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Early postpartum resting-state functional connectivity for mothers receiving buprenorphine treatment for opioid use disorder: A Pilot Study

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Abstract

Objective: Between 1999 and 2014, the prevalence of opioid use disorder (OUD) among pregnant women quadrupled in the U.S. The standard treatment for peripartum women with OUD is buprenorphine. However, the Maternal Behavior Neurocircuit, that regulates maternal behavior and mother-infant bonding, has not been previously studied for human mothers receiving buprenorphine treatment for OUD (BT). Rodent research shows opioid effects on reciprocal inhibition between maternal care and defense maternal brain subsystems: hypothalamus and periaqueductal gray respectively. We conducted a longitudinal functional magnetic resonance imaging (fMRI) pilot study in humans to specifically examine resting-state functional connectivity (rs-FC) between periaqueductal gray and hypothalamus, and explore associations with maternal bonding for BT.

Methods: We studied 32 mothers who completed fMRI scans at 1-month (T1) and 4-months postpartum (T2), including 7 mothers receiving buprenorphine for OUD and 25 non-OUD mothers as a comparison group (CG). The participants underwent a 6-minute resting-state fMRI scan at each time point. We measured potential bonding impairments with the Postpartum Bonding Questionnaire to explore how rs-FC with periaqueductal gray is associated with bonding impairments.

Results: As compared to CG, BT mothers differed in periaqueductal gray-dependent rs-FC with the hypothalamus, amygdala, insular cortex, and other brain regions at T1 and many of these differences disappeared at T2, suggesting potential therapeutic effects of continuing buprenorphine treatment. Also, the "rejection and pathological anger" subscale of Postpartum Bonding Questionnaire at T1 and T2 were associated with the T1-to-T2 increases in periaqueductal gray-dependent rs-FC with the hypothalamus and amygdala.

Conclusion: Preliminary evidence links maternal bonding problems for mothers with OUD early in the postpartum to connectivity between specific care and defense maternal brain circuits, which may be mitigated by buprenorphine treatment. This exploratory study supports a potential mechanism to study both therapeutic benefits and risks of opioids for maternal care and bonding with infants.

Introduction

The opioid epidemic in the U.S. directly affects millions of women – with about 20% of all pregnant women prescribed opioids and 2.5% chronically using (1), and the number of pregnant women with opioid use disorder (OUD) more than quadrupled from 1999 to 2014 (from 1.5 per 1,000 delivery hospitalizations to 6.5) (2). The standard of care for OUD among pregnant women is opioid maintenance therapy with buprenorphine. Buprenorphine treatment for OUD (BT) (3-6) is a common choice of treatment to mitigate the physiological stress associated with repeated cycles of maternal / fetal intoxication and withdrawal, preserving uterine stability via receptor occupancy (7-9). Compared to detoxification (10, 11) and possibly methadone treatment (12-20), BT may result in milder neonatal abstinence syndrome (NAS) (6, 21-28). However, the effects of buprenorphine treatment on the human maternal brain affected by OUD are not well understood (29). So far there is just one study of mothers with polysubstance addictions showing reduced activation in reward regions of the brain in response to their own infant's face cues (30); and one of mothers with BT for OUD vs. depressed controls showing increased own vs. other baby cry responses in multiple motivation brain regions (31).

It is known, however, that maternal substance use in general places their infants at fourfold increased risk for abuse or neglect, contributing to as much as 80% of child maltreatment cases (32) and 60% of infant out-of-home placements (33). Opioid-induced deficits in homologous maternal behaviors have also been established in animal models (34-36). These effects appear to be mediated in part by activation of mu-opioid receptors in the hypothalamic medial preoptic area (mPOA) (37-39). More recent findings have implicated the activation of select opioid receptor subtypes within the periaqueductal gray (PAG) in dysregulating maternal behavior.

The PAG plays a pivotal role in switching from maternal caregiving behaviors to defensive/aggressive behaviors such as predation in rodents (40) that are known to be regulated by mu and kappa opioid receptor activity (41, 42). Indeed, infusion of a mu-opioid receptor agonist into the ventrolateral PAG disrupts the balance of maternal care and aggression and kappa-opioid receptor antagonism, shifting behaviors from maternal caring to predation behaviors in rodents (41). These recent results are particularly relevant for mothers receiving

buprenorphine for OUD (BT) because buprenorphine is both a partial mu-opioid receptor agonist and kappa-opioid receptor antagonist. The hypothalamus and PAG are key parts of a Maternal Behavior Neurocircuit (MBN, Figure 1) that is necessary for the survival of mammals from well-developed rodent models (43-49) . In this scheme, maternal caregiving and defensive/aggressive systems are balanced with reciprocal inhibition of the hypothalamic mPOA and PAG respectively. (50). Thus, mothers would not be distracted from sensitive maternal care in the absence of threat or exhibit insensitive or aggressive behavior toward their infant.

