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Development and Temporal Organization of Repetitive Behavior in an Animal Model

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Abstract

Despite repetitive behaviors being a common feature of a number of clinical disorders and ubiquitous in normative development, little attention has been given to their ontogeny or temporal dynamics. We characterized these features in a mouse model of repetitive behavior to identify discrete trajectories of development and developmental changes in temporal dynamics. Three qualitatively distinct trajectory groups were identified which allowed for an examination of the interaction between temporal organization and developmental trajectory. Significant differences in temporal dynamics were found across development and among trajectory groups. Significant interactions of trajectory group and developmental period on temporal organization were also found. The combination of group-based trajectory modeling and a novel method for analysis and graphic depiction of temporal organization allowed for the exploration of the interplay between these two fundamental behavioral processes. Such methods may be useful tools in the assessment and treatment of repetitive behavior in clinical populations.

Keywords

neurodevelopmental disorders; autism; deer mice; stereotypy; developmental trajectory; successive difference plots

INTRODUCTION

Repetition of behavior that appears to serve no obvious purpose has long been considered an important dimension of psychopathology. These behaviors variously termed stereotypies, compulsions, and rituals are seen in a variety of neuropsychiatric and neurodevelopmental disorders (Frith & Done, 1990; Lewis & Bodfish, 1998; Ridley, 1994). For example, in autism for which repetitive behavior is diagnostic (DSM-IV-TR, American Psychiatric Association, 2000), affected individuals exhibit stereotyped motor movements, compulsions, rituals and routines, an insistence on sameness, and circumscribed interests (Lewis & Bodfish, 1998). These same repetitive behaviors are also a common feature of a number of neurodevelopmental disorders, some of which have an identifiable genetic defect (e.g., Rett, Fragile X, and Prader–Willi syndromes; Carter et al., 2009; Moss, Oliver, Arron, Burbidge,

& Berg, 2009; Symons, Butler, Sanders, Feurer, & Thompson, 1999). Repetitive behaviors are also part of the clinical phenotype of obsessive-compulsive disorder, Tourette syndrome, and fronto-temporal dementia (Cath et al., 2001; Mateen & Josephs, 2009; Muller et al., 1997).

Various forms of aberrant repetitive behavior also occur in a significant percentage of individuals without cognitive impairment or neurological or psychiatric disorder (Castellanos, Ritchie, Marsh, & Rapoport, 1996; Rafaeli-Mor, Foster, & Berkson, 1999; Singer, 2009). At the same time, there is ample documentation that repetitive motor behavior, compulsions, and rituals are features of normative development as well (e.g., Evans et al., 1997; Thelen, 1980). For example, throughout early childhood, children engage in a number of stereotyped motor acts (e.g., swaying, rocking, flapping) and later display a variety of compulsive and ritualistic behaviors (e.g., insistence on certain clothing or foods, bedtime rituals). These behaviors are typically less frequent or intense than those seen in clinical populations, and decline with age.

Despite the importance of repetitive behavior in a variety of clinical disorders and their expression in typical development, little is known about how such behaviors develop. As Symons, Sperry, Dropik, and Bodfish (2005) have indicated, very few investigations have addressed the developmental course of repetitive behaviors in children at risk for disorders such as autism. Esbensen, Seltzer, Lam, and Bodfish (2009) examined a wide range of ages (2–62 years) in individuals with autism using a modified cross-sectional design. They reported that repetitive behaviors decreased across age regardless of subtype of repetitive behavior. In a recent study of children with autism and developmental delay, Richler, Huerta, Bishop, and Lord (2010) assessed the development of restricted repetitive behaviors longitudinally at 2, 3, 5, and 9 years of age. They measured the two major subtypes of repetitive behaviors (repetitive sensorimotor behavior and insistence on sameness) and reported decreased sensorimotor repetitive behaviors and increased insistence on sameness behaviors with increasing age. Importantly, using a group-based trajectory modeling procedure, they found three sub-groups with qualitatively distinct developmental trajectories. Studies such as these, particularly those aimed at identifying heterogeneous developmental trajectories, begin to provide the knowledge base necessary for the design of efficacious early intervention and prevention strategies.

