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Social Order: Using The Sequential Structure of Social Interaction to Discriminate Abnormal Social Behavior in the Rat

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Abstract

Social interactions form the basis of a broad range of functions related to survival and mating. The complexity of social behaviors and the flexibility required for normal social interactions make social behavior particularly susceptible to disruption. The consequences of developmental insults in the social domain and the associated neurobiological factors are commonly studied in rodents. Though methods for investigating social interactions in the laboratory are diverse, animals are typically placed together in an apparatus for a brief period (under 30 min) and allowed to interact freely while behavior is recorded for subsequent analysis. A standard approach to the analysis of social behavior involves quantification of the frequency and duration of individual social behaviors. This approach provides information about the allocation of time to particular behaviors within a session, which is typically sufficient for detection of robust alterations in behavior. Virtually all social species, however, display complex sequences of social behavior that are not captured in the quantification of individual behaviors. Sequences of behavior may provide more sensitive indicators of disruptions in social behavior. Sophisticated analysis systems for quantification of behavior sequences have been available for many years; however, the required training and time to complete these analyses represent significant barriers to high-throughput assessments. We present a simple approach to the quantification of behavioral sequences that requires minimal additional analytical steps after individual behaviors are coded. We implement this approach to identify altered social behavior in rats exposed to alcohol during prenatal development, and show that the frequency of several pairwise sequences of behavior discriminate controls from ethanol-exposed rats when the frequency of individual behaviors involved in those

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sequences does not. Thus, the approach described here may be useful in detecting subtle deficits in the social domain and identifying neural circuits involved in the organization of social behavior.

Keywords

Prenatal Alcohol Exposure; Fetal Alcohol Spectrum Disorders; Play Fighting; Aggression

Introduction

Social interactions are complex and comprised of many constituent behaviors with distinct roles that support a broad range of functions including bonding, play, establishment of dominance hierarchies, and communication. The dynamic nature of social interactions present major challenges for social species, and to researchers engaged in the analysis of social behavior. For example, rough and tumble play is characterized by dynamic interactions that are topographically similar to genuine fighting, such that distinguishing play and fighting depends upon potentially subtle signals. In the rat, play behavior can be distinguished from other forms of behavior based on the target of “attacks”, with the nape of the neck being the primary target of play compared to more posterior targets for aggressive behaviors (Pellis & Pellis, 1987, 2007). Although play fighting may appear to be stochastic, these behaviors are highly organized into sequences characterized by reciprocity among the individual animals (Pellis & Pellis, 1987) and both participants must engage in the appropriate sequential behaviors (S. M. Himmler, Himmler, Pellis, & Pellis, 2016). Such sequential processes are ubiquitous in the social domain and among social species. For example, verbal interactions among humans are typically characterized by reciprocity and complex temporal sequences of behavior (Levinson, 2016).

Normal social interactions require awareness of multiple factors, including the status of others (such as social dominance or sex) and sensitivity to contextual factors including temporal context and the behavior of conspecifics (S. M. Himmler et al., 2016). The neural circuitry involved in social behavior is commensurately complex and distributed, including limbic, subcortical, and neocortical regions including the prefrontal cortex (Numan, 2015). In rodents, the regions of the frontal cortex have been linked with abnormalities in social behavior (Bell, Pellis, & Kolb, 2010; Hamilton et al., 2010; B. T. Himmler, Pellis, & Kolb, 2013; Kolb, 1974; Pellis et al., 2006; Schneider & Koch, 2005) and the capacity for adaptation in the social domain. The hippocampus has also been implicated in the appropriate sequencing of social behavior (Maaswinkel, Gispen, & Spruijt, 1997). In addition to dependence on intact neural circuitry, social competencies depend critically upon adequate social experience during development (Pellis et al., 2006; Pellis & Pellis, 2007).

Owing to the complexity of neurobiological and experiential factors, abnormalities in social behavior are common consequences associated with disorders of the nervous system. During the past decade research in our laboratory has investigated the effects of moderate prenatal alcohol exposure (PAE) in a rat model of Fetal Alcohol Spectrum Disorders (FASD). FASD is an umbrella term that includes disorders associated with a broad range of negative consequences resulting from exposure to alcohol during prenatal development, including

Fetal Alcohol Syndrome (FAS), partial FAS, and alcohol related neurodevelopmental disorders (ARND) (May et al., 2014; Riley, Infante, & Warren, 2011). The prevalence of FASD is approximately 2–5% in the United States (May et al., 2014). The consequences of heavy developmental alcohol exposure include facial dysmorphologies and severe deficits in cognition and behavior (Riley et al., 2011). The consequences of moderate PAE are more subtle, yet persistent, in humans and non-human animal models (Conry, 1990; Marquardt & Brigman, 2016; Streissguth et al., 1991; Streissguth, Barr, & Sampson, 1990; Valenzuela, Morton, Diaz, & Topper, 2012). Deficits in social behavior have been repeatedly observed in children with FASD (Disney, Iacono, McGue, Tully, & Legrand, 2008; Greenbaum, Stevens, Nash, Koren, & Rovet, 2009; Larkby, Goldschmidt, Hanusa, & Day, 2011) and in non-human animal models of FASD across a broad range of exposure doses (Cullen, Burne, Lavidis, & Moritz, 2013; Hamilton et al., 2010; Kelly & Tran, 1997; Middleton, Varlinskaya, & Mooney, 2012; Mooney & Varlinskaya, 2011; Parker et al., 2014; Tunc-Ozcan, Ullmann, Shukla, & Redei, 2013; Varlinskaya & Mooney, 2014; Wellmann, George, Brnouti, & Mooney, 2015). The detection of social behavior deficits with more moderate (e.g., Blood ethanol concentrations (BECs) of ~60–80mg/dl) (Hamilton, Barto, et al., 2014) or low exposure (e.g., BECs < ~40mg/dl) (Cullen et al., 2013) can be more challenging tasks compared to detecting effects of heavy exposure (e.g., BECs > ~200mg/dl).

