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Alcohol exposure in utero disrupts cortico-striatal coordination required for behavioral flexibility

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Abstract

Deficits in behavioral flexibility are a hallmark of multiple psychiatric, neurological, and substance use disorders. These deficits are often marked by decreased function of the prefrontal cortex (PFC); however, the genesis of such executive deficits remains understudied. Here we report how the most preventable cause of developmental disability, *in utero* exposure to alcohol, alters cortico-striatal circuit activity leading to impairments in behavioral flexibility in adulthood. We utilized a translational touch-screen task coupled with *in vivo* electrophysiology in adult mice to examine single unit and coordinated activity of the lateral orbital frontal cortex (OFC) and dorsolateral striatum (DS) during flexible behavior. Prenatal alcohol exposure (PAE) decreased OFC, and increased DS, single unit activity during reversal learning and altered the number of choice responsive neurons in both regions. PAE also decreased coordinated activity within the OFC and DS as measured by oscillatory field activity and altered spike-field coupling. Furthermore, PAE led to sustained connectivity between regions past what was seen in control animals. These findings suggest that PAE causes altered coordination within and between the OFC and DS, promoting maladaptive perseveration. Our model suggests that in optimally functioning mice OFC disengages the DS and updates the newly changed reward contingency, whereas in PAE animals, aberrant and persistent OFC to DS signaling drives behavioral inflexibility during early reversal sessions. Together, these findings demonstrate how developmental exposure alters circuit-level activity leading to behavioral deficits and suggest a critical role for coordination of neural timing during behaviors requiring executive function.

Keywords

local field potential; executive control; in vivo electrophysiology; touchscreen

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1. Introduction

Deficits in executive functions can lead to intransigent patterns of behavior and poor decision making that are hallmarks of neuropsychiatric disorders (Elliott, 2003; Holmes and Wellman, 2009; Chudasama, 2011). Fetal alcohol spectrum disorders (FASD), caused by prenatal alcohol exposure (PAE), are the leading cause of preventable developmental disability (Ethen et al., 2009) and are increasingly characterized by deficits in executive function, together with confirmed exposure (Chudasama, 2011). Findings in children with FASD suggest that difficulties in planning, cognitive flexibility, and inhibition are better predictors of behavioral problems than intelligence-based measures (Mattson et al., 1999; Kodituwakku et al., 2001).

Reversal learning is a widely used paradigm for assessing behavioral flexibility across species. Such tasks require a subject to learn a stimulus-outcome association, and then switch to a different reward association after the initial association has been well-learned. In rodent models, PAE consistently impairs reversal learning irrespective of alcohol route of administration, dose, and assessment modality (Wainwright et al., 1990; Thomas et al., 2004; Marquardt et al., 2014; Atalar et al., 2016; Marquardt and Brigman, 2016; Waddell and Mooney, 2017). While effects on behavioral flexibility are consistent, there are few studies describing the neural basis of these executive disruptions following PAE, and even fewer utilizing translational measures of behavior.

The formation of a stimulus-outcome association is mediated by the dorsal striatum (DS), whereas the orbital frontal cortex (OFC) mediates reversal of these learned associations (Brigman et al., 2010; Graybeal et al., 2011). Single unit studies demonstrate that the DS mediates associative learning via integration of state-action-outcome associations (Yin et al., 2009; Corbit et al., 2012; Brigman et al., 2013; Bergstrom et al., 2018). In contrast, the OFC encodes response outcomes and tracks changes in choice value across reversal learning in order to exert top-down control of action (Schoenbaum et al., 2003; Bissonette et al., 2008; Moorman and Aston-Jones, 2014; Marquardt et al., 2017). These distinct processes can be quantified in real time by synchronization of local field potentials (LFP) in both the OFC and DS (DeCoteau et al., 2007b; Tort et al., 2008; Pennartz et al., 2011). Yet, the influence of developmental exposure to alcohol on these individual signals is still not described. Further, the interaction between OFC and DS required to make appropriate choices as associations change is still not fully understood.

In the current study, we tested the hypothesis that PAE impairs behavioral flexibility by directly altering the activity of both the OFC and DS, and the connectivity between these regions. Utilizing a rodent model of FASD we analyzed single unit activity in the OFC and DS during a touch-screen visual discrimination reversal paradigm sensitive to developmental alcohol exposure. Next, we utilized a dual-region recording paradigm to test whether OFC-DS intra- and inter-region coordination was significantly decreased by PAE. We show that PAE significantly altered rates of firing in OFC and DS neurons, decreased coordinated activity within OFC and DS, and altered OFC-DS connectivity. Diminished spike-field coordination and inter-region functional connectivity suggest that increased perseveration may be caused by an inability to overcome established patterns of activity in the OFC,

disrupting coordination of the OFC-DS cortico-striatal circuit required to facilitate learning of the new associations.

2. Materials and Methods

2.1 Prenatal Alcohol Exposure

Male and Female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were housed singly in a temperature- and humidity-controlled vivarium under a reverse 12 h light/dark cycle (lights off 0800 h). Female mice underwent a limited access voluntary prenatal alcohol exposure paradigm shown not to alter maternal care or gross pup development, as previously described (Brady et al., 2012; Brady et al., 2013; Marquardt et al., 2014; Olguin et al., 2019). Two hours into the dark cycle, female mice were given access to either 0.066% (w/v) saccharin or an ethanol solution (5% w/v for two days, then 10% w/v) sweetened with 0.066% (w/v) saccharin. Access was given daily for four hours (from 1000 to 1400 hr) during the dark cycle. Voluntary consumption was recorded by weight of solution consumed per kg of female weight. After one week of drinking 10% ethanol + saccharin or the water-saccharin control solution, individual females were placed into the cage of a singly housed male overnight at 1400 hr, immediately following the drinking period. Males never had access to ethanol. Females continued to consume ethanol and saccharin solutions throughout the consecutive five-day mating period. Pregnancy was positively determined by monitoring weight gain every 3–4 days. Dams were weaned off the alcohol solution (or saccharin alone) beginning on post-natal day 0 using a step-down procedure over 6-days, halving ethanol concentration every other day. PAE and saccharin (SAC) offspring were weaned at approximately 4 weeks of age and housed in groupings of 2–4 per cage in a temperature- and humidity-controlled vivarium under a reverse 12 h light/dark cycle (lights off 0800 h) and tested during the dark phase. All behavior was conducted on adult male offspring (n=26–28 per treatment). Beginning at 6–7 weeks of age, offspring were food-restricted to 85% of their free-feeding body weight. Operant training began once mice reached food-restricted weight and all mice were behavioral tested between 8–14 weeks. All experimental procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