Similar to the literature on clinical disorders, relatively little is known about the developmental trajectories of normative repetitive behavior. A notable exception is the work of Thelen (1979) who showed that specific rhythmical stereotyped movements in infants had a clear onset, peak, and decline coincident with emerging voluntary motor control. In large measure, however, the factors that influence the persistence or decline of repetitive behaviors with age remain unidentified.

In addition to development, the temporal structure of repetitive behavior has received scant attention despite the fact that stereotypy is generally considered to be characterized by temporal regularity rather than variability or complexity. In addition, as Newell, Mayer-Kress, Hong, and Liu (2009) have pointed out, the temporal dynamics of repetitive behavior may be more informative and robust than the more typically used mean frequency (number of responses in a specified time period) or duration measures. Early work by Lewis, MacLean, Johnson, and Baumeister (1981) identified ultradian rhythms in the stereotyped motor behavior of individuals with developmental disabilities. In one of the few additional efforts to characterize the temporal dynamics of repetitive behavior, Peterson and Leckman (1998) analyzed the distribution of tics in Tourette syndrome patients. These investigators showed that intervals between tics were not random and that the temporal structure in the tic time series could be uncovered using several statistical approaches or models. In an

intriguing set of studies, Newell et al. have shown increased regularity in movements associated with upright postural stability (Bodfish, Parker, Lewis, Sprague, & Newell, 2001), sitting (Newell & Bodfish, 2007), and spontaneous blinking (Lee, Bodfish, Lewis, & Newell, 2010) in individuals with neurodevelopmental disorders who engaged in stereotyped behavior versus matched controls. Finally, as far as we are aware, there has been no attempt to address the intriguing question of whether such temporal characteristics interact with developmental trajectory.

Appropriate animal models could provide a wealth of information about how repetitive behaviors develop. This would seem particularly true for rodents given their abbreviated developmental period. In addition, the ability to extensively monitor laboratory animals provides the opportunity for assessing temporal dynamics and further addressing the important question of how the temporal structure of such behaviors systematically changes with developmental trajectory.

Stereotyped motor behaviors can be induced in animals following a variety of perturbations including early social deprivation, environmental restriction, exposure to specific pharmacological agents, and specific CNS insults including genetic mutations (see Lewis, Tanimura, Lee, & Bodfish, 2007 for a review). Despite these models, as Rushen and Mason (2006) pointed out, the lack of longitudinal studies of stereotypy development remains a substantial problem. With regard to temporal organization, although circadian variations in stereotypy have been examined (e.g., Mason, 1993), little work has addressed the temporal structure of stereotypy in animals. Our laboratory has employed a deer-mouse (*Peromyscus maniculatus*) model of aberrant repetitive behavior (e.g., Hadley, Hadley, Ephraim, Yang, & Lewis, 2006; Powell, Newman, Pendergast, & Lewis, 1999; Tanimura, Vaziri, & Lewis, 2010). When housed under standard laboratory conditions, deer mice exhibit excessive repetitive vertical jumping and/or backward somersaulting. These behaviors occur at a high rate, persist across much of the life of the animal, and appear relatively early in development, sometimes as early as weaning. These behaviors do not require social isolation, specific cues or contexts, pharmacological agents, or specific CNS insult for induction. Consistent with stereotypy in deer mice having its origins in environmental restriction, housing deer mice in larger, more complex environments (environmental enrichment) following weaning markedly attenuates the development and expression of these behaviors (Hadley et al., 2006; Powell et al., 1999). An early assessment of the developmental trajectory of stereotypies in deer mice (Powell et al., 1999) indicated that stereotypies increased in frequency and reached adult levels at about 2 months after weaning at which point the frequency plateaued.

In our assessments of adult deer mice, we have consistently observed a high degree of variability in the expression of repetitive behavior. Thus, in the present paper, we sought to track the development of stereotypy in deer mice and determine whether multiple trajectories best characterize our sample. Newell (1996) has suggested that key to understanding the emergence of stereotypies is the change in dynamics and the loss of behavioral adaptability. Thus, we also sought to characterize the temporal organization of repetitive behavior in deer mice. We hypothesized that repetitive behavior would become more predictable or regular over development. The interaction between temporal structure and developmental trajectory has not previously been examined and so we have sought to examine the interplay between temporal dynamics and developmental trajectory.