Analyses of rodent social behavior typically include quantification of the frequency and duration of behaviors of interest. For example, in our previous studies we have utilized analysis of video recordings to code behaviors of interest using specialized software that creates a record of the precise onset and offset time of each coded behavior (Barto, Bird, Hamilton, & Fink, 2016). From these records, the overall frequency and total time spent engaged in each behavior are calculated for statistical analyses (Bird et al., 2017; Hamilton et al., 2010; Hamilton, Barto, et al., 2014; Hamilton, Magcalas, et al., 2014; Rodriguez et al., 2016). Similar approaches to quantification of social behavior are commonly utilized in the field. Considering that social behavior abnormalities following neurobiological or other experiential manipulations can be difficult to detect, analyses of the sequential structure of social behaviors could provide a more sensitive metric for detection of abnormalities. For example, Maaswinkel et al. (1997) observed alterations in the sequential structure of social behavior following hippocampal damage in the absence of gross changes in the frequency of individual behaviors. Analysis of behavioral sequences could also provide insight into the function of potentially ambiguous behaviors. For example, we demonstrated that adult male rats prenatally exposed to moderate levels of alcohol display increased wrestling behavior, however, attempts to disambiguate whether this reflects play or aggression based on the topography of the behavior (Hamilton, Barto, et al., 2014) or ultrasonic vocalizations (Bird et al., 2017) have yielded mixed results. Quantification of behavioral sequences involving wrestling could contribute critical information needed for this disambiguation.

Several approaches exist for characterizing the sequential structure of behavior. For example, the Eshkol-Wachman Movement Notation method (Eshkol & Wachman, 1958) is a system in which body position in space and time is coded, providing a record of the temporal sequence of movements and their organization. These records can be performed separately on interacting organisms, and utilized to examine the relative spatiotemporal structure of behavior between individuals (Carrier, Leca, Pellis, & Vasey, 2015; Norman, Pellis, Barrett,

& Henzi, 2015; Pasztor, Smith, MacDonald, Michener, & Pellis, 2001; Pellis, 1982). A major advantage of this system is that the topography of specific behaviors can be characterized in great detail. A downside to the use of this system is that it requires considerable training and time to perform the analyses, which may be impractical or impossible for studies that utilize large sample sizes. The purpose of the current paper is to evaluate a sequential analysis technique to characterize abnormal social behavior in the rat, using previously published data on the effects of moderate PAE. We employed an analysis of the sequential structure of social and non-social behaviors that can be easily applied to any data set in which individual behavior timing and duration are coded. The approach involves using an arbitrary alphanumeric code for each behavior of interest, and creating code strings that comprise the overall sequence of behaviors during a social interaction session. The frequency of specific sequences (e.g., pairs, triplets) of behaviors can be quantified for analysis. Using this approach, we were able to distinguish the behavior of animals exposed to alcohol prenatally from control animals based on paired sequences of behavior, when the frequency of individual behaviors did not discriminate treatment groups. Although this approach was applied to alcohol effects here, it could be applied to any situation in which the requisite behavioral coding is available.

Methods

Data Source and Behavior Quantification

The behavioral data utilized for the present analyses have previously been reported (Hamilton, Barto, et al., 2014; Rodriguez et al., 2016). All experimental procedures included in this manuscript adhered to the Public Health Service policy on humane care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of New Mexico (IACUC protocol numbers MCC-101106 and 101166). All data were obtained from adult male Long-Evans rats that were exposed to moderate levels of alcohol (PAE) or saccharin (SAC) during gestational development via daily voluntary consumption of a 5% ethanol solution or a saccharin solution by the dam as described in Hamilton, Magcalas, et al. (2014). The level and pattern of voluntary ethanol consumption achieved with the exposure method has not yielded significant effects on maternal weight gain, offspring birth weight, litter size, placental wet weight, offspring weight at behavioral testing, or maternal care (Hamilton, Magcalas, et al., 2014; Staples, Rosenberg, Allen, Porch, & Savage, 2013). Due to the latter, and our prior observations of social behavior effects of moderate prenatal alcohol exposure with or without cross-fostering, pups were maintained with the dam until weaning. At that time all animals were pair-housed with a partner from the same prenatal treatment condition at weaning and tested during adulthood (> 90 days of age). Rats were acclimated to a test apparatus (95 cm × 47 cm × 43 cm) in pairs during 30 minute sessions on three consecutive days. All rats were habituated to the apparatus in the same pairings in which the social behaviors were carried out (Hamilton, Magcalas, et al., 2014). At the end of the second session, animals were housed in isolation for 24 hours and reunited in the test apparatus for a 12 minute social interaction session. Video recordings of the social interaction sessions were analyzed offline using the Simple Video Coder (SVC) (Barto et al., 2016) by a blind rater. Using the SVC software, the precise temporal onset and offset times for behaviors of interest were recorded for each individual