We postulate that human mothers have a homologous MBN**, (**Figure 1) (51-53), including opposing maternal ***care*** and ***defense*** subsystems, that reciprocally inhibit each other to balance maternal sensitive infant care behaviors with need for defensive/aggressive behaviors that maintain safety (31). The care subsystem includes the mPOA in hypothalamus, ventral tegmental area (VTA), nucleus accumbens (NAc), and ventral pallidum (VP) that may regulate maternal caregiving behaviors such as licking and grooming for rodents - sensitive, attuned care, gentle gaze, voice, touch and movement for humans (54). In the defense subsystem, the PAG may activate maternal defensive behaviors, such as aggression toward intruders and predatory behaviors. Among humans, PAG has been activated a baby distress cry (55), with such activity related to mental state talk (56) – this is interesting since a mother would need to balance caring and defensive behaviors in response to a cry which may solicit care or signal emergent concerns for safety (57). The regulation of other regions of the two subsystems of the MBN to orchestrate parenting has been demonstrated with human functional magnetic resonance imaging (fMRI) studies, including amygdala (58-60), insular cortex (61, 62), orbitofrontal cortex (OFC) (63) and hypothalamus (64-66) in response to infant cues. These regions may also be affected by addiction and polysubstance use (30, 31, 67, 68) suggesting the need for more research on task-based brain physiology in the maternal brain. There is growing recognition, however, that high-order cognitive and emotional function, such as required for human parenting, may depend on coherent activity between key brain regions within neurocircuits, rather than single regions.

Resting state functional connectivity (rs-FC) is a method used in functional magnetic resonance imaging (fMRI) to examine regional interactions during a resting or task-negative state, during which an explicit task is not being performed (69, 70). Resting brain state conditions are observed through changes in blood-oxygen-level dependent (BOLD) signals. Despite limitations which low temporal resolution and regional variations in neurovascular

physiology, the resting, the resting state approach is useful to explore the brain's functional organization in terms of functional connectivity (71). It has revealed networks of synchronous activity which are consistently found in healthy subjects, different stages of consciousness, mental disorders and across species (72, 73). As in the present study, rs-FC may be particularly to pursue *a priori* hypotheses in established circuits (74).

So far, there are just one rodent and two human maternal brain studies using resting state connectivity methods described above. Rat dams with early life chronic social stress showed depressed maternal care specifically during early lactation, and changes in functional connectivity in regions associated with sensory processing, maternal and emotional responsiveness, memory, and the reward pathway, including reduced PAG-anterior cingulate connectivity (75). Among human mothers, 8 with depression vs. 9 healthy at 9 weeks postpartum, there was decreased functional connectivity in numerous corticolimbic circuits, including coupling within the default mode network (DMN), including anterior cingulate cortex (ACC), inferior parietal cortex and precuneus, as well as between DMN nodes and amygdala (76). In another human study at 10 weeks postpartum, 14 depressed vs. 23 healthy mothers showed decreased DMN activity using a PCC seed region (77). These results may be interpreted to reflect diminished self-reflection and social cognition (78) as well as thoughts about duties and responsibilities to others (79) important to parenting. Additional research related to the content of maternal thoughts during the resting phase may be important to clarify the relationship between maternal thoughts moods and caregiving in health and mental illness (80).

In the current study, we hypothesize that the specific functional connectivity between hypothalamus and PAG, which are two functionally opposing regions in MBN (50), may be decreased in mothers receiving buprenorphine for OUD and relate to impaired bonding, consistent with rodent deficits in maternal behavior with opioids (34, 35). We thus examined rs-FC between the PAG and hypothalamus in BT mothers in reference to a comparison group (CG) of mothers who did not have OUD or receive any psychotropic medication. Furthermore, to examine the roles of functional connectivity between PAG and hypothalamus in possible dysregulation of maternal behaviors, we also explored how the PAG-dependent rs-FC may be related to symptoms of mother-infant bonding impairments, measured by the Postpartum Bonding Questionnaire (PBQ) (81), in BT and CG mother in the early postpartum period. The PBQ was chosen for this purpose because its subscales of Impaired Bonding (IB), Rejection and

Pathological Anger (RPA), and Infant-Focused Anxiety (IFA) have been linked to maternal psychological abuse of infants and maternal depression in multiple cultures (82-85). We know that depression is common comorbidity of OUD (86, 87) and that depressive symptoms, even if not meeting clinical threshold (88-90), can seriously risk adverse child outcomes across cognitive, behavioral and social domains (91-96).