GENERAL METHODS

Subjects

All deer mice (*Peromyscus maniculatus*) were obtained from the breeding colony maintained in our laboratory, and kept on a 16:8-hr light/dark cycle with lights off at 10:00 a.m. Rodent chow and water were available ad libitum. The room was maintained at 20–25°C and 50–70% humidity. Mice sharing the same weaning date were group-caged (5–6 mice/cage) at weaning (PND21) in standard rodent cages (48 × 27 × 15 cm) and they remained in the same cage group throughout the experiment. All procedures were performed in accordance with the guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Florida Institutional Animal Care and Use Committee.

Stereotypy Assessment

Rates of spontaneous stereotypy (hindlimb vertical jumping or backward somersaulting) were assessed using a modified automated photocell detection apparatus (Columbus Instruments). Photocells were placed such that vertical activity (jumping and somersaulting) resulted in beam breaks (stereotypy counts) whereas rearing did not. For Experiment 2, each occurrence of a detected photocell interruption was registered with a real-time value using Labview (National Instruments). This allowed us to construct a time series of the intervals between individual consecutive stereotyped responses. The session consisted of the 8 hr of the dark cycle. Mice were individually placed in testing cages (22 × 28 × 25 cm) made of Plexiglas and habituated for at least 1 hr prior to the beginning of the dark cycle. Food and water were provided. All sessions were digitally video-recorded for identification of behavioral topographies and accuracy of the automated counters.

EXPERIMENT 1: CHARACTERIZATION OF THE DEVELOPMENT OF SPONTANEOUS STEREOTYPY

Methods and Data Analysis

Fifty-two mice (female: $n = 23$; male: $n = 29$) were randomly selected from the colony and tested for their rates of stereotypy as described previously. Starting at 1 week post-weaning (PND26–28), the frequency of stereotypy for one complete dark cycle was measured weekly for at least 8 consecutive weeks. We selected 1 week post-weaning to insure that the repetitive behavior would be of sufficient amplitude to be measured reliably by our automated apparatus.

Group-Based Trajectory Modeling—In order to determine if there were qualitatively different developmental trajectories of repetitive behavior, we subjected the data from Experiment 1 to a group-based trajectory modeling procedure. We chose the Trajectory Procedure (Proc Traj), which is designed to run on the SAS platform (Jones & Nagin, 2007; Jones, Nagin, & Roeder, 2001). Proc Traj is a specialized mixture model that estimates the trajectories of multiple groups within the population. This provides an important advantage over conventional regression or growth curve procedures that model only one mean within the population. Proc Traj is a particularly useful strategy for our purposes where the number and structure of the trajectories were largely unknown. This group-based trajectory modeling allowed for the calculation of the probability of membership in each discrete trajectory for every subject. The number of discrete developmental trajectories that best accounted for individual data was determined by comparing the values of the Bayesian Information Criterion (BIC) between different models, where smaller values indicate a better fit (see Jones et al., 2001). There was considerable between-animal variability as well

as week-to-week variability in the frequency of stereotyped responses. Hence, we performed a log transformation of the stereotypy counts, a commonly used procedure to reduce non-homogeneity of variance of data related to growth.

RESULTS

From Figure 1, it is apparent that stereotyped activity reached asymptotic levels by around 6 weeks post-weaning. This was also confirmed by a polynomial fitted to the whole data set. When the log (10 based) counts y was fit to the time t (in weeks) for all the data, the following equation

$$y = -1.95 + 0.78t - 0.063t^2$$

had the best fit versus any other order polynomials. Based on this model, stereotyped behavior was judged to reach a plateau at $t = 6.22$ weeks with the mid-point of development being at about $t = 3.5$ weeks.