2.2 Recording Methods

2.2.1 Operant Testing: All operant behavior was conducted in a custom made acrylic chamber measuring $21.6 \times 17.8 \times 12.7$ cm covered in matte white contact paper, based on the Med Associates design (model # ENV-307W, Med Associates, St. Albans, VT). The chamber was housed within a sound- and light-attenuating box (Med Associates, St. Albans, VT). A pellet dispenser delivering reward (14 mg dustless pellets; #F05684, BioServ, Frenchtown, NJ) into a magazine, a house-light, tone generator and an ultra-sensitive lever was located at one end of the chamber. At the opposite end of the chamber there was a touch-sensitive screen (Conclusive Solutions, U.K.) covered by a black acrylic aperture plate allowing two 2×5 cm touch areas separated by 0.5 cm and located at a height of 6.5 cm from the floor of the chamber. Stimulus presentation in the response windows and touches

were controlled and recorded by the K-Limbic Software Package (Conclusive Solutions, U.K.).

Mice were habituated to the operant chamber and to eating out of the pellet magazine by being placed in the chamber for up to 30 min with pellets available in the magazine. Mice retrieving 10 pellets within 30 min were moved onto pre-training. Mice were given a three-stage pre-training regimen. First, mice were trained to obtain reward by pressing a lever within the chamber on a Fixed Ratio 1 schedule. Mice pressing and collecting 30 rewards in under 30 min were moved to touch training. During this stage, a lever press led to the presentation of a white (variously-shaped) stimulus in 1 of the 2 response windows. Throughout the paradigm, images on the touch-screen were spatially pseudorandomized preventing side bias and ensuring location was not an informative variable. The stimulus remained on the screen until a response was made. Touches in the blank response window had no effect, while a touch to the white stimulus resulted in reward delivery, immediately cued by a tone and illumination of the magazine light on the opposite side of the operant chamber from the touch-screen. Mice initiating, touching and retrieving 30 pellets within 30 min were moved to the final stage of pre-training. This stage was identical to touch training except that responses at the blank window during stimulus presentation produced an immediate 10 sec timeout, signaled by illumination of the house light, to discourage indiscriminate screen responding. Errors on this, and all subsequent stages, were followed by correction trials in which the same stimuli and left/right position was presented until a correct response was made. Mice making 75% (excluding correction trials) of their responses at a stimulus-containing window over a 30-trial session were implanted with a fixed electrode array.

2.2.2 Electrode Array Implantation: After completing pre-training and at least two consecutive days of free-feeding, all mice were anesthetized with isoflurane and placed in a stereotaxic alignment system (Kopf Instruments, Tujunga, CA) for implantation of a microelectrode array bilaterally in either the OFC or DS (n= 7–8 per treatment and region). A second cohort was implanted with a dual array targeting both OFC and DS in the right hemisphere (n= 12 per trt). Each array (Innovative Neurophysiology, Durham, NC) was comprised of 16 individual 35 μ m-diameter tungsten microelectrodes arranged into 2 bundles of 2 $\times$ 4 electrodes (Fig. 1A; 150 μ m row/column spacing). The bilateral OFC group arrays were spaced 2.75 mm laterally between bundles, targeting OFC using coordinates: AP +2.60, ML +1.375, DV –2.60 (Fig. 1C). The bilateral DS group arrays were spaced 4.2 mm laterally and targeted DS using coordinates: AP +0.75, ML +2.04, DV –2.80 (Fig. 1F). Dual OFC-DS arrays had a custom offset between bundles with targeting coordinates for OFC: AP +2.60, ML +1.375, DV –2.60; and targeting coordinates for DS: AP +0.75, ML +2.04, DV –2.80. After 7 days of recovery, body weight reduction resumed and all mice were given a post-surgery reminder consisting of the last pre-training session to ensure retention of pre-training criterion.

2.2.3 Discrimination Reversal Paradigm: Following array implantation, all mice were tested on a pairwise discrimination reversal paradigm as previously described (Marquardt et al., 2017; Marquardt et al., 2019). Mice were first trained to discriminate two

novel, approximately equi-luminescent stimuli, presented spatially pseudorandomized across a possible 30-first presentation trials (not including correction trials) per daily session. As in pre-training, responses at the correct stimulus resulted in reward, immediately cued by the onset of a 1 sec tone; responses to the incorrect stimulus resulted in timeout, immediately cued by a 10 sec house-light followed by correction trials until a correct response was made. Due to the pseudorandomized location of the stimuli, correct performance during a session could not exceed 50% if a side bias was present. Correct stimuli were balanced across mice. Discrimination criterion was 85% correct responding (excluding correction trials) over two consecutive sessions. Reversal training began on the session immediately after discrimination criterion was attained, independently for each mouse. Here, the designation of correct versus incorrect stimuli was reversed for each mouse. Mice were trained on 30-trial daily sessions (same as for discrimination) to a criterion of 85% correct responding (excluding correction trials) over two consecutive sessions. In order to measure performance differences across distinct sessions, percent correct responses, total errors, reaction time (time from lever press initiation to screen touch) and magazine latency (time from screen touch to reward retrieval) were analyzed.

2.2.4 In vivo Electrophysiological Recording: Neuronal activity was continuously recorded during touch-screen behavior using a multichannel acquisition processor (OmniPlex, Plexon, Dallas, TX) as previously described (Brigman et al., 2013; Marquardt et al., 2017; Marquardt et al., 2019). Single unit oscillatory activity was captured during the following stages in OFC and DS electrode implanted mice: **D^{early}** = early session of discrimination where performance was at chance (% correct = 50%), **D^{late}** = session of discrimination criterion attainment (% correct > 85%), **R^{early}** = first session of reversal when perseverative responding is highest (% correct < 20%), **R^{mid}** = session of reversal where chance performance was re-attained (% correct = 50%), and **R^{late}** = session of reversal where criterion is re-attained (% correct > 85%). To focus specifically on time of behavioral impairment, single unit and oscillatory activity was captured during the following concurrent states in dual OFC-DS electrode implanted mice: **D^{late}**, **R^{S1}** = first session of reversal when perseveration is highest, **R^{S3}** = third session of reversal when perseverative responding begins to decrease, **R^{S4}** = fourth sequential session of reversal, and **R^{mid}**. If mice obtained chance criterion (50% correct) during the third or fourth sessions of reversal, the recording session was labeled chance reversal and mice were not recorded further. Continuous spike signal was sampled at 40 kHz and waveforms were manually sorted during recording, based on a manually set voltage threshold. Local field potentials were sampled from the same electrodes at 1 kHz and automatically low pass filtered at 200 Hz. Neuronal recording data were timestamped by responses from K-Limbic software by TTL pulse to reward tone and punishment house light. At the completion of testing, array placement was verified by electrolytic lesions made by passing 100 μ A through the electrodes for 20 sec using a current stimulator (S48 Square Pulse Stimulator, Grass Technologies, West Warwick, RI). Brains were removed post perfusion with 4% paraformaldehyde, 50 μ m coronal sections cut with a vibratome (Classic 1000 model, Vibratome, Bannockburn, IL), stained with cresyl violet and placement verified with reference to a mouse brain atlas (Paxinos and Franklin, 2001).