animal. In our prior reports, these behavioral records have been used to quantify the total number, duration, and latency to first occurrence of individual behaviors. These behaviors include social interactions such as wrestling (including pinning), anogenital sniffing, other sniffing of the partner's body (social sniffing), and allogrooming (grooming of the partner). For analysis purposes wrestling was defined as any interaction during which the partners were engaged in tumbling/rolling or pinning the partner down. Anogenital sniffing was defined as any instance of one animal making snout contact with the anogenital region of the partner. Social sniffing was coded as any other contact between the snout and the body of the partner (with the exception of the nape of the neck, as this is a target for attacks during play and wrestling). Allogrooming was coded as any grooming/licking of the partner. Self-directed and individual behaviors (self-grooming, body shaking), and environment-directed behaviors (sniffing/digging in the bedding, rearing) were also quantified. Video examples of exemplar behaviors are available in (Hamilton, Magcalas, et al., 2014).

The frequency of each behavior was quantified for each rat. Because the mean frequency of scratching, crossing over/under, and boxing was less than one for the entire population of rats these variables were not included in the analyses. Representative ethograms illustrating the timing and duration of the eight analyzed behaviors for each prenatal treatment group are shown in Figure 1. For sequential analyses of paired behaviors, the onset and offset times were imported into Matlab. Each individual behavior was symbolized using a letter, thus representing a sequence of behaviors for each rat (e.g., RRRRWWWGGGRR…). These behavioral codes were nominal in that each behavior was symbolized by exactly one letter independent of the duration of the behavior. Search queries were then generated for each possible pairwise sequential combination of the eight behaviors included in the analyses. This allows for analysis of each target behavior and its preceding or following behaviors. For example, the occurrence of the pattern of a rear preceding a social sniff would be "RS." Finally, these search queries were run on each rat's behavior sequence to assess the frequency of all possible pair-wise sequences for each individual target behavior such that the frequency of sequences where the target behavior either followed or preceded each behavior was quantified for each rat. A sample data file and Matlab script illustrating generation of the nominal code strings and search functions is provided in the supplementary materials.

Statistical Analyses

Separate independent samples t-tests were conducted to compare SAC and PAE rats on measures of frequency of individual behaviors and behavioral sequences. We report significant results at $p < 0.05$ and $p < 0.0016$, corrected for multiple comparisons. Measures of effect size (Cohen's *d*) were calculated for each test and standard guidelines for characterization of effects as small (> 0.20), medium (> 0.50), and large (> 0.80) were adopted (Cohen, 1988). To evaluate the relative ability of individual and sequential behaviors to discriminate prenatal treatment groups, two types of stepwise discriminant analyses were performed; the frequency of the eight individual behaviors were included in one discriminant analysis, and separate discriminant analyses were conducted for each of the eight target behaviors. The purpose of the latter analyses was to determine if the frequency of behavioral sequences provided greater discrimination than the frequency of individual

behaviors. These analyses included the individual behavioral frequency and the frequency of all sequences involving that behavior. Thus, each of the eight discriminant analyses included 16 behavioral measures (1 individual frequency and the 15 unique sequential frequencies). A threshold of greater than + 0.30 for structure matrix coefficients (loadings) on individual variables was adopted to identify variables that provided robust discrimination of groups. All statistical analyses were performed in SPSS (version 24) for the Mac.

Results

Behavior Frequency

Mean frequencies for individual behaviors in the SAC and PAE treatment groups are presented in Table 1A and mean frequencies for behavioral sequences are presented in Tables 1B (SAC) and 1C (PAE). Values from independent samples t-tests are represented in Figure 2. Measures of effect size and precise p-values associated with independent samples t-tests are shown in Tables 2A (individual behaviors) and 2B-C (behavioral sequences).

For individual behavior frequencies, only the frequency of wrestling was significantly different (PAE > SAC; see Figure 2A) with a large effect size (> 0.80; see Table 2A). The effect size for digging (PAE > SAC) slightly surpassed the threshold for a medium effect size (> 0.50), however, this effect did not approach significance. All other effect sizes for individual behaviors were < 0.47.

For behavioral sequences there were five effects significant at $p < 0.05$, and two effects with p values below the corrected alpha level of 0.0016 (see Figure 2B). Of these, five effects involved wrestling and each of these occurred at higher rates in PAE rats. The rate of wrestling followed by a separate instance of wrestling was increased in PAE rats. Social (trunk) sniffing preceding wrestling at a higher rate in PAE rats, but anogenital sniffing did not.

Discriminant Analyses

Structure matrix coefficients for the stepwise discriminant analyses are represented graphically in Figure 3 and numerical values are provided in Supplementary Table 1 and discriminant function statistical outcomes are provided in Supplementary Table 2. For the discriminant analysis conducted on measures of individual behavior frequencies, only wrestling had a coefficient > 0.3 (Figure 3A). Figures 3B–C represent coefficients for separate discriminant analyses that were organized around each target behavior and all possible sequences of other behaviors and the target behavior. For example, the linear discriminant analysis for wrestling behavior included the frequency of wrestling as well as frequencies for sequences that included all possible sequences of other behaviors that preceded (Figure 3B) or followed (Figure 3C) wrestling. This approach was selected because it allowed sequential behaviors involving a specific behavior to be evaluated against measures of the individual target behavior, and because the sample size was insufficient to support discriminant analyses performed on all 72 variables simultaneously. Because the discriminant analyses were performed for each target behavior in this way, the resulting coefficients should be evaluated within each row spanning the three matrices of Figure 3.