Using Proc Traj, the developmental trajectories were best classified into three groups based on the BIC as shown in Figure 2. There are two curves for each cluster, one from the estimated mean and one from the smoothed 4th order polynomial, an option provided by Proc Traj. The first trajectory (Traj 1, the bottom two curves) representing a small percentage of subjects (11.2%) confirms what we have known for some time, namely, a small group of mice that did not develop high levels of repetitive behavior despite being reared in standard cages. The second group (Traj 2: 41.1% of mice, the middle curves) showed a gradual progression from low levels of stereotypy at 1 week post-weaning to high levels by 6 weeks post-weaning. The third group (Traj 3: 47.7% of mice) is noteworthy in their already high levels of stereotypy by 1 week post-weaning. Interestingly, despite a different developmental course, the Traj 2 and 3 groups end-up at about the same level of repetitive behavior by 8 weeks post-weaning (Fig. 2).

In this sample of 52 mice representing 15 litters, only 9 exhibited backward somersaulting. Of these nine mice (five male, four female), five were grouped in Traj 2 whereas four were grouped in Traj 3. A Fisher's exact test showed no relationship between topography and trajectory ($p = .66$). Interestingly, for eight of the nine, somersaulting replaced jumping, as early as approximately 4 weeks and as late as 8 weeks post-weaning. At any one time, however, each mouse exhibited only one of the two topographies of stereotypy. Additionally, Traj 2 had an equal number of male and female mice whereas Traj 3 was 44% male. Traj 1 had more males than females but the total number of mice in this trajectory was small. There was no significant association between sex and trajectory group ($p = .52$).

EXPERIMENT 2: TEMPORAL ORGANIZATION OF SPONTANEOUS STEREOTYPY FOR DIFFERENT TRAJECTORY GROUPS

Methods and Data Analysis

In a separate set of mice, temporal organization was evaluated using all stereotypic responses counted during a single dark cycle at 1, 3.5, and 6 weeks post-weaning. We used these responses to assess hour-by-hour variation in frequency and the temporal dynamics of the 8-hr time series. We chose 1 week (PND26-28), 6 weeks (PND62-64), and the middle point 3.5 weeks post-weaning (PND43-45) as the times to measure stereotyped responding based on the analysis described for Figure 1. The distributions of stereotypy counts by hour across the dark cycle at each developmental period are depicted in Figure 3.

Based on Experiment 1 results, mice categorized by cluster analysis as belonging to the Traj 1 ($n = 13$), 2 ($n = 9$), or 3 ($n = 7$) groups were used for analyses of temporal organization of behavior during each session. The three clusters matched well with the results presented in Figure 2. The frequency of stereotyped responses was used to determine developmental trajectories and trajectory groups in Experiment 1. Frequency is only the first (mean) order of the stereotypy pattern, however, and does not provide any information about the temporal dynamics or complexity of the repetitive behaviors. To assess temporal organization, we examined the second order property of stereotyped responses, that is, the difference between consecutive inter-response intervals. Lee et al. (2010) used the same approach to identify the temporal structure in spontaneous blinking using adjacent interblink intervals. This analysis showed decreased complexity of eye-blinking in individuals with intellectual disability versus controls.

If $x_1, x_2, \dots, x_t, \dots$ are the intervals between successive stereotyped responses, then the second order property may be measured by $D_t = x_t - x_{t-1}$, or the change or difference between adjacent inter-response intervals. The magnitude of D_t is difficult to interpret, however, because it is affected by the relative duration of the time between stereotyped responses or behaviors. Consequently, it is greatly affected by the frequency of stereotypy. Thus, we chose to “normalize” D_t by the magnitude of the durations of x_t and x_{t-1} , or

$$\delta_t = \frac{D_t}{x_t + x_{t-1}} = \frac{x_t - x_{t-1}}{x_t + x_{t-1}} \quad (1)$$

The numerator of (1) is similar to the standard deviation and its denominator is similar to the mean. Thus (1) is related to the coefficient of variation, which has been used to measure the regularity of cluster formation (see Cressie, 1991, p. 590). Moreover, δ_t is unit free and $-1 \leq \delta_t \leq 1$.