Materials and Methods

Procedures

Participants were recruited from community health clinics, primary care clinics, and the University of Michigan Medicine Centers. They received an initial screening in the third trimester of pregnancy and completed questionnaires before undergoing two fMRI scanning sessions, at 1-month postpartum (T1) and 4-month postpartum (T2). Participants with OUD were prescribed buprenorphine treatment (BT) as part of routine medical care and there were no infant admissions for medical complications after initial NAS. All procedures were approved by the Human Subject Institutional Review Board at University of Michigan.

Participants

For the sample in the current study (n = 32), we included group of mothers who were maintained on buprenorphine replacement therapy for their OUD (BT), *n*=7, years of age: *M* = 29.86, *SD*= 6.62) and a comparison group (CG) of mothers who reported no opioid or other substance uses or any psychotropic medications (CG, n = 25, years of age: *M*= 27.62, *SD*= 8.46). The early postpartum mothers' infant's year of age were *M*= 0.08, *SD*= 0.01 for BT and *M*= 0.07, *SD*= 0.27 for CG. The BT mothers received buprenorphine medication during pregnancy and continued throughout the time of the study. The average dose recorded every 2 weeks postpartum was (*M*= 14.18 mg, *SD*= 3.35) with six out of 7 mothers stabilizing between 12 and 16 mg.

Self-reported Measure

Postpartum Bonding Questionnaire (PBQ): The PBQ is a 25-item self-report questionnaire developed to screen early postpartum women for potential parent-infant bonding disorders (97). The PBQ consists of 4 subscales to indicate factors of clinical relevance, namely

Impaired Bonding (IB, subscale 1 with sensitivity of 0.82 for detecting mothers with bonding disorders), e.g., "I feel distant from my baby"; Rejection and Pathological Anger (RPA, subscale 2 with sensitivity of 0.88 for infant rejection and 0.67 for severe anger), identifies mothers with serious infant-oriented hostility, e.g., "I resent my baby"; Infant-Focused Anxiety (IFA, subscale 3), which may be useful in anxious mothers, e.g., "My baby makes me feel anxious"; and subscale 4, which signals the presence of incipient abuse that would require urgent intervention, e.g., "I have done harmful things to my baby". None of the participants indicated abuse from subscale 4 and only the first three subscales (IB, RPA, and IFA) were examined in the current study.

Functional and Structural Magnetic Resonance Imaging

Imaging Data Acquisition

During the fMRI scanning session, each participant was positioned in a supine orientation with her head positioned in a head coil. Visual stimuli (a cross at the center) were presented via a goggle system and Nordic NeuroLab audio system. The fMRI scanning was performed with a 3.0 Tesla Philips magnetic resonance imaging scanner using a standard 8-channel radiofrequency SENSE head coil with the following acquisitions: (1) A high-resolution T1 scan was acquired to provide precise anatomical localization (TR of 9.8 ms, TE = 459 ms, FA = 8°, FOV of 256 mm, slice thickness of 1.0 mm, 180 slices with 288 x 288 matrix per slice). (2) A run of T2*-weighted BOLD (blood oxygenation level dependent) EPI sequence volumes were acquired for whole-brain fMRI during the resting-state task (180 frames, TR = 2000 ms, TE = 30 ms, FA = 90°, FOV = 220 mm, 42 contiguous axial slices, slice thickness = 2.8 mm with 64 x x64 matrix per slice, voxel size = 3.44 x 3.44 x 2.8 mm^3), after discarding the first five initial frames to allow for equilibration of scanner signal.

Imaging Data Processing and Analysis

Functional MRI data were pre-processed and analyzed using statistical parametric mapping software (SPM8; Welcome Department of Imaging Neuroscience, London UK). Slice timing correction was performed using a middle slice as a reference (slice 21) (98). After slice time correction, images within each run were realigned to the mean image of the first run to correct for movement. Realigned functional images and structural images were spatially