For this reason, the coefficients for identical sequences will not be identical because they were determined in the context of different variables. For example, the value for the sequence shaking (target behavior) followed by wrestling in Figure 3B (column 1, row 6), though comparable, is not identical to the value obtained from a discriminant analysis in which wrestling is the target behavior and shaking is the preceding behavior (Figure 3C row 1, column 6). Frequencies of the same behaviors in sequence (e.g., wrestling followed by wrestling) provide the only cases for which the coefficients correspond because the variables are identical and evaluated within the same discriminant analysis.

For the discriminant analyses performed on sequential behaviors, several measures had values > 0.3. In addition to wrestling frequency, which provided good discrimination between prenatal treatment groups, the frequency of sequential behaviors including wrestling also provided good discrimination. Half of the 16 unique coefficients for behavior sequences that surpassed the 0.3 threshold involved wrestling. Specifically, the frequency of wrestling followed by wrestling or rearing, and the frequency of wrestling preceded by social sniffing, allogrooming, and shaking provided good discrimination between groups. It is important to emphasize that the frequency of individual behaviors for the latter three variables did not provide good discrimination between groups, thus, the sequential structure of these social behaviors and their relationship to wrestling provide better group discrimination above that possible using individual behavior frequencies alone. Several other sequential behaviors provided good discrimination when the individual behavioral frequencies did not discriminate, and direct statistical comparisons did not yield significant group effects. These include allogrooming preceding shaking, following social sniffing, and either preceding or following rearing (SAC > PAE). Self-grooming after anogenital sniffing (SAC > PAE), digging either before or after anogenital sniffing, and sequences of environment-directed behaviors (rearing following or preceding digging; PAE < SAC) also provided good discrimination.

General Discussion

The primary goal of the present paper was to evaluate the utility of quantitative data on the frequency of paired behavioral sequences during social interaction to identifying abnormal social behavior. The approach utilized here involved counting the number of all possible sequences of individual behavior pairs from a set of behaviors selected to assess social interaction, self-directed behavior (e.g., grooming), and environment-directed behaviors (e.g., rearing). These quantitative data were obtained using a simple search algorithm performed on data coded from video records, which included the precise timing and duration of each behavior. The coded data were converted to sequential strings of letters corresponding to behaviors, which were then searched from all possible paired sequences. For each individual behavior, this analysis provides information regarding the antecedent and subsequent behaviors that occasion each behavior. Using this approach, abnormal social behavior in rats that experienced a neurodevelopmental insult (prenatal alcohol exposure; PAE) could be discriminated from control rats based on the sequential structure of social behaviors, even when the frequency of individual behaviors involved in the sequences did not differ between groups. Because a broad range of neurodevelopmental insults result in

altered social behaviors, this approach could be useful in identifying potentially subtle behavioral alterations in the social domain.

Though characterization of PAE effects on social behavior was not our primary goal, we briefly discuss the principal findings obtained with the sequential structure analysis in the context of evaluating the utility of this approach. For individual behavior frequencies, PAE rats engaged in significantly higher numbers of wrestling compared to controls. This observation has been highlighted in previous reports findings from our laboratory (Bird et al., 2017; Hamilton et al., 2010; Hamilton, Barto, et al., 2014; Rodriguez et al., 2016) and a discriminant analysis revealed that wrestling was the only individual behavior that distinguished control from PAE rats. Separate discriminant analyses further revealed several behavioral sequences that discriminated groups. The frequency of wrestling followed by wrestling, wrestling followed by rearing, and the frequency of wrestling preceded by social sniffing, allogrooming, and shaking provided good discrimination between groups. Of primary significance to the goal of the present paper, none of the other behaviors involved in these sequences discriminated groups on the basis of individual behavior frequencies, with the exception of the wrestling-wrestling sequences. Thus, the quantification of sequential structure was more sensitive to detection of altered social behavior than individual behavior frequencies.

Previously we observed greater rates of social sniffing in PAE rats when partners were novel, or not experienced recently (e.g., the preceding 7 days) (Hamilton et al., 2010), whereas SAC rats displayed increased anogenital sniffing. These differences were taken to reflect differential social investigation strategies, with social sniffing being more readily performed “opportunistically” when close in proximity to a partner, whereas anogenital sniffing requires greater effort. In the present analysis the “less-committal” form of social sniffing, though not occurring at different rates in PAE and SAC rats, preceded wrestling behavior more frequently in PAE rats. Similarly, body shaking occurred at greater rates prior to wrestling in PAE rats. This observation, as well as the pattern of sniffing preceding wrestling in PAE rats, could provide some clues as to the function of wrestling. Although it is unlikely that wrestling in these analyses represents bona fide play behavior, as all rats were well into adulthood at the time of behavioral assessment, ruling out play as an alternative to agonistic factors has been difficult. Conspicuous signs of fighting or related behaviors have not been observed, although previous analyses indicated that PAE rats directed attacks toward the rump more than the nape of the neck (Hamilton, Barto, et al., 2014), which is the primary target of play behavior (Pellis & Pellis, 1987). Analysis of ultrasonic vocalizations also failed to clearly implicate agonistic factors in increased wrestling as 22kHz vocalizations did not occasion the encounters of interest (Bird et al., 2017). Because body shaking is a sign of being uncomfortable, these observations provide further evidence that wrestling behaviors in PAE rats reflect agonistic encounters rather than play. Rearing prior to and after wrestling was also increased in PAE animals, indicating that PAE rats were more likely to engage in environment-directed behavior prior to or after wrestling at higher rates than SAC controls.