From our plots of successive differences, with δ_t on the x -axis and δ_{t+1} on the y -axis, it was clear that in adult animals, the data points in the scatter plot formed a characteristic pattern resembling the letter Y . We thus measured the temporal pattern of stereotyped behavior relative to its conformity to the Y -shape pattern by defining the Y -score of a point (x, y) in the scatter diagram as its minimum distance to the Y pattern. More specifically,

$$\begin{aligned} \varphi &= \min(d_1, d_2, d_3), \text{ where} \\ d_1 &= |x|, \text{ if } y \geq 0; d_2 = |y|, \text{ if } x \leq 0; \text{ and} \\ d_3 &= \frac{x+y}{\sqrt{2}}, \text{ if } x > 0 \text{ and } y < 0. \end{aligned}$$

The average φ for all the (x, y) pairs in a given time interval is defined as the Y -score of this interval. We were interested in changes in Y -scores as a function of different developmental periods and whether there were systematic differences in Y -scores across the three developmental trajectories. The two factors considered are; the trajectory group (three levels; Traj 1, 2, and 3) and the developmental period (3 levels, 1, 3.5, and 6 weeks post-weaning). To consider possible non-stationarity within the same dark cycle (see Fig. 3), Y -scores were estimated in each developmental period by dividing each dark cycle into three consecutive sections. SAS PROC MIX was used for the data analysis. The mice were considered as a random factor.

RESULTS

Y Plots

The utility of *Y*-plots in representing the relative temporal organization of repetitive behavior is illustrated by Figure 4. In this example, if all individual stereotyped responses were equally spaced in time, all the points would cluster at the 0,0 coordinate (Panel A; *Y*-score would equal 0). If all stereotyped responses were clustered in bouts and the inter-bout intervals were all equivalent and if all stereotyped responses within a bout were equally spaced, the plot would be made up of four points at the following coordinates: $(-x, 0)$; $(0, +y)$; $(0,0)$; and $(+x, -y)$; (Panel B). When connected these 4 points would form a *Y*. Finally, if the distribution of stereotyped responses was temporally unorganized, the plot would reflect points made up of randomly distributed coordinates (Panel C). Two special cases, one with fixed inter-bout intervals and random within-bout intervals is simulated in D; the other one with random inter-bout intervals and fixed within-bout intervals is simulated in E. In practice, however, it is usually very difficult to locate the bout boundaries.

Figure 5 depicts representative *Y* plots from three mice representing each of the three developmental trajectories and, for each individual mouse, each of the three developmental time points. All the plots are based on the same number of stereotyped responses ($n = 850$). The *Y*-scores of the patterns in Figure 5 are given in each panel. They are the averages of from all the data pairs. The smaller the *Y*-score, the more regular are the stereotyped responses with a random temporal pattern having the highest *Y*-score which is equivalent to .371. As these plots indicate, at 1 week post-weaning, Traj 3 shows a much lower *Y*-score and a clear *Y*-shaped plot. Little difference can be seen between Traj 1 and 2 with little evidence for a *Y*-shaped pattern. At 3.5 weeks post-weaning, the *Y*-score has dropped for the Traj 2 mouse consistent with a now clear *Y*-shaped pattern of coordinates. Little change can be seen in Traj 1 and 3 with the former remaining poorly organized or more random and Traj 3 maintaining a high level of organization. At 6 weeks post-weaning the Traj 2 mouse has the lowest of its three scores and exhibited a clear *Y*-shaped pattern. Little change was observed in the Traj 1 and 3 mice.

Thus, for the Traj 3 mice, the relation between t (*x*-axis) and $t+1$ (*y*-axis) forms a *Y*-shaped pattern at each of the three developmental time periods. For the Traj 2 mice, this relation resembled a random pattern early in development and a clear *Y*-shaped pattern later in development. For the Traj 1 mice, this relation showed little evidence of a *Y*-shaped pattern and does not systematically change over development. Table 1 summarizes the mean *Y*-scores for animals at different developmental periods within each trajectory group. The numbers in the parentheses are standard errors for the mean estimation. The significance among the factors is given in the analysis of variance (ANOVA) table (Tab. 2). From this table, it can be seen that the *Y*-score is strongly affected by the interaction between trajectory and developmental period as well as by each factor independently.

