modeled with maternal sensitivity, number of transitions, auditory counts and visual counts, but dropped below significance when tactile counts were added to the model, perhaps because of the reduced power due to smaller sample size at 6.5-years.

**Table S-4** Interrelation among measures of quality or kind of caregiving

| | 1 | 2 | 3 |
|---|---|---|---|
| 1. Maternal Sensitivity | - | - | - |
| 2. Auditory Count | 0.32** | - | - |
| 3. Visual Count | -0.10 | .14 | - |
| 4. Tactile Count | -0.13 | .09 | .18* |

*$p$<.05, **$p$<.01

**Table S-5** Relation between measures of quality or kind of caregiving and child MDI and delayed recall score

| | MDI | | Delayed Recall | |
|---|---|---|---|---|
| | t | β | t | β |
| Maternal Sensitivity | 3.44** | .30 | .53 | .07 |
| Auditory Count | 2.29* | .179 | -.74 | -.11 |
| Visual Count | .087 | -.04 | .45 | .06 |
| Tactile Count | -2.46* | -.19 | -.28** | -.38 |

*$p$<.05, **$p$<.01; MDI and delayed recall as dependent measures and with covariates as identified in primary analyses predicting child cognitive outcomes and described in the text.