normalized using DARTEL method in SPM8 (99). The normalized functional images were re-sliced to 2×2×2mm voxels. Images were then spatially smoothed using a Gaussian filter with a full-width half-maximum value of 8mm. To assess and mitigate movement artifact, we calculated the frame-wise displacement (FD), defined as the sum (in millimeters) of the absolute values of frame displacement in successive functional volumes, based on six translation and rotation parameters from the realignment file (100). Rotational displacements were converted from degrees to millimeters moved on the surface of a 50 mm sphere. A frame-scrubbing routine was used to censor out any frame with FD > 0.5 mm from the regression analysis described below, yielding a scrub ratio for each subject, with a cutoff of 60% censored-out frames for sample exclusion, according to the literature showing that as many as 60% of frames can be removed and still yield analyzable results (100, 101). There was no BT vs CG difference in the averaged FD before scrubbing (BT: $M = 0.379$, *s.e.* = 0.063; CG: $M = 0.300$, *s.e.* = 0.028; $F(1,40) = 1.284$, $MS_{error} = 0.056$, $p = 0.264$). This suggests no significant head movement as a potential confounding factor between groups. Three-compartment segmentation of the high-resolution structural image from the DARTEL normalization were applied to the functional time series to extract cerebral spinal volume (CSF) and white matter (WM) compartments, which then were subjected to a principal component analysis to identify the top 5 components in each (102), which should correspond to heart rate and respiratory effects on the BOLD signal (103). Adjusted time courses were derived from sequential regressions of the time series with the following regressors: Linear trend of 6 motion parameters, their temporal derivatives, the quadratics of these 12 parameters, 5 components from the PCA of CSF, 5 components of PCA of WM, followed by band pass filtering from 0.01 – 0.1 Hz, and then motion scrubbing. The seed mask of PAG, as a region-of-interest (ROI), in rs-fMRI analysis was generated using PickAtlas software (WFU Pickatlas, http://fmri.wfubmc.edu/software/pickatlas) as a box-shaped mask centered at the ventrolateral PAG (MNI x = 0, y = −27, z = −8, with 3 × 1 × 1 mm extensions), according to the literature (104, 105). In the first-level analysis, adjusted time courses were averaged across all voxels in the seed mask. The average time course in the seed was correlated with time courses from all other voxels in the brain, Fisher r-to-Z transformed, and taken to second level, between-group analyses. In the second-level analysis, statistical parametric maps in the first model were submitted to General Linear Models (GLMs) to test for the effects of interest. The effects of interest included the group difference (BT vs Controls) in the sample of

32 mothers and each of the three PBQ subscales in a subsample of BT and CG mothers (n = 5 in each group). All the second level models were controlled for whole-brain multiple comparison at the cluster level for voxel-wise intensity threshold at $p < 0.001$, which is considered to yield reliable statistical inference (106). We also used Threshold-Free Cluster Enhancement (TFCE) method (107) to perform family-wise small volume correction (*s.v.c.*) in a ROI mask of the hypothalamus (MNI x=±4, y=-2, z=-12, with a 4mm radius) to examine the PAG-Hypothalamus functional connectivity according to the hypothesis that the reciprocal inhibition between PAG and hypothalamus plays a key role in regulating maternal behaviors.

Behavioral Data Analysis

We tested the associations between the PAG-hypothalamus functional connectivity and three subscales of PBQ (IB, RPA, and IFA) at T1 and T2 in the early postpartum mothers, using bivariate Pearson's *r* correlation analysis in SPSS v.24 (IBM Corp. Armonk NY).

Results

Behavioral Results

The participants in BT and CG did not differ in their age ($F_{(1,30)}$=0.081, MS_{error}=70.25, p=0.78), number of offspring ($F_{(1,30)}$=0.16, MS_{error}=0.45, p=0.69), and the youngest child's age in the early postpartum mothers ($F_{(1,8)}$=1.00, MS_{error}=0.01, p=0.35). Please see table 1 for demographics. In a mixed model of GLM, we tested the main effects of group (BT vs CG), Time (T1 and T2) and Time by Group interaction effect in three subscales of PBQ. We found that there were no significant group main effects for any of the PBQ subscales (IB: $F_{(1,6)}$=0.002, MS_{error}=35.90, p=0.97; RFA: $F_{(1,6)}$=0.026, MS_{error}=9.63, p=0.88; IFA: $F_{(1,6)}$=1.49, MS_{error}=5.06, p=0.27); there was a significant main effect of Time in IFA ($F_{(1,6)}$=21.00, MS_{error}=0.15, p=0.004), but not other subscales (IB: $F_{(1,6)}$=2.45, MS_{error}=15.98, p=0.17; RPA: $F_{(1,6)}$=3.10, MS_{error}=3.96, p=0.13); there were no significant Time by Group interaction effects for any of the subscales, though a marginal trend for IFA, (IB: $F_{(1,6)}$=0.066, MS_{error}=15.98, p=0.45; RFA: $F_{(1,6)}$=0.001, MS_{error}=3.96, p=1.00; IFA: $F_{(1,6)}$=3.86, MS_{error}=0.15, p=0.10). See Figure 2 for the Time by Group line charts of PBQ subscales.

Neuroimaging Results

Main effects of Group at T1 and T2, Time, and Time by Group Interactions

We examined the main effects of Group at timepoint, the main effects of Time, and the Time by Group interaction effects on the PAG-dependent resting-state functional connectivity (rs-FC) in SPM8. There were no Time main effects observed in the PAG-dependent rs-FC.