Figure 6 gives the multiple comparisons of the *Y*-scores for each trajectory group-developmental period combination. Any two values circumscribed within the same parallelogram are not statistically distinguishable based on our analyses. The overall significance level was set at .05 after Bonferroni correction. Thus, the criterion for statistical significance for any pair was set at .05/36 or .0014 as there are 36 pairs in the comparison. From this figure, we can see that for the Traj 2 group, their repetitive behavior becomes increasingly organized or regular across the three developmental periods. For the Traj 1 and 3 groups, however, no significant changes in *Y*-scores are observed over the developmental periods.

Within a developmental period, the repetitive behavior of the Traj 3 mice was found to be significantly more organized or regular at 1 week post-weaning than the repetitive behavior of the Traj 1 or 2 mice which did not differ and which approached random. At 3.5 weeks post-weaning, the organization of repetitive behavior did not differ between the Traj 2 and 3 mice and was significantly more organized or regular than the behavior of the Traj 1 mice. Finally, by 6 weeks post-weaning, the temporal organization of repetitive behavior in the Traj 2 and 3 groups was virtually identical whereas the Traj 1 mice remained relatively unorganized in the distribution of their stereotyped responses over time.

The similarity of the conclusions from Figure 2 based on frequency and Figure 6 based on the Y -score makes one suspect the Y -score and frequency provide similar or redundant information. The data showed the opposite, however. Since the frequency of stereotypy varies between mice, the normalized inter-response interval using 1 for the third developmental period (6 weeks post-weaning) for each mouse was used for comparison. The R^2 for Y -scores and frequency was .11, and if the inter-response intervals were not normalized for the individual mouse, the R^2 was only .03. Thus, the Y -score contains a substantial amount of new information beyond frequency.

DISCUSSION

In the present paper, we sought to characterize the ontogeny of stereotypy in deer mice and determine whether these animals follow discrete trajectories of development. We also sought to characterize the temporal organization of repetitive behavior in deer mice. We hypothesized that repetitive behavior would become more predictable or regular over development. We then sought to examine temporal organization within the context of different trajectories of development. The interaction between temporal structure and developmental trajectory has not previously been examined and so we sought to explore the interplay between these fundamental behavioral processes.

Our findings indicate that, as a group, deer mice develop adult levels of repetitive behavior by about 6 weeks post-weaning. This is in substantial agreement with our earlier report despite the less sophisticated data acquisition and analysis methods used in that study (Powell et al., 1999). Given the considerable heterogeneity associated with the group curve depicted in Figure 1, however, we employed a group-based trajectory modeling procedure (Proc Traj), which yielded three distinct trajectories as the optimal solution. These groups consisted of animals that showed a monotonic increase in the frequency of stereotypy (Traj 2) across the 6-week period, a group that expressed uniformly low levels of stereotypy across development (Traj 1) and the last group which exhibited high levels of stereotypy by 1 week post-weaning with relatively little increase observed subsequently (Traj 3). Identification of the Traj 1 group is consistent with our prior observations of typically having a small number of animals that, despite being reared in standard cages, do not ever develop appreciable levels of stereotypy. The Traj 3 group is also consistent with our previous anecdotal observations that some of our animals displayed stereotypy as early as weaning. Thus, a drawback of the current study is lack of inclusion of animals younger than 1 week post-weaning. Future experiments will determine if the developmental trajectory of the Traj 3 mice is qualitatively different when such observations are added.

Abnormal repetitive behaviors are commonly displayed in animals housed in restricted environments (e.g., zoo, farm, and laboratory). Indeed, repetitive behaviors are the most common category of abnormal behavior observed in confined animals (Würbel, 2001). Despite this, there has been little attention paid to the development of these behaviors. Proc Traj allowed us to examine the heterogeneity of stereotypy trajectories and identify differences that would not have been seen using conventional cross-sectional or longitudinal

analyses. Although this group-based trajectory modeling has been used in human developmental studies including autism (e.g., Anderson, Oti, Lord, & Welch, 2009; Richler et al., 2010), it has not been used in animal studies or applied to the problem of repetitive behavior in animals. Identification of discrete trajectories provides a number of opportunities for further investigation. For example, we can now pursue identification of variables that might predict trajectory membership. We can explicitly test the strength of both time-independent and time-dependent covariates. Of particular importance would be investigating potential mechanisms that protect the Traj 2 mice from developing high levels of repetitive behavior despite being reared in standard cages. Mapping trajectories will also allow us to pursue identification of neurobiological mechanisms that play a role in the differential timing of the expression of stereotypy. Finally, we can now examine various experiential and biological manipulations for their impact on altering the development of repetitive behavior. Such studies would have important implications for treatment efforts in neurodevelopmental disorders such as autism.