2.3 Data Analysis

2.3.1 Waveform Analysis: Waveforms were re-sorted offline using principal component analysis of spike clusters and visual inspection of waveform and inter-spike interval <1% shorter than 2 ms using Offline Sorter (Plexon Inc, Dallas, Texas). Epochs of firing rate spanning 1 sec pre-choice to 3 sec post-choice in averaged bins of .05 sec were created with NeuroExplorer software (NeuroExplorer; NEX Technologies, Littleton, MA) for both correct and incorrect choice trials. The 3 sec post-choice analysis window overlapped with immediate tone, during correct choice, and the first 3 sec of house-light during incorrect trials allowing for analysis of immediate response to secondary associative cues (Fig 1B). This time window has previously been shown to capture post-event activity while reducing event overlap (Brigman et al., 2013; Marquardt et al., 2017; Marquardt et al., 2019). If reward retrieval did occur prior to the end of the 3 sec analysis window on correct trials, the epoch for that trial was truncated to prevent overlap with reward signaling. Less than 5% of neurons were categorized as fast-spiking interneurons, based on baseline firing rate of >15 Hz (during 1 sec pre-event), and these were excluded from analysis (Brigman et al., 2013; Marquardt et al., 2019). Within DS, all units recorded had spike durations of <0.06 ms on average, and were characterized as putative medium spiny neurons. Neurons with average baseline-firing rate of 0 spikes/bin (<3%) were also excluded from the analysis. For all other neurons, normalized firing rates were calculated by Z-scoring 3 sec post-choice activity to 1 sec pre-choice baseline. Patterns of firing rate changes were examined for the period during immediate cue delivery (choice → 1 sec post), movement time (1 → 2 sec post) and magazine approach (2 → 3 sec post) using repeated-measures ANOVA followed by the Fisher's post-hoc test. In addition to spike-firing activity changes, the proportion of the population of neurons that significantly increased their firing rate in the post-event period (compared to individual pre-event baseline using Student's t-test; also known as behaviorally responsive neurons) were analyzed across choice-type, treatment and discrimination reversal session using chi square test of independence. All event-responsive neurons were analyzed, independent of significant response to other timestamped events.

2.3.2 Local Field Potential Analysis: Inter-trial phase consistency (ITPC) quantifies the variability of a frequency-specific signal at each point in time across a behavioral measure. ITPC values vary from 0 to 1, where 0 indicates random phases at that time-frequency point across trials, and 1 indicates identical phase values at that time-frequency point across trials. Time-frequency analyses were adapted to depth recordings using methods previously described (Marquardt et al., 2019) and computed using custom Matlab (TheMathWorks, Natick, MA) scripts. All LFP recordings were grounded with a cerebellar screw. Each regional bundle (OFC vs. DS) was referenced to the within-region recording electrode most distal from the second-region bundle to minimize volume conduction contribution (Marquardt et al., 2019). All analyses were conducted independently for each non-reference electrode. Since there were no significant differences in phase coherence between electrodes within each regional bundle within a single treatment group, data were averaged together over electrodes to characterize a single OFC and a single DS recording. Epochs were aligned to the time of choice (−1000 to 3000 ms).

Single trial data for each trial-type were convolved with a set of complex Morlet wavelets, defined as a Gaussian-windowed complex sine wave $e^{(i2\pi ft)} * e^{-t^2/(2 * \sigma^2)}$, where t is time, f is frequency (from 1 to 80 Hz in 80 logarithmic spaced steps to maximize lower frequency visualization), and σ is the width of each frequency band set at $4/(2\pi f)$ (Cavanagh et al., 2009; Cohen, 2014). Inter trial phase consistency (ITPC) was quantified as the length of the average of unit-length vectors that were distributed according to their phase angles (Lachaux et al., 1999). Since ITPC is highly dependent upon trial number, mice with fewer than 18 epochs of a given event on a given session were excluded from analysis for that session. To control for uneven trial number between subjects and conditions, 18 randomly selected trials were used to compute ITPC and averaged over 250 permutations for an average ITPC per subject unbiased by completed trial number.

A time-frequency region of interest (TF-ROI) was identified as event-dependent alterations in the ITPC spectra that were significantly greater than a randomly shuffled dataset combined across treatment, subjects and sessions. Notably, this TF-ROI selection was orthogonal to all hypothesis tests of treatment (PAE vs. SAC), session and region. A linear mixed model (R, lme4), which is robust against missing data, was used to statistically compare the average ITPC magnitude within the TF-ROI. Session was defined as a within-subjects factor and treatment as a between-subjects factor within a linear mixed model ANOVA, with *post-hoc* Tukey's test adjusted by Bonferroni's correction for multiple comparisons.

2.3.3 Spike-Field Coupling Analysis: Spikes and LFPs were recorded on the same array. The low frequencies addressed in this report are robust to spike-induced voltage contamination, which primarily affects gamma-band activity (Zanos et al., 2011). Spike-field coupling was analyzed by taking instantaneous phase angles of the LFP at the time of single-unit spikes during the one second after tone epoch (Vinck et al., 2012). Spikes during correct and incorrect choice types were analyzed separately, and mice completing fewer than 18 of the choice type were excluded from that session. Spikes occurring during an epoch were windowed into four separate 1000 ms bins (−1000 ms pre-choice to +3000 ms post-choice) (spike number average per epoch per subject: SAC DS = 298; SAC OFC = 602; PAE DS = 200; PAE OFC = 440). Instantaneous phase angles were calculated as in ISPC, except that frequency was only analyzed up to 40 Hz in linear spaced steps to evenly represent frequencies. Within each bin, averaged over 500 bootstraps, 200 angles were used to compute the paired phase consistency (PPC₁) value (Vinck et al., 2012) as the cosine of the angular difference between each angle within the vector:

$$PPC = \frac{\sum_{j=1}^N \sum_{k=j+1}^N \left| \text{mean} \left(e^{i(\theta_j - \theta_k)} \right) \right|}{N(N-1)}, \text{ where } \theta \text{ is the angle in radians and } N \text{ is defined}$$

by length of LFP epoch and number of spikes. PPC₁ removes angle comparisons from same trials, reducing trial bias. Due to results from ITPC the 1000 ms epoch beginning at tone offset was the time of interest analyzed and plotted SFC magnitude by frequency. Data were averaged into three frequency bins for statistical analysis, low (5–15 Hz), medium (16–25 Hz), and high (26–40 Hz), and analyzed using a linear mixed model autoregression (R,

lme4). Statistically significant contributions of factors and interactions were determined by ANOVA and post-hoc Tukey's tests.