The main effects of Group (BT vs CG) on PAG-dependent rs-FC at T1 are summarized in Table 2 and Figure 3. At T1, BT vs CG mothers showed greater PAG-dependent rs-FC in the right inferior temporal gyrus, dorsal caudate nucleus/septal area, and the right amygdala/hippocampus complex; when examining *a prior* ROI using TFCE, we also found that the PAG-dependent rs-FC with the hypothalamus was stronger in BT as compared to CG mothers at T1(MNI: [4, -4, -10], 25 voxels, $TFCE = 27.00$, $p = 0.026$ *s.v.c.*). Conversely, BT vs CG mothers showed lesser PAG-dependent rs-FC in the right supramarginal gyrus, bilateral middle temporal gyrus (MTG), precuneus/posterior cingulate cortex, bilateral pericentral gyrus, and right insular cortex at T1.

The main effects of Group (BT vs CG) on PAG-dependent rs-FC at T2 are summarized in Table 3 and Figure 4. At T2, BT mothers showed PAG-dependent rs-FC that was greater than those in CG mothers in the hypothalamus ROI (MNI: [-6, -4, -10], 3 voxels, $TFCE = 19.61$, $p = 0.041$ *s.v.c.*). Conversely, BT mothers showed lesser PAG-dependent rs-FC in the dorsal and ventral ACC (dACC and vACC).

For the Time by Group interaction effects, the results are summarized in Table 4 and Figure 5. We found that, as compared to CG mothers, BT mothers showed greater T1-to-T2 increases of the PAG-dependent rs-FC with the right MTG and insular cortex; conversely, greater T1-to-T2 decreases in the PAG-dependent rs-FC with the dorsal precuneus, left OFC, septal area, and dACC.

PBQ Associations with PAG-dependent rs-FC

We explored the associations between three subscales of PBQ (IB, RPA, and IFA) and the PAG-dependent rs-FC with the brain regions reported in the results above in the early postpartum mothers, i.e., the hypothalamus, right amygdala/hippocampus, right MTG, right insular cortex, septal area/caudate nucleus, dACC, left OFC, and dorsal precuneus (see Figure 6 for the line charts of the parameter estimates of these regions by group and time). Among the regions reported above, only the hypothalamus and right amygdala's PAG-dependent rs-FC were

associated with some of the subscales in PBQ, as described below. The BT and CG mothers did not differ in the associations between any of the PBQ subscales and PAG-dependent rs-FC.

Across both groups, the T1-to-T2 changes in the PAG-dependent rs-FC with the hypothalamus was associated with T1 PBQ's IB ($r = 0.70$, $p = 0.025$) and RPA ($r = 0.76$, $p = 0.011$), but not IFA ($r = 0.31$, $p = 0.38$), and with T2 PBQ's RPA ($r = 0.81$, $p = 0.014$), but not IB ($r = 0.46$, $p = 0.25$) and IFA ($r = -0.097$, $p = 0.82$) (see Figure 7). The T1-to-T2 changes in the PAG-dependent rs-FC with the right amygdala was associated with T1 PBQ's RPA ($r = 0.72$, $p = 0.019$) and IFA ($r = 0.65$, $p = 0.043$), but not IB ($r = 0.48$, $p = 0.17$), and with T2 PBQ's IB ($r = 0.82$, $p = 0.013$) and RPA ($r = 0.73$, $p = 0.039$), but not IFA ($r = 0.41$, $p = 0.32$) (see Figure 8).

Discussion

In the context of an unprecedented opioid crisis in the U.S, many peripartum women with OUD are treated with buprenorphine maintenance treatment (3), despite concerns for parenting among affected human mothers (29). Rodent research indicates that opioids can disrupt maternal behavior by acting on the PAG and other maternal brain circuit regions including the hypothalamus (34-36). We tested whether a group of women with OUD and buprenorphine treatment (BT mothers) differed from a comparison group (CG) of non-OUD mothers during the early postpartum, in the resting-state functional connectivity associated with the PAG (PAG-dependent rs-FC) in the maternal brain neurocircuit (48, 49). We found that at 1-month postpartum (T1), as compared to CG, BT mothers showed greater PAG-dependent rs-FC in the right inferior temporal gyrus, septal area/caudate nucleus, right amygdala/hippocampus, and hypothalamus; they also showed lesser PAG-dependent rs-FC in the supramarginal gyrus, posterior cingulate cortex/precuneus, postcentral gyrus, middle cingulate cortex, insular cortex, precentral gyrus, and dorsal precuneus. At 4-month postpartum (T2), as compared to CG, BT mothers showed a marginally greater PAG-dependent rs-FC in the hypothalamus and significantly lesser PAG-dependent rs-FC in the ventral and dorsal anterior cingulate cortex. In terms of Time by Group interaction, BT mothers, as compared to CG, showed greater T1-to-T2 increases in the PAG-dependent rs-FC in the right middle temporal gyrus and insular cortex, due to a reversal of these regions' initial negative rs-FC with PAG; they also showed greater T1-to-T2 decreases in the PAG-dependent rs-FC in the septal area/caudate nucleus, dorsal anterior

cingulate cortex, left orbital frontal cortex, and dorsal precuneus, due to a reversal of these regions' initial positive rs-FC with PAG.