Stereotyped behavior is typically thought of as behavior characterized by regularity or relative lack of variability. Longer time series of stereotyped behavior do show systematic variability, however (e.g., Lewis et al., 1981). In the present study, we showed a temporal distribution of stereotyped behavior across the 8-hr dark cycle with a decrease in frequency over the first 2 hr of the dark cycle and a prominent increase in frequency over the last 2–3 hr of the dark cycle. Interestingly, when animals were sorted by trajectory group, this pattern could be seen at all three developmental time points in the Traj 1 and 3 groups but was slow to develop in the Traj 2 group. The temporal variation in frequency observed over the dark cycle provides an opportunity for testing whether specific physiological variables (e.g., corticosterone) vary systematically with frequency.

Similar results were obtained when the data were subjected to a statistical model that assessed the relative organization of stereotyped behavior. This analysis showed that the stereotypy of the Traj 2 mice became significantly more organized or less random across development, whereas the Traj 1 and 3 mice showed no significant change in temporal organization of stereotypy across development. We also found trajectory group differences in temporal organization across developmental periods. The stereotyped behavior of the Traj 1 and 2 mice was significantly less organized (more random) at 1 week post-weaning than the Traj 3 mice. At 3.5 and 6 weeks post-weaning the repetitive behavior of the Traj 1 mice remained largely unorganized whereas the Traj 2 and 3 mice were significantly more organized. The absence of increased temporal organization in the behavior of the Traj 1 mice is noteworthy in that it shows that despite 5 weeks of engaging in the same behavior many times per day, its expression did not become more fixed or regular. Whether the failure to become more organized is important in the failure of these animals to develop appreciable levels of stereotypy is a matter of conjecture.

The changes across development and among trajectories were seen not only statistically but also graphically in the successive difference or *Y* plots. These plots are instructive in that they depict the degree to which repetitive behavior is organized into bouts and the degree to which individual stereotyped responses are organized within bouts. For example, in the Traj 2 mice tested at 1 week post-weaning, there is little evidence of stereotyped responses being clustered in bouts and little evidence for regularity in individual stereotyped responses within a bout. That organization clearly is in evidence by 3.5 weeks post-weaning, however. The Traj 3 group conversely showed considerably more structure, in terms of responses being clustered in bouts and exhibiting regularity of responding within a bout, at 1 week post-weaning than observed in the Traj 2 mice. It has been known for some time that repetitive behavior tends to occur in bouts but little, if any, attention has been given to individual variability in such structure or the developmental timing of such structure.

A number of statistical approaches using either frequency domain (e.g., spectral analysis) or time domain (e.g., Auto Regressive Integrated Moving Average or ARIMA) models can be used to uncover the structure of a time series (see Asher et al., 2009; Bokil, Tchernichovsky, & Mitra, 2006; Seely & Macklem, 2004; for reviews see also Maye, Hsieh, Sugihara, & Brembs, 2007; Nakamura et al., 2008). Using successive differences to generate plots of the temporal structure of repetitive behavior provided a very straightforward graphical or pictorial representation of temporal organization. Based on this plot, we generated an index or coefficient of organization from the plot, which importantly was nearly independent of frequency. Finally, for our own data even within the same time series, lack of stationarity happens quite often. In this respect, *Y*-scores were more stable than the frequency. The coefficients of variation for *Y*-score and frequency within the same time period for the same mouse were .15 and .67, respectively.