The other two significant group effects for behavioral sequences involved allogrooming and rearing (in each order) and were expressed at higher rates in SAC animals. One possibility is

that these behaviors occur together because of their topographical similarities. In allogrooming, the grooming partner tends to make contact with the partner using the forepaws, placing the animal in a position similar to rearing. The fact that both orders were observed suggests that control rats also transitioned from rearing into allogrooming as well. Previously we have observed greater levels of allogrooming in SAC rats (Hamilton, Barto, et al., 2014). Although reductions in PAE rats in this aspect of social behavior were not robust in the combined dataset utilized here, the sequential structure of this behavior relative to rearing was reduced in PAE rats in the absence of substantial differences in the baseline frequency of the individual behaviors. The effect sizes for group differences in these behavioral sequences were medium to large (Cohen's $d = 0.74–0.80$). As with the wrestling effect noted above, because rearing is a high frequency activity, it is highly likely that rearing is involved in behavioral sequences that also occur at higher rates.

Sequential data may also provide contextual cues relevant to understanding the purpose or motivation of the behavior. A single behavior never occurs in isolation; the events that precede and follow the target behavior offer information regarding the motivation of the organism (Bijou, Peterson, & Ault, 1968). The examination of behavior pairings can also clarify the locus of behavior between environment-directed, self-directed, or social-directed. For example, that wrestling is occasioned by body shaking suggests that the wrestling behavior is agonistic rather than reflecting play or affiliative behavior. Considering that wrestling as an individual behavior discriminated treatment groups, it is perhaps not surprising that several sequences involving wrestling also discriminated groups well. Several behavioral sequences, however, discriminated treatment groups even though neither of the individual behavior frequencies discriminated groups. These observations indicate that the sequential analyses can detect alterations in temporal configurations of behavior that would otherwise be missed. In the dataset utilized here, pairings of social behaviors, self-directed behaviors environment-directed behaviors discriminated groups, including allogrooming preceding shaking, allogrooming following social sniffing, allogrooming preceding or following rearing, self-grooming following anogenital sniffing, and digging before or after anogenital sniffing. Importantly, each of these behavioral pairings involved at least one social behavior. Collectively, these observations indicate that the sequential structure of social behaviors is altered by PAE, and these measures provide unique sources of information for identifying group differences in social behavior.

The techniques utilized here to quantify behavioral sequences can be considered as intermediate to the standard approach of quantifying the frequency and duration of individual behaviors during a single session, and more sophisticated analyses of behavioral sequences, such as that achieved with the Eshkol-Wachman notation system (Eshkol & Wachman, 1958). Presuming that a record of individual behaviors and their timing is available (e.g., from coding of video records), the ease with which sequential behaviors can be quantified is among the major advantage of this approach. The Eshkol-Wachman system can provide a highly detailed analysis of movement sequences for an individual or multiple individuals, which is of great utility if the structure of particular behaviors is needed. The precision afforded by the Eshkol-Wachman system, however, requires additional training for personnel and more time for analyses to be completed. The latter is a particularly important consideration when large numbers of cases need to be analyzed and high-throughput

assessments are required. In cases where analysis of the precise structure of individual behaviors is not required, the approach described here can provide robust and sensitive measures capable of discriminating normal and abnormal social behavior in rats. This approach offers several advantages in that it does not rely on complex technologies or training, and the only expertise needed is how to code the targeted behaviors as would be performed for identification of individual behaviors.

There are several limitations of the approach and its implementation in the present paper. The precise temporal relationships among the behaviors involved in a sequence were not considered. With the available data, therefore, we cannot conclude whether or not a behavior influenced the onset of a behavior directly or had an influence on other intervening behaviors that were not coded. As the number of coded behaviors increases, this approach will tend to yield counts of behavioral sequences that primarily reflect behaviors that occurred close together in time. As the number of coded behaviors decrease, the variance in inter-behavioral intervals would be expected to increase and the possible combinations of behaviors would decrease. Temporal aspects of behavioral sequences could, of course, be easily included in analyses and would be necessary to fully characterize the sequences of interest. The current approach also did not include consideration of when, within a session, the sequences occurred, which can reasonably be expected to contribute to the expression of behaviors. As implemented, the approach also only considered pairwise sequences of behavior, however, once the individual behaviors are coded searches for higher-order patterns can be performed. An additional consideration of some importance is that the voluntary ethanol consumption model utilized to obtain the present data only yield a limited range of blood ethanol concentrations (~60–80 mg/dL) based on the amount of ethanol rats will consume. Thus, we do not address the sensitivity of the analytical method using systematic dose-response curves. Other approaches that do not involve voluntary self-administration, such as gavage, injections, or vapor exposure would be needed to address dose-response relationships. Because social behavior alterations are observed with a broad range of ethanol exposure models that utilize different exposure timing, duration, routes, and doses, we are hopeful that other groups will evaluate the utility of the approach described here for discriminating ethanol-exposed from non-exposed animals.