2.3.4 Granger Prediction: We performed Granger bivariate autoregression analysis for OFC→DS and DS→OFC broadband directional coupling. Due to its high time resolution and spatial precision, intracranial LFP data are well suited for Granger autoregression analysis (Barnett and Seth, 2014). Granger bivariate autoregression analysis was adapted from the BSMART toolbox (Cui et al. 2008), Granger causality connectivity analysis tool box (GCCA) (Seth, 2010) and Analyzing Neural Time Series Data (Cohen, 2014), as previously described (Marquardt et al., 2019). Raw LFP data from both OFC and DS were downsampled to 200 Hz from 1000 Hz sampling rate. The ERP was subtracted from each raw data epoch prior to analysis. Each trial type was analyzed independently on a sliding time scale from −1 sec pre-choice to +3 sec post-choice, windowed into 500 ms blocks with 250 ms overlap. Each time segment was de-trended for improved stationarity and z-scored. As with ITPC, mice completing fewer than 18 of the defined trial type were excluded from the analysis for the corresponding recording session. Prior to Granger calculation, model order was calculated utilizing Bayesian Information Criterion (BIC) independently for each trial type, session, treatment and subject (Schwarz, 1978). The average model order was rounded to the nearest whole number for a final order number of 7, allowing 35 ms of past data to be incorporated in to the Granger prediction, which is comparable to previous intracranial LFP data (Zavala 2014). Granger prediction defined as $\ln\left(\frac{\sigma(Ex)}{\sigma(Exy)}\right)$, with $\sigma(Ex)$ and $\sigma(Exy)$ variance error terms from univariate and bivariate autoregression models, respectively. Both OFC → DS and DS → OFC directions were analyzed, although cortico-striatal anatomy suggests the OFC→DS projection would be the only direct monosynaptic pathway (Zingg et al., 2014). Autoregression coefficients were convolved with complex sine waves across focused logspaced frequencies from 1 to 15 Hz and applied to the model error variance via transfer function for Granger analysis across frequency and time (Cui et al., 2008).

Differences in Granger autoregression magnitude have been shown to be distinguishable between low-frequencies, however, for conservative Granger prediction, this analysis was completed on a broad low-frequency (<15Hz) time-range of interest (TF-ROI). The average Granger Predication magnitude within this low-frequency TF-ROI was calculated for each subject and was used for statistical comparison as described with ISPC. Information transfer direction and choice response types were treated independently. DS → OFC directionality magnitude was 10x lower than OFC→DS, and was below the threshold to differentiate signal from noise, which is consistent with directional connectivity of corticostriatal loops (Alexander and Crutcher, 1990). Therefore, only OFC → DS results were analyzed here.

3. Results

We successfully captured 865 single units during the 5 learning stages of the touch-screen task. In the OFC we captured 427 putative pyramidal neurons (SAC = 205, 233 = PAE) while in the DS we captured 438 putative medium spiny neurons (SAC = 205; PAE = 233). In order to examine the recruitment and activity of neurons after PAE, we compared both the

firing rate and percentage of recorded neurons that significantly altered firing rate after a correct choice across groups.

3.1 Cortical Spike Firing and Recruitment is Altered by PAE

Consistent with our previous findings, PAE treatment did not affect the number of sessions that bilateral OFC implanted mice took to reach initial discrimination criterion (SAC 17.2 ± 5.5 , PAE 12.3 ± 4.6 , ns, $p=0.46$). Following successful discrimination, when CS+/CS- designations were reversed to test behavioral flexibility, PAE mice reached 50% chance reversal (SAC 8.2 ± 0.8 , PAE 5.4 ± 0.5 , ns, $p=0.34$), and re-attained criterion in similar number of sessions as SAC controls (SAC 25.0 ± 4.9 , PAE 16.4 ± 2.0 , ns, $p=0.30$). However, PAE mice committed significantly more correction trials on the first day of reversal (Fig. 1D; Session x Treatment interaction $F_{4,88}=3.002$, $p=0.02$). This increase in correction trials was not accompanied by increases in first presentation error trials (i.e. non-correction errors; ns, $p=0.72$), revealing a selective deficit in behavioral flexibility in PAE mice, similar to deficits reported previously (Marquardt et al., 2014). There were no effects of treatment on latency to respond to visual stimuli or retrieve rewards following correct choices (Fig 1E).

Following a correct choice, OFC single-units significantly increased firing rate 2–3 sec post-correct choice across learning sessions (Fig 2A; Main Effect of Stage D^{early}: $F_{2,297}=18.472$, $p=0.0001$; D^{late}: $F_{3,222}=16.448$, $p=0.0001$; R^{early}: $F_{3,231}=16.465$, $p=0.0001$; R^{mid}: $F_{3,252}=12.336$, $p=0.0001$; R^{late}: $F_{3,228}=20.258$, $p=0.0001$). OFC firing rate responses were stable across sessions and were not significantly altered in PAE animals, except when the response was well learned. Specifically, PAE treatment significantly decreased firing rate during presentation of the associate tone when animals were performing at criterion during both D^{late} and R^{late} (D^{late}: Time x Treatment Interaction $F_{19,1406}=2.302$, $p=.001$; R^{late}: $F_{19,1444}=1.883$, $p=.01$). Analysis of single unit activity of the OFC following an incorrect choice revealed a consistent and sustained firing during the post-choice during sessions where the response required was ambiguous (Figure 2B; D^{early}: $F_{3,288}=6.638$, $p=.001$;; R^{early}: $F_{3,237}=3.422$, $p=.032$; R^{mid}: $F_{3,252}=5.029$, $p=.001$). PAE treatment significantly reduced this sustained increase following an incorrect response both during early discrimination learning (D^{early} Time x Treatment Interaction $F_{19,1862}=2.220$, $P=0.01$) and also during middle and late stage reversal learning (Figure 2B; R^{mid}: Main Effect of Treatment $F_{1,1596}=74.711$, $p=0.006$; Time x Treatment Interaction $F_{19,1596}=2.075$, $p=.04$; R^{late}: Main Effect of Treatment $F_{1,1425}=5.691$, $p=.031$).