We also explored whether the PAG-dependent rs-FC in any of the brain regions, showing group differences, were associated with deficits in maternal behaviors, as measured by the Postpartum Bonding Questionnaire (PBQ). While the BT and CG mothers did not differ in the subscales of PBQ, i.e., impaired bonding (IB), rejection and pathological anger (RPA), and infant-focused anxiety (IFA), we found that the T1-to-T2 changes in PAG-dependent rs-FC with the hypothalamus and right amygdala were positively correlated with some of the subscales of PBQ at T1 and T2. In contrast with group difference analysis, correlation analysis may have detected difference because of increased power coming with data pooling across BT and CG groups. For the PAG-hypothalamus rs-FC, the IB and RPA, but not IFA, at T1 were correlated with the T1-to-T2 increases in the PAG-hypothalamus rs-FC; further, the T1-to-T2 increases in the PAG-hypothalamus rs-FC were correlated with the RPA, but not IB and IFA, at T1. For the PAG-amygdala rs-FC, the RPA and IFA, but not IB, at T1 were correlated with the T1-to-T2 increases in the PAG-amygdala rs-FC; further, the T1-to-T2 increases in the PAG-amygdala rs-FC were correlated with the IB and RPA, but not IFA, at T1. In sum, we found that, among the three subscales of PBQ, RPA was related to the PAG-dependent rs-FC the most. As RPA reflect outward expression of maternal aggression towards the infants, we found preliminary evidence for the roles of PAG-hypothalamus and PAG-amygdala rs-FC in the dysregulation of maternal aggression, which may reflect an impairment in the reciprocal inhibition between PAG and hypothalamus and their roles in maternal aggression and caregiving behaviors, respectively.

Among human mothers, PAG regional responses have been related to observing pictures of a mother's own child vs. an unknown child (108, 109). These task-based studies have not analyzed the reciprocal inhibition between PAG and maternal care regions such as the hypothalamus. It is possible that PAG activation in these studies was part of a balanced response to own infant cues, in which mothers may have been preparing to exhibit caring or defensive behaviors depending on other circumstances. In support, a recent study of mothers (110), PAG connectivity with OFC during own vs. other baby picture task was inversely related to parenting stress. Perhaps in this study, stress reduces OFC regulation of defensive and aggressive drives from the PAG, in accord with circumstances in which a stressed mother must prepare to defend their infant. Future studies may be able to assess the timing of regional brain activity, in order to

describe the order by which brain regions influence each other. In the only rs-FC rodent study of the homologous maternal brain, connectivity between PAG and ACC was decreased for dams with early life chronic social stress and interpreted as a decrease the reward salience of pups, resulting in depressed maternal care during early lactation. There was also an improvement in connectivity from early to late lactation (similar levels between groups during late lactation), considered to be the result of cumulative exposure to pup related stimuli, like what is observed when nulliparous rats are induced to express maternal care by exposure to foster pups (111). We will next discuss the possible maternal brain roles for regions that we report to have PAG-dependent rs-FC differences between BT and CG at T1 and T2.

Connectivity of PAG with other regions of the care and defense subsystems of the MBN to orchestrate parenting in humans is consistent with task-based fMRI responses to infant stimuli in the amygdala (58-60), insular cortex (61, 62), orbitofrontal cortex (OFC) (63) and hypothalamus (64-66). In rodent studies, oxytocin neurons in the in mPOA of the hypothalamus regulate dopamine function in the ventral tegmental area and mediate the reward salience of pups (112, 113). In another rat model, disrupted hypothalamus connectivity with PAG was related to impaired maternal care (114). Furthermore, rats that display low levels of maternal care exhibit lower oxytocin receptor binding in the mPOA of the hypothalamus (115), which strengthens the importance of this region in regulating sensitive maternal care. The ACC also plays a key role in mediating appropriate responses to infant stimuli. In response to infant cry, human mothers with lower depressive symptoms have increased activity in the dorsal ACC (116, 117). Perhaps depression interferes with ACC-related empathy that has been demonstrated with human neuroimaging metanalyses (118, 119) and parental brain studies (65, 120). In further accord, depressed mothers vs. healthy controls have been shown to exhibit decreases in the ACC-dependent rs-FC (76).