A number of potential uses and future research questions are suggested. With respect to assessments of PAE effects, the analysis of sequential structure of behaviors could be utilized to identify more subtle effects of PAE (Rodriguez et al., 2016) and manipulations designed to ameliorate the effects of PAE (Waddell, Yang, Ho, Wellmann, & Mooney, 2016; Wellmann et al., 2015). More generally, the analysis of social behavioral sequences could be useful in a broad range of animal models for which social behaviors are altered. This approach might be particularly useful in cases where subtle effects on behavior are suspected. Analysis of behavioral sequences may hold utility for exploring the neural bases of social behavior (Siviy & Panksepp, 2011). Further, although not done here, a broad characterization of normal social behavior sequences would be needed to provide normative data for different species and strains. Of course, future efforts involving the application of this approach need not be limited to the social domain, as any sequences of discrete behaviors could be quantified equally well.

In summary, the present paper describes a simple approach to the analysis of the sequential structure of social behavior based on coding of individual behaviors from video recordings. Behavioral sequences were quantified and measures obtained in normal control rats and rats with impaired social behavior (following prenatal alcohol exposure) revealed robust differences in groups on social behavior sequences that were more sensitive than measures of individual behavior. Thus, the described approach provides an easily implemented method that is potentially useful for identifying alterations in social behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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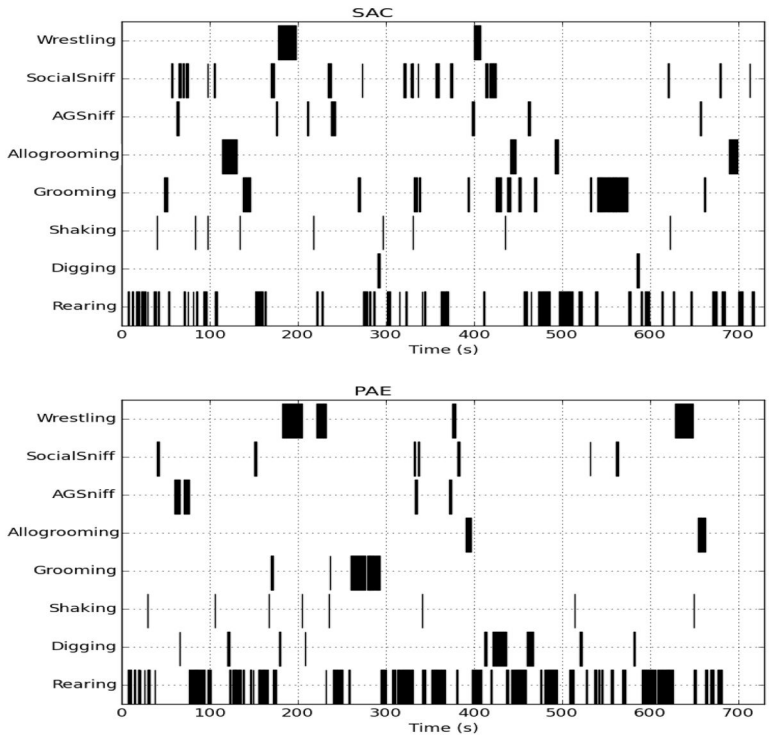

Figure 1.
Representative ethograms from one SAC and one PAE rat for each target behavior.

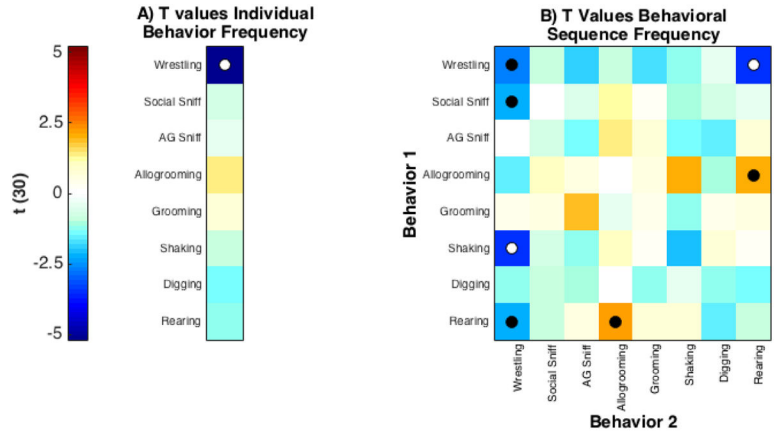

Figure 2.

Pseudocolor plots representing the magnitude of t values for comparisons (SAC-PAE) on individual target behavior frequencies (A) and for behavioral sequences (B). Negative (blue) values indicate higher frequencies in PAE rats. Positive values (red) indicate higher frequencies in SAC rats. Black circles indicate $p < 0.05$; white circles indicate $p < 0.0016$

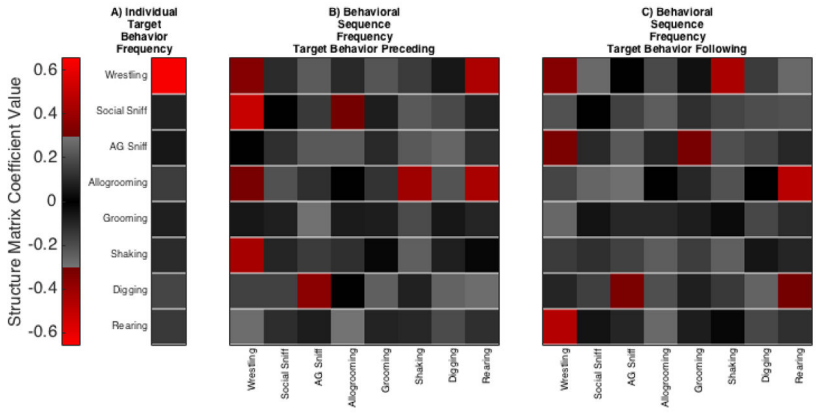

Figure 3.