A simple graphical and statistical method to examine temporal organization of repetitive behavior provides a very useful tool for subsequent investigations. Behavior is often characterized or quantified in terms of frequency and duration and not typically in terms of temporal structure. Assessing temporal organization provides information about complexity, variability, and flexibility (Asher et al., 2009). Thus, temporal organization may prove to be a more useful variable than frequency or duration in terms of how resistant the behavior may be to intervention. The strikingly predictable or regular dynamics found in the repetitive behavior of most adult deer mice is in sharp contrast with the complexity, variability, and relative unpredictability that characterizes adaptive, functional behavior. In the “dynamical disease” model of Glass and Mackey (1988), medical illness (e.g., cardiovascular disease) can be characterized by periodic or predictable dynamics in the relevant physiological systems, whereas healthy systems are complex and variable. Indeed, within the field of dynamical diseases, the most repetitive and predictable dynamics are associated with the most severe pathological conditions (e.g., Cheyne–Stokes breathing), a phenomenon referred to as the “law of stereotypy” (Goldberger, 1997). Abnormal repetitive behavior may be viewed from this perspective as representing a loss or lack of complexity. Loss of complexity or variability in behavior would seem to reflect adverse neuroadaptations in the CNS that occur over development (Hadders-Algra, 2008). Thus, developmental trajectory and temporal organization may provide more powerful correlates of neurobiological variables than frequency or duration of behavior. In addition, the methods used in the current study may be useful tools in the assessment and treatment of repetitive behavior in clinical populations such as autism and related neurodevelopmental disorders.

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FIGURE 1.
The development of stereotypy post-weaning (mean frequency per hour + SEM).

FIGURE 2. Three distinct developmental trajectories yielded by SAS Proc Traj (log of the mean frequency per hour; raw data and smoothed curves).

FIGURE 3.

Repetitive behavior during the 8-hr dark cycle. (A) Traj 1 at each of three developmental periods (1, 3.5, 6 weeks post-weaning); (B) Traj 2 at each of the same three developmental periods; (C) Traj 3 at each of the same three developmental periods.

FIGURE 4.

Model successive difference plots. (A) behavioral responses equally spaced in time yield points clustered at the (0,0) coordinate; (B) behavioral responses with fixed inter-response and inter-bout intervals yield four points; (C) unorganized behavioral responses yield randomly distributed coordinates. Their normalized (t , $t+1$) are given in the right diagrams. (D) Behavioral responses with fixed inter-bout intervals and random within-bout intervals; (E) behavioral responses with variable inter-bout intervals and fixed within-bout intervals, respectively.

FIGURE 5.

Y-plots from three mice representing each of the three developmental trajectories at three developmental time-points. Each plot contains $n = 850$ behavioral responses. The top three panels are from a Traj 1 mouse, middle panels from a Traj 2 mouse and bottom from a Traj 3 mouse. The values in the parentheses are the Y-scores.

FIGURE 6. Multiple comparisons of mean *Y*-scores for each trajectory group at each developmental period. Values circumscribed within the same parallelogram are not statistically different.

Table 1The Mean *Y*-Scores at Three Developmental Periods Within Each Trajectory Group

	Traj 1 (<i>n</i> = 13)	Traj 2 (<i>n</i> = 9)	Traj 3 (<i>n</i> = 7)
Developmental period 1 (1 week)	.252 (.012)	.260 (.013)	.116 (.014)
Developmental period 2 (3.5 week)	.240 (.012)	.166 (.012)	.123 (.014)
Developmental period 3 (6 week)	.218 (.011)	.132 (.012)	.127 (.014)

Table 2ANOVA Table From SAS PROC Mixed With *Y*-score as the Response Variable

Effect	Num DF	Den DF ⁺	<i>F</i>	<i>p</i> -Value
Trajectories	2	139	27.02	<.0001
Developmental periods	2	139	27.65	<.0001
Interaction	4	139	15.89	<.0001

⁺, Represents numerator and denominator degrees of freedom for the F -test of the fixed effects in the mixed model. Due to the three repeated measures per dark cycle, the Den DF take the large number of observations into account. Similar examples can be found in Littell, Milliken, Stroup, Wolfinger, and Schabenberger (2006); Chapter 5.