The current study is preliminary and with notable limitations. First, given that the CG mothers were not affected by OUD at all and there was no placebo control, we could not control for the influences of previous opioid uses in confounding the effects of buprenorphine per se. Given the ethical and feasibility barriers to a placebo control trial with OUD subjects, future studies may be able to approach the pure effects of opioids with longitudinal research in which subjects may be their own controls at different doses and time points. Future work will also require full characterization of participants with OUD, including the quantity and frequency of

all prescription, licit and illicit drug using a "time-line follow back" interview with calendar prompts and other memory aids to facilitate accurate recall of drug use (121-123). It will be also important to consider receptor occupancy as an important aspect of opioid dosing. Additional consideration will be required of the possible medical consequences of OUD, and sociodemographic and medical backgrounds – especially since we already know that parental stress, poverty, anxiety and postpartum depression affect the parental brain (80, 124-126). Indeed, stress response dysregulation has been established in other substance use disorders (127-130). As raised in the introduction, changes rs-FC must be interpreted with caution because they do not capture directionality of effect, rather basic associations of brain activity at rest, that are themselves under study. Next, due to the small sample size in the samples with PBQ, the results in testing the group difference in PBQ and the associations between the PBQ subscales and PAG-dependent rs-FC warrant replication studies in the future. Furthermore, there are complex caveats around the measurement of parental thoughts and behaviors. In this study, we only used the PBQ, which is a self-report measure that may not capture aspects of parenting about which participants may be embarrassed or unaware. Future work would benefit from more comprehensive interviews such as the working model of the child interview (131) and parental interview of thoughts and behaviors (66, 132) as well as video based objective measures of parenting sensitivity, intrusiveness, interfering, forcing, overriding, anger/hostility, and criticizing of their child (133-135). Finally, the translational validity of the MBN across species requires more ethologically valid neuroimaging paradigms connected to real-world behavioral condition.

Though preliminary, the current study probes potential buprenorphine effects on mothers affected by OUD. BT is currently the best practice for pregnant women suffering OUD, despite the potential adverse effects of any exogenous opioids on maternal behavior. It is therefore important to examine whether buprenorphine treatment for OUD, as a partial mu-opioid agonist and kappa-opioid antagonist, exerts beneficial or harmful effects on maternal brain and behavior during the postpartum. On one hand, our work may have identified a brain mechanism for the potential benefits of buprenorphine treatment on reversing abnormality of maternal brain function and behavior: the initial group differences in PAG-dependent resting-state functional connectivity dissipated by four months postpartum - perhaps representing postpartum adjustments in this sample of mothers with healthy infants who were receiving ongoing medical

care. On the other hand, our work may have identified a potential link between excessive PAG-hypothalamus functional connectivity and bonding impairments, on which the buprenorphine effects remain undetermined. In future, this work may be combined with brain models of other conditions that interfere with parenting like parental stress, poverty, anxiety and postpartum depression (80, 124-126) and potential brain mechanisms at work in parenting interventions (136) to inform and optimize comprehensive interventions for BT mothers and fathers and elucidate potential links between parental brain function and child outcomes (137). In sum, our preliminary work calls for more attention to PAG-dependent functional connectivity in the MBN as a possible brain mechanism to better assess opioid-sensitive parental brain functions in the context of parenting behaviors and parent-child bonding.

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Table 1

Demographics

	BT	CG
Age		
mean	29.86	27.62
s.d.	6.62	8.46
Infant age at T1 scan		
mean	0.08	0.07
s.d.	0.01	0.027
Number of Child		
mean	1.71	1.60
s.d.	0.76	0.66
Race		
European American	6	13
African American	0	12
Native American	1	0
Social Economic Status		
Low (under $20K household income)	4	14
Middle	3	11

Table 2

BT vs CG main effects on PAG-dependent rs-FC at T1

Brain Region	Side	MNI Coordinates X	Y	Z	No. of Voxels	Z score
BT > CG						
Inferior temporal gyrus	R	68	-28	-24	119	5.21
	R	62	-12	-28	174	4.95
Septal area/Caudate nucleus	R/L	-4	16	4	478	5.05
Amygdala/Hippocampus	R	24	-14	-12	220	4.88
Hypothalamus*	R/L	4	-2	-12	44	2.42
BT < CG						
Supramarginal gyrus	R	58	-46	30	1111	5.55
Middle temporal gyrus	L	-46	-58	8	487	5.14
	R	40	-70	22	356	5.09
Posterior cingulate cortex/Precuneus	R	14	-46	12	655	4.94
Postcentral gyrus	L	-68	-14	26	108	4.61
Middle cingulate cortex	R	18	-36	40	298	4.59
Insular cortex	R	48	6	-2	122	4.57
	R	36	16	4	122	4.54
Precentral gyrus	R	48	-2	50	86	4.51
Precuneus, dorsal	R/L	-6	-56	60	160	4.49

*Except this *a priori* ROI, all regions listed above were cluster-level whole brain corrected (FWE<0.05) at voxel-wise $p = 0.001$