Pseudocolor plot representing the magnitude of structure matrix coefficients (loadings) from stepwise discriminant analyses. Values represented in red exceed the accepted magnitude of +0.3 threshold for loadings on individual variables. All numerical values for structure matrix coefficients are provided in Supplementary Table 1. The first 8×1 matrix (A) represents the structure matrix coefficients for a linear discriminant analysis on the frequency the 8 individual target behaviors during the 12 minute social interaction session. Only wrestling exceeded the 0.3 threshold. Eight separate linear discriminant analyses were conducted on the frequency of behavior sequences for each individual target behavior; Each linear discriminant analysis included the frequency of the individual target behavior and each possible sequence of target behaviors preceding (B) or following (C) other candidate behaviors. Thus, each row spanning the matrices represents the structure matrix coefficients from a single linear discriminant analysis. For this reason, comparisons of coefficient magnitudes should only be performed within rows as the values represented across rows represent the results of different linear discriminant analyses. For matrices B and C, the behaviors listed on the horizontal axes represent the behaviors that following or preceded the target behaviors, respectively. Although only wrestling discriminated prenatal treatment groups with respect to the frequency of individual behaviors, the frequency of behavioral sequences involving all other behaviors except self grooming yielded structure matrix coefficients above 0.3. For example, the frequency of ‘Body Shaking’ (row 6) did not discriminate prenatal treatment groups, however, the frequency with which this behavior preceded wrestling behavior provided good discrimination among groups.

Table 1

Mean (SEM) frequency of A) individual behaviors in SAC and PAE rats, and behavior sequences in SAC (B) and PAE (C) rats. For sequential behaviors (B and C) the initial behaviors are represented in rows and the subsequent behaviors are in columns. Values in gray indicate a significant treatment group effect at $p < 0.05$.

A. Mean (SEM) for Individual Behavior Frequencies		
	SAC	**PAE**
Wrestling	1.25 (0.25)	4.38 (0.55)
Social Sniff	11.63 (1.11)	13.38 (2.25)
AG Sniff	3.00 (0.49)	3.44 (0.81)
Allogrooming	2.25 (0.92)	0.94 (0.38)
Grooming	9.88 (1.34)	8.50 (1.45)
Shaking	6.88 (1.07)	8.07 (0.77)
Digging	5.50 (1.34)	9.38 (2.32)
Rearing	40.63 (2.52)	46.25 (4.01)

B. SAC: Mean (SEM) Sequential Behavior Frequencies									
					Behavior 2				
		Wrestling	**Social Sniff**	**AG Sniff**	**Allogrooming**	**Grooming**	**Shaking**	**Digging**	**Rearing**
Behavior 1	Wrestling	0.13 (0.09)	0.13 (0.09)	0 (0)	0.07 (0.07)	0 (0)	0.19 (0.11)	0.13 (0.09)	0.5 (0.13)
	Social Sniff	0.38 (0.13)	2.25 (0.4)	1.07 (0.22)	0.38 (0.18)	0.38 (0.16)	1.32 (0.4)	0.75 (0.27)	4.25 (0.44)
	AG Sniff	0.19 (0.14)	0.63 (0.18)	0.07 (0.07)	0.19 (0.14)	0.25 (0.15)	0.5 (0.19)	0.07 (0.07)	0.82 (0.3)
	Allogrooming	0 (0)	0.19 (0.19)	0.13 (0.09)	0.13 (0.13)	0.44 (0.23)	0.44 (0.19)	0 (0)	0.82 (0.23)
	Grooming	0.19 (0.11)	0.69 (0.2)	0.38 (0.16)	0.32 (0.16)	4.38 (1.03)	0.32 (0.12)	0.19 (0.14)	3.13 (0.64)
	Shaking	0 (0)	1.19 (0.38)	0.19 (0.11)	0.07 (0.07)	1.32 (0.26)	0.07 (0.07)	0.82 (0.36)	3 (0.6)
	Digging	0.07 (0.07)	0.57 (0.23)	0 (0)	0 (0)	0.19 (0.11)	0.63 (0.33)	0.88 (0.36)	2.94 (0.68)
	Rearing	0.25 (0.12)	5.25 (0.65)	0.94 (0.24)	0.63 (0.24)	2.57 (0.43)	3.32 (0.58)	2.69 (0.65)	23.5 (2.25)

C. PAE: Mean (SEM) Sequential Behavior Frequencies									
					Behavior 2				
		Wrestling	**Social Sniff**	**AG Sniff**	**Allogrooming**	**Grooming**	**Shaking**	**Digging**	**Rearing**
Behavior 1	Wrestling	0.75 (0.22)	0.25 (0.12)	0.19 (0.11)	0.19 (0.14)	0.25 (0.15)	0.44 (0.19)	0.19 (0.11)	1.57 (0.28)