Consistent with our previous findings, the proportion of choice responsive neurons significantly varied across learning stages ($\chi^2=35.95$, $p=0.001$). In contrast to selective reductions in firing rate following choice behavior, PAE significantly *increased* the proportion of recorded neurons that significantly altered their response following a correct choice. PAE mice had significantly increased choice-responsive neurons recruited during D^{early}, R^{early} and R^{late} (Fig 2C; D^{early}: $\chi^2=9.63$, $p=.002$; R^{early}: $\chi^2=4.338$, $p=0.04$; R^{late}: $\chi^2=15.47$, $p=0.001$). Similarly, PAE treated mice showed an increase in incorrect choice responsive units, but only during the criterion phase of reversal (R^{late}: $\chi^2=4.74$, $p=0.029$).

3.2 Dorsal Striatum Spike Firing and Recruitment is Increased by PAE.

As in OFC implanted mice, DS bilateral recorded mice showed no significant differences between SAC control and PAE treated animals in acquisition of the visual discrimination (SAC 11.5 ± 1.9 , PAE 9.6 ± 1.0 sessions, ns, $p=0.46$), or re-attainment of new contingencies post-reversal (SAC 17.0 ± 2.5 , PAE 19.4 ± 3.0 sessions, ns, $p=0.30$). PAE significantly and selectively increased the number of perseveration errors (Session x Treatment interaction $F_{4,88}=3.002$, $p=0.02$) made during the first session of reversal (Fig 1G), with no significant alteration in latency to respond to stimuli or retrieve a reward following a correct response during any session (Fig 1H).

In SAC control animals DS firing rate significantly increased above pre-choice baseline during the 2–3 sec epoch after a correct choice consistently across all sessions (Fig 3A; Main Effect of Time D^{early} : $F_{3,312}=16.492$, $p=0.0001$; D^{late} : $F_{3,204}=3.559$, $p=0.02$; R^{early} : $F_{3,255}=5.366$, $p=0.001$; R^{mid} : $F_{3,237}=6.618$, $p=0.0001$; R^{late} : $F_{3,228}=6.659$, $p=0.0002$). Although elevated early on, DS firing rate in PAE animals was not significantly different from SAC controls during discrimination stages (D^{early} : ns, $p=0.23$; D^{late} : ns, $p=0.18$). However, PAE animals had a significantly elevated DS firing rate immediately after tone cessation during R^{early} and R^{mid} (Time x Treatment Interaction R^{early} : $F_{19,1615}=1.610$, $p=0.046$; R^{mid} : $F_{19,1501}=1.645$, $p=0.040$). PAE increases in firing rate persisted through the R^{late} stage, where it was significantly increased above control levels continually from after the tone to immediately prior to reward retrieval (Main Effect of Treatment $F_{19,1444}=8.012$, $p=0.006$; Time x Treatment Interaction $F_{19,1444}=1.600$, $p=0.049$).

Following an incorrect response, DS units showed a pattern similar to the OFC with a slow but consistent ramping across the post-choice epoch when the house light was on. As in the OFC, this was consistent for all stages except D^{late} and R^{late} when animals were at criterion performance (Figure 3B; Main Effect of Time D^{early} : $F_{19,1615}=1.769$, $p=0.041$; R^{late} : Main Effect of Time $F_{19,1615}=2.458$, $p=0.03$). During R^{early} , single-unit response to an incorrect choice was significantly different than all other sessions (Main Effect of Session $F_{4,84}=4.184$, $p=0.012$, followed by post-hoc test), with response to an incorrect choice showing a continued ramping across the 3-second post-choice (Figure 3B; Main Effect of Time $F_{3,264}=8.189$, $p=0.001$, followed by post-hoc test). Unlike in the OFC, in the DS, PAE mice had increased firing following an incorrect response during initial learning in D^{early} (Time x Treatment Interaction $F_{19,1615}=1.850$, $p=0.033$). Most strikingly, PAE treatment significantly reduced firing rate following an incorrect response during this first session of reversal (Time x Treatment Effect $F_{19,1615}=2.855$, $p=0.031$).

Analysis of the proportion of DS behaviorally responsive neurons following a correct choice found that consistent with OFC patterns, PAE mice had significantly increased proportion of choice-responsive neurons, specifically early reversal in R^{early} and R^{mid} (Fig 3C; R^{early} : $\chi^2=21.85$, $p=0.001$; R^{mid} : $\chi^2=27.62$, $p=0.0001$). Similarly, analysis of neurons significantly altering firing to incorrect choice found that PAE treated mice had a greater percentage during both D^{late} and R^{late} (Figure 3C; D^{late} : $\chi^2=10.2$, $p=0.016$; R^{late} : $\chi^2=19.9$, $p=0.0002$).

3.3 Inter Trial Phase Consistency is decreased in OFC and DS after PAE

To determine if neuronal coordination within and between the OFC and DS was impacted by PAE, we recorded from a cohort of mice with dual OFC-DS arrays. Mice performed the discrimination reversal protocol with *in vivo* electrophysiology during discrimination criterion (D^{late}), and focused recordings were performed during early reversal sessions to better address inter- and intra-region coordination during sessions where behavior is impaired by PAE (Marquardt et al., 2014). As in single region animals, PAE mice made significantly more correction trials during these early reversal recording sessions than SAC controls ($F_{1,21}=4.716$, $p=0.0427$).

Analysis of correct choice behavior showed that ITPC increased significantly above background levels in the OFC and DS immediately following tone cessation for both SAC and PAE mice (Fig 4 & 5). Two separate TF-ROI were observed from this response, an upper TF-ROI from 1.75 to 7Hz, and a second, lower, TF-ROI from 1 to 1.75Hz, occurring 500 ms later. Average 1–1.75Hz TF-ROI ITPC magnitude across sessions was very different between OFC and DS in SAC control animals (Region x Treatment Interaction $F_{1,141}=16.10$, $p=.00009$, followed by Tukey's post-hoc). Average 1–1.75Hz TF-ROI ITPC magnitude remained comparable to SAC control levels in the OFC of PAE mice compared to controls (Fig. 4C), but was significantly decreased in the DS of PAE animals during R^{S3} , R^{S4} and R^{mid} (Fig 5C; Region x Treatment Interaction $F_{1,141}=16.10$, $p=.00009$, Treatment x Session Interaction $F_{4,141}=9.936$, $p<.00001$).