Table 3

BT vs CG main effects on PAG-dependent rs-FC at T2

Brain Region	Side	MNI Coordinates X	Y	Z	No. of Voxels	Z score
BT > CG						
Hypothalamus*	R/L	-8	-2	-12	5	2.01
BT < CG						
Anterior cingulate cortex, dorsal	R/L	2	32	2	198	4.48
Anterior cingulate cortex, ventral	L	-12	42	-10	89	3.82

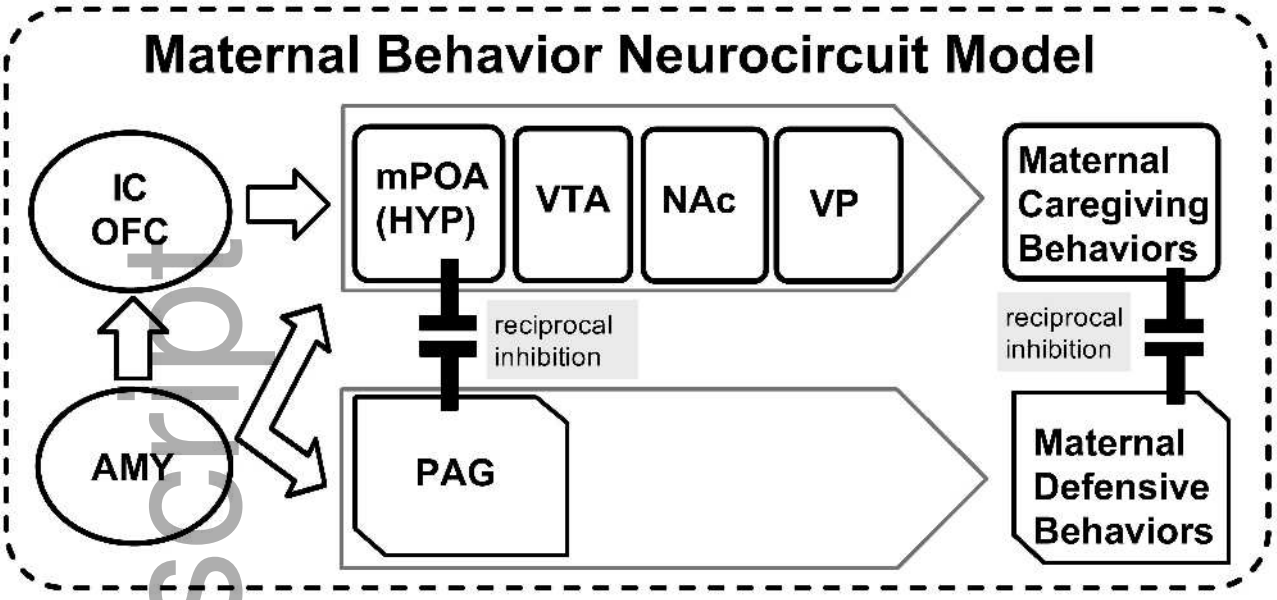
*Except this *a priori* ROI, all regions listed above were cluster-level whole brain corrected (FWE<0.05) at voxel-wise $p = 0.001$

Table 4

Group (BT vs CG) by Time interaction effects on PAG-dependent rs-FC

Brain Region	Side	MNI Coordinates X	Y	Z	No. of Voxels	Z score
BT > CG in T1-to-T2 increases						
Middle temporal gyrus	R	60	-40	4	211	4.78
Insular cortex	R	48	10	-6	110	4.51
BT > CG in T1-to-T2 decreases						
Precuneus, dorsal	L	-26	-74	50	137	5.03
Orbital frontal cortex	L	-18	32	-18	169	4.84
Septal area/Caudate nucleus	R/L	-10	0	6	271	4.78
Anterior cingulate cortex, dorsal	L	-2	30	10	175	4.58

All regions listed above were cluster-level whole brain corrected (FWE<0.05) at voxel-wise $p = 0.001$



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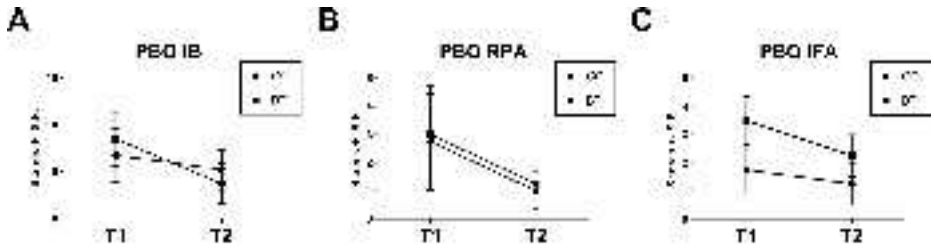

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