C. PAE: Mean (SEM) Sequential Behavior Frequencies

						Behavior 2				
		Wrestling	**Social Sniff**		**AG Sniff**	**Allogrooming**	**Grooming**	**Shaking**	**Digging**	**Rearing**
	Social Sniff	1.07 (0.3)	2.25 (0.61)		1.32 (0.34)	0.13 (0.09)	0.32 (0.12)	1.88 (0.42)	1.19 (0.48)	4.63 (0.89)
	AG Sniff	0.19 (0.11)	0.88 (0.31)		0.38 (0.23)	0 (0)	0.13 (0.13)	0.94 (0.27)	0.32 (0.16)	0.57 (0.16)
	Allogrooming	0.13 (0.09)	0 (0)		0.07 (0.07)	0.13 (0.09)	0.25 (0.2)	0.07 (0.07)	0.07 (0.07)	0.25 (0.15)
	Grooming	0.13 (0.13)	0.57 (0.16)		0.07 (0.07)	0.44 (0.23)	3.69 (1.02)	0.57 (0.16)	0.13 (0.09)	2.69 (0.32)
	Shaking	0.63 (0.18)	1.63 (0.44)		0.44 (0.19)	0 (0)	1.25 (0.25)	0.44 (0.19)	0.5 (0.28)	2.88 (0.33)
	Digging	0.25 (0.15)	0.88 (0.31)		0.07 (0.07)	0 (0)	0.44 (0.19)	0.82 (0.28)	1.69 (0.57)	4.94 (1.29)
	Rearing	0.88 (0.28)	6.25 (0.93)		0.75 (0.2)	0.07 (0.07)	2.13 (0.44)	2.75 (0.41)	4.94 (1.4)	27.19 (3.27)

Table 2

Effect sizes and p values for prenatal treatment comparisons (SAC-PAE) shown in Fig. 2; A) Frequencies of individual behaviors, B) Effect sizes for behavioral sequences, C) p values for behavioral sequences. Values in gray indicate a significant treatment group effect at $p < 0.05$.

A. Effect sizes (p values) for Individual Behavior Frequencies (SAC – PAE)		
	Cohen's d	**P values**
Wrestling	−1.84	<0.0001
Social Sniff	−0.25	0.4895
AG Sniff	−0.16	0.6449
Allogrooming	0.47	0.1956
Grooming	0.25	0.4893
Shaking	−0.32	0.3735
Digging	−0.51	0.1576
Rearing	−0.42	0.2440

B. Effect sizes (Cohen's d) for Prenatal Treatment Comparisons of Behavioral Sequence Frequencies (SAC – PAE)									
					Behavior 2				
		Wrestling	**Social Sniff**	**AG Sniff**	**Allogrooming**	**Grooming**	**Shaking**	**Digging**	**Rearing**
Behavior 1	Wrestling	−0.96	−0.31	−0.66	−0.30	−0.61	−0.43	−0.17	−1.24
	Social Sniff	−0.76	0.00	−0.22	0.44	0.11	−0.35	−0.28	−0.13
	AG Sniff	0.00	−0.25	−0.48	0.49	0.23	−0.48	−0.54	0.27
	Allogrooming	−0.52	0.35	0.21	0.00	0.22	0.69	−0.35	0.74
	Grooming	0.14	0.17	0.66	−0.16	0.17	−0.45	0.14	0.22
	Shaking	−1.23	−0.27	−0.43	0.35	0.06	−0.69	0.25	0.06
	Digging	−0.42	−0.29	−0.35	0.00	−0.43	−0.15	−0.43	−0.49
	Rearing	−0.75	−0.31	0.22	0.80	0.25	0.28	−0.52	−0.33

C. P values for Prenatal Treatment Comparisons of Behavioral Sequence Frequencies (SAC – PAE)									
					Behavior 2				
		Wrestling	**Social Sniff**	**AG Sniff**	**Allogrooming**	**Grooming**	**Shaking**	**Digging**	**Rearing**
Behavior 1	Wrestling	0.0110	0.3813	0.0726	0.4102	0.0935	0.2386	0.6395	0.0014
	Social Sniff	0.0403	1.0000	0.5366	0.2186	0.7516	0.3353	0.4289	0.7072

C. P values for Prenatal Treatment Comparisons of Behavioral Sequence Frequencies (SAC – PAE)

						Behavior 2				
		Wrestling	**Social Sniff**	**AG Sniff**	**Allogrooming**	**Grooming**	**Shaking**	**Digging**	**Rearing**
	AG Sniff	1.0000	0.4813	0.1842	0.1781	0.5177	0.1848	0.1355	0.4566
	Allogrooming	0.1536	0.3253	0.5592	1.0000	0.5304	0.0606	0.3253	0.0455
	Grooming	0.6998	0.6250	0.0710	0.6456	0.6364	0.2156	0.6998	0.5427
	Shaking	0.0016	0.4550	0.2386	0.3253	0.8619	0.0606	0.4920	0.8559
	Digging	0.2426	0.4108	0.3253	--------	0.2386	0.6653	0.2293	0.1802
	Rearing	0.0419	0.3813	0.5398	0.0303	0.4797	0.4296	0.1527	0.3600