The 1.75–7Hz TF-ROI average ITPC magnitude response was highly dynamic in SAC control animals, responding significantly different between sessions (Fig 4 & 5; Main Effect of Session $F_{4,141}=4.78$, $p=.0012$) and between regions (Main Effect of Region $F_{1,141}=19.10$, $p=.00002$). Within this TF-ROI, PAE mice had significantly decreased ITPC magnitude in *both* OFC and DS during R^{S1} and R^{S3} (Fig 4D & 5D); Main Effect of Treatment $F_{1,141}=8.56$, $p=.004$; Treatment x Session Interaction $F_{4,141}=3.33$, $p=.01$).

Analysis of incorrect choice behavior found similar increases above background levels for both PAE and SAC. However, no significant differences were seen in either TF-ROI either by recording session or treatment with no significant interactions in the OFC or DS (Fig S1 & S2 main effect of treatment ns, $p=0.20$; ns; main effect of session ns, $p=0.62$; ns; main effect of region ns, $p=0.19$; ns). Overall, these findings indicate that intra-region coordination was significantly decreased following a correct choice only, primarily in the 1.75 to 7Hz range in both the OFC and DS during early sessions of reversal in PAE animals. This decreased coordination continued until animals exited the perseverative phase of reversal.

3.4 Spike-Field Coupling is Dysregulated by PAE

Based on our findings of decreased striatal and cortical phase alignment in tandem with aberrant recruitment of single units in PAE, we next examined whether coordination of single-units with local oscillations was impaired via spike-field coupling analysis within the OFC and DS. As no significant differences in field ITPC were seen across sessions or regions following incorrect choice responses, we focused the analysis on correct choice

behavior. In both the OFC and DS, spike-field coupling in the low frequency range (5–15 Hz) was significantly different from medium (16–25 Hz) and high (26–40 Hz) spike-field coupling magnitude. Medium and high frequency range spike-field coupling did not significantly differ from each other (OFC: Main Effect of Frequency $F_{2,253}=18.57$, $P<.01$; DS: Main Effect of Frequency $F_{2,253}=4.47$, $p=.01$), therefore further analysis focused on 5–15 Hz spike-field coupling alterations.

OFC spike-field coupling decreased in SAC control animals on the first session of reversal before increasing across early reversal sessions, re-attaining a similar spike-field coupling magnitude by the attainment of behavioral chance performance (Fig 6A). PAE animals had significantly elevated spike-field coupling during the first session of reversal followed by a gradual drop-off across reversal sessions in the OFC (Fig 6B; Main Effect of Treatment $F_{1,253}=8.01$, $p=.005$; Treatment x Session Interaction $F_{4,253}=2.52$, $p=.04$).

DS 5–15Hz spike-field coupling in SAC control animals was high during D^{late} and dropped off dramatically during the first sessions of reversal, before peaking during R^{S4} (Fig 6C). PAE treated animals had a blunted low-frequency spike-field coupling across frequencies, but no changes were significant (Fig 6D). As a measure of coordination between oscillations and spike firing, spike-field coupling further shows that in the OFC during the behaviorally impaired R^{S1} , cortical timing and coordination are altered after PAE.

3.5 PAE alters OFC-DS connectivity

OFC→DS function connectivity calculated by Granger prediction identified two significantly different time periods of connectivity (Main Effect of TF-ROI $F_{1,155}=6.58$, $p=.01$). “Choice” TF-ROI encompassed the time point of behavioral screen touch (choice), while the second “Tone” TF-ROI immediately followed the cessation of the reward-associated tone cue during a similar time of increased ITPC response.

In SAC control mice, functional connectivity during the Choice TF-ROI successively increased across reversal sessions peaking by R^{S4} (Fig 7A & C). In contrast, average functional connectivity during the Tone TF-ROI was low across sessions (Fig 7D). PAE treated mice had a significantly different functional connectivity profile compared to controls (Fig 7B; Main Effect of Treatment $F_{1,155}=5.69$, $p=.02$). Connectivity levels during Choice TF-ROI were not significantly different from controls during any session (Fig 7B & C), however, PAE animals had significantly elevated Tone TF-ROI connectivity on R^{mid} (Fig 7B & D; Treatment x Time Interaction $F_{1,155}=5.55$, $p=.02$). These results suggest that PAE animals had greater functional communication mid-reversal, further indicating alterations in signal timing and coordination.

4. Discussion

Utilizing an alcohol exposure model that has been previously shown to impair behavioral flexibility, we found that moderate PAE led to persistent changes in neuronal firing during early reversal and significantly altered both regional coordination of spike-timing and cortico-striatal communication. Specifically, PAE decreased OFC firing rates following a correct choice during early learning and late reversal, and following incorrect responses

during the critical mid-reversal learning characterized by exploratory behavior (Marquardt et al., 2017). PAE also increased DS firing rates following a correct response throughout reversal, and decreased DS responding following an incorrect response during early reversal. Overall, neuronal recruitment as measured by choice-responsive neurons in both the DS and OFC was significantly increased by PAE regardless of response type. Together, these results suggests that PAE disorganizes cortical and striatal signaling required to properly track choice feedback during learning and reversal. Consistent with these findings, both OFC and DS showed disrupted regional activity to correct responses, as measured by decreased inter-trial phase consistency as well as altered functional connectivity between the regions during early reversal sessions. This aberrant continuation of coordinated activity suggests that impaired flexibility after PAE may be due to inefficient information updating from cortex to striatum. It is possible that as contingencies change, decreased firing and coordination requires that PAE animals receive repeated instances of cortico-striatal signaling before they are able to update choice behavior.

Overall, OFC firing rates to correct responses were remarkably stable across learning sessions. PAE mice had subtle, but significant, decreases in firing when choices were well learned. While incorrect choice OFC responding was more dynamic, PAE mice had decreased firing activity during early learning, and also during late reversal learning sessions. When we analyzed the proportion of units contributing to rate changes following choice behavior, we found that PAE mice recruited significantly more choice responsive neurons in both regions, and to both choice types, even when firing rates were reduced. Together, these results suggest that PAE may have subtly altered reward prediction signaling as OFC firing in response to positive reward expectation generally followed patterns described in non-treated rodents and non-human primates, but was reduced during criterion performance when associations were well-learned (Thorpe et al., 1983; Schoenbaum and Eichenbaum, 1995; Schoenbaum et al., 2003). Firing following an incorrect response was also altered after PAE in a region specific manner. While initially elevated above control, PAE mice showed a significant reduction in DS firing during the first session of reversal, while PAE OFC neurons were consistently dampened during later stages. Based on waveform characteristics, the DS units analyzed in the current study were categorized as putative medium spiny neurons, which comprise 95% of all neurons in the striatum. Since striatal medium spiny neurons have an inhibitory influence over tonically active inhibitory basal ganglia output nuclei, firing of striatal neurons facilitates information transfer through the cortico-basal ganglia-thalamo-cortical loop (Alexander and Crutcher, 1990; Mink, 1996; Frank, 2005). Together with the increased recruitment of choice-responsive neurons in the DS and OFC in PAE animals after both response types, prenatal exposure seems to alter post-choice dynamic firing across cortico-striatal circuits, making both reward and error feedback more difficult to separate and increasing perseveration. Compared to chronic interment ethanol exposure in adulthood (DePoy et al., 2013), the effects seen after PAE on firing rate and recruitment were less robust, suggesting that developmental exposure may alter both individual activity, but also more regional activity and coordination as well. While the current study focused on the role of pyramidal OFC and MSN in the DS, cholinergic tonically active neurons (TANs) are also well established to play a role in reversal learning (Matamales et al., 2016). While only comprising 2–5% of all striatal neurons, TANs and are

posited to help optimize learning of reward outcomes via long bursting periods followed by silent periods (Aosaki et al., 1994; Franklin and Frank, 2015). These neurons have been shown to be altered by alcohol, and future studies utilizing tools to target cholinergic interneurons are needed to examine how prenatal exposure alters TAN function and influences reward learning and flexible behavior (Blomeley et al., 2011).

Data from dual OFC-DS recordings revealed that PAE led to non-coordinated striatal and cortical regional oscillatory activity. Specifically, following PAE, phase alignment following a correct-choice was significantly decreased during the first 3 sessions of reversal in both OFC and DS. In tandem with the increased recruitment of single units shown during early reversal sessions, these results may suggest that PAE impaired coordination between single-units and local oscillations (Womelsdorf and Fries, 2006; Gregoriou et al., 2009). Therefore, we conducted analysis of spike-field coupling and found that despite decreased OFC ITPC magnitude, spike-field coupling was elevated in the OFC of PAE across early reversal. We have previously shown that spike-field coupling correlates with behavioral strategy. As animals proceed through reversal, the switch from a perseverative, to an exploratory and then new associative pattern of behavior is accompanied by shifts in coupling at different frequencies (Marquardt et al., 2017). The consistently elevated low-frequency coupling after PAE suggests that after developmental exposure, mice show a delayed switch in the typical signaling that is necessary to promote efficient reversal. One possibility is that the absence of proper phase-consistency on the OFC failed to provide adequate windows for spike recruitment. This loss of signal then delayed the ability to update stimulus-response mappings downstream in the DS, as seen in the loss of phase-consistency in this region as well. This maladaptive pattern seen in the DS: increased recruitment of behaviorally responsive neurons and decreases in spike-field coupling, is particularly interesting. As in previous studies of rapid associative learning after adult binge drinking, this pattern suggest a continued reliance on previously learned behavioral patterns due to inefficient OFC updating during reversal (DePoy et al., 2013). These results also suggest that restoring phase-consistency in the OFC following choice behavior may improve downstream signaling, and rescue the deficits in behavioral flexibility seen after PAE.

Consistent with results in other tasks, the majority of DS spike-field coupling occurred in the beta (13–39 Hz), not alpha (8–13 Hz) band (Berke et al., 2004). Beta-coupling was seen in control mice in the transition from perseverative to learning phases of reversal where choice values are ambiguous and likely driven by top-down processing from frontal regions (Okazaki et al., 2008; Engel and Fries, 2010). In contrast, choices become more stimulus driven during later reversal, and becomes associated with higher frequency activities (>30Hz) which are involved in bottom-up processing (Cardin et al., 2009). Beta (13–39 Hz) frequency coupling in the DS was essentially absent in PAE mice during reversal, suggesting that the DS remains stimulus-driven, leading to the natural consequence of perseverative responding.

Disruption of coordinated signaling may further be promoted by discordant communication *between* regions. Synchronous activity between the OFC and DS is likely required to rapidly learn action-outcome expectations, playing a particularly important role when outcomes change (DeCoteau et al., 2007a). For example, rats chronically exposed to cocaine show

impaired reversal and decreased synchrony in the OFC - nucleus accumbens circuit (Goto and Grace, 2005; Calu et al., 2007; McCracken and Grace, 2013). While it is unlikely that OFC signaling to the DS alone initiates changes in behavior (Bissonette et al., 2008; Bissonette et al., 2013), our data suggest that OFC may inform future choices in a way that is complimentary to error signaling from the medial prefrontal cortex. Analysis of coordination between the OFC and DS in dual implanted mice showed that PAE significantly increased functional connectivity between the OFC and DS. While perhaps surprising, the persistent increase in OFC-DS connectivity into later reversal where connectivity is already decreasing in control, suggests that PAE may weaken outcome signaling from the OFC when contingencies change, resulting in more trials needed in order for animals to alter behavior. Our data also suggest that maladaptive perseveration after PAE may be due alteration in cortico-striatal firing following choices coupled to an overall increase in event-responsive neuronal firing. This increased “noise”, or lack of differentiated firing to expected and unexpected rewards during early reversal, may be compounded by deficient OFC network coordination as measured by ITPC. This suggests that these features lead to inefficient “top-down” influence over the DS which then results in sustained connectivity across regions past what is required in optimally functioning animals.

Together, the current findings reveal additional complexity regarding the effects of alcohol on developing circuits beyond altering firing rates. Alterations in neuronal firing, as well as timing and coordination of cortical and striatal regions may underlie impairments in executive function seen in FASD, as well as other neuropsychological disorders. The current model provides a framework for examining the activity from the level of individual units to interactive networks, although future studies are required to elucidate how developmental alcohol exposure alters synaptic structure and function leading to the changes in activity seen here. Specifically, alcohol is well known to alter both glutamatergic, and GABAergic function and activity in the cortex, and current studies are examining how both level and function of these receptors is altered in our exposure paradigm (Carpenter-Hyland et al., 2004; Skorput and Yeh, 2016). It is hoped that these integrative approaches can help elucidate the neural mechanisms underlying cognitive-behavioral deficits following neurodevelopmental insults, and provide a foundation for the development of novel prophylactic or therapeutic treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Examined how prenatal alcohol exposure (PAE) alters behavioral flexibility.
- Utilized single and dual region in vivo recording coupled with a touch-screen task
- Moderate PAE disrupts cortical and striatal spike-firing activity in vivo.
- PAE alters also alters inter-region cortico-striatal coordination during reversal.
- Insight into how a prevalent developmental insult impairs executive function.

Figure 1. Recording Paradigm and Behavior Results After PAE.

(A) Diagram of bilateral array with integrated LED headcap and electrode spacing for dual and single region recording. (B) Experimental trial timeline with highlighted recording window encompassing stimulus sampling (blue), correct choice with correct tone cue (yellow) and magazine approach epoch. (C) Center placement of orbitofrontal cortex targeted electrode arrays in PAE (red) and SAC control (white) mice. (D) PAE treatment did not significantly alter discrimination performance, but significantly increased the number of correction errors made during the first session of reversal. (E) PAE did not affect the time to respond to stimuli, and did not alter latency to retrieve reward across sessions. (F) Center placement of bilateral dorsal striatal targeted electrode arrays in PAE (red) and SAC control (white) mice. (G) PAE treated mice performed similarly to controls during discrimination, but had significantly increased numbers of correction errors during the first session of reversal. (H) PAE treatment did not significantly affect time to screen response, nor latency to retrieve reward during any session.

Figure 4. PAE alters OFC Phase Alignment to a Correct Choice Cue.

(A) Inter-trial Phase Consistency (ITPC) increased above background levels in higher frequencies immediately following tone across trials, particularly during early reversal (RS1-RS4) before decreasing back to discrimination levels. (B) ITPC was significantly reduced in PAE animals versus SAC in higher frequencies. (C) No significant changes across sessions or between treatments in the Low frequency region of interest in the OFC. (D) PAE treatment decreased average phase alignment for upper frequency of interest after correct choice on RS¹ and RS³ in the OFC. (E) Center placement of ipsilateral dual arrays in orbital frontal cortex PAE (red) and SAC control (white) mice. $n = 12$ per trt. Closed squares indicate treatment means with $\pm$ SEM. Open shapes are individual subject values, border color indicating treatment SAC (black), PAE (red) and shape indicating region OFC (O), DS (). * = $P < .05$, difference between treatments.

Figure 5. PAE alters DS Phase Alignment to a Correct Choice Cue.

(A) Inter-trial Phase Consistency (ITPC) increased above background levels immediately following tone across trials, and robustly increased across frequencies during early reversal (RS1-RS4) before decreasing back to discrimination levels. (B) ITPC was similarly significantly reduced in PAE animals versus SAC. (C) RPAE treatment decreased average phase alignment for upper frequency of interest after correct choice on RS¹ and RS³ in the OFC. (D) In the DS, low frequency region of interest average phase alignment is decreased across reversal sessions RS³, RS⁴ and R^{mid} in PAE mice. (E) PAE treatment decreased average phase alignment for upper frequency of interest after correct choice on RS¹ and RS³ in the DS. (E) Center placement of ipsilateral dual orbital frontal cortex and dorsal striatal targeted electrode arrays in PAE (red) and SAC control (white) mice. n = 12 per trt. Closed squares indicate treatment means with $\pm$ SEM. Open shapes are individual subject values, boarder color indicating treatment SAC (black), PAE (red) and shape indicating region OFC (O), DS (). * = $P < .05$, difference between treatments.

Figure 6. PAE Increases Spike-Field Coupling in the OFC during Early Reversal.

(A) Orbital frontal cortex spike-field coupling across sessions, from left to right Discrimination Criterion (D^{late}), First session of reversal (R^{S1}), Reversal session three (R^{S3}), Reversal session four (R^{S4}), and Reversal chance (R^{mid}). Low-frequency (5–15 Hz) spike-field coupling is significantly different from mid (16–25 Hz) and high (>25 Hz) frequency ranges during all sessions. PAE treated mice had significantly greater low-frequency spike-field coupling on R^{S1} compared to controls. (B) Orbital frontal cortex low-frequency average spike-field coupling across sessions. Spike-field coupling decreases on R^{S1}, but returns to discrimination criterion levels by R^{mid}. PAE treatment significantly increases spike-field coupling on R^{S1}. (C) Dorsal striatum spike-field coupling across sessions, from left to right Discrimination Criterion (D^{late}), First session of reversal (R^{S1}), Reversal session three (R^{S3}), Reversal session four (R^{S4}), and Reversal chance (R^{mid}). Low-frequency (5–15 Hz) spike-field coupling is significantly different from mid (16–25 Hz) and high (>25 Hz) frequency ranges during all sessions. PAE treatment decreased mid frequency and high frequency spike-field coupling on R^{S4} and R^{mid}, respectively; however changes were not significant. (D) Dorsal striatum low-frequency average spike-field coupling across sessions. Spike-field coupling is high during D^{late} and peaks again on R^{S4}. PAE reduces response on R^{S4}, but not significantly. Vertical dashed lines indicate boundaries between low (5–15 Hz), medium (15–25 Hz) and high (25–40 Hz) frequency ranges. Horizontal lines indicate random chance derived from 5000 iterations of shuffled data within session. Spike-field by frequency graphs (A-B) are means $\pm$ SEM of bootstrapped spikes. Low frequency graphs (C-D) are means $\pm$ SEM between subjects. **=P<.01, difference between treatments; †=P<.05, difference between frequencies.

Figure 7. PAE Prolongs Functional Connectivity during a Correct Choice.

(A) Granger Causality in OFC to DS direction in SAC control mice. Functional connectivity is maintained across all sessions, from left to right Discrimination Criterion (D^{late}), First session of reversal (R^{S1}), Reversal session three (R^{S3}), Reversal session four (R^{S4}), and Reversal chance (R^{mid}). Choice (–) and tone (...) regions of interest were defined by significant difference from random shuffled data. (B) Granger Causality in PAE animals, across sessions. Outlined tone region of interest is significantly greater in PAE treated mice compared to controls. (C) Choice region of interested averaged granger prediction across sessions. (D) Tone region of interested averaged granger prediction across sessions. PAE significantly increases average functional connectivity during the tone region of interest. Closed squares indicate treatment means with $\pm$ SEM. Open shapes are individual subject values, boarder color indicating treatment, SAC (black), PAE (red). * = $P < .05$, difference between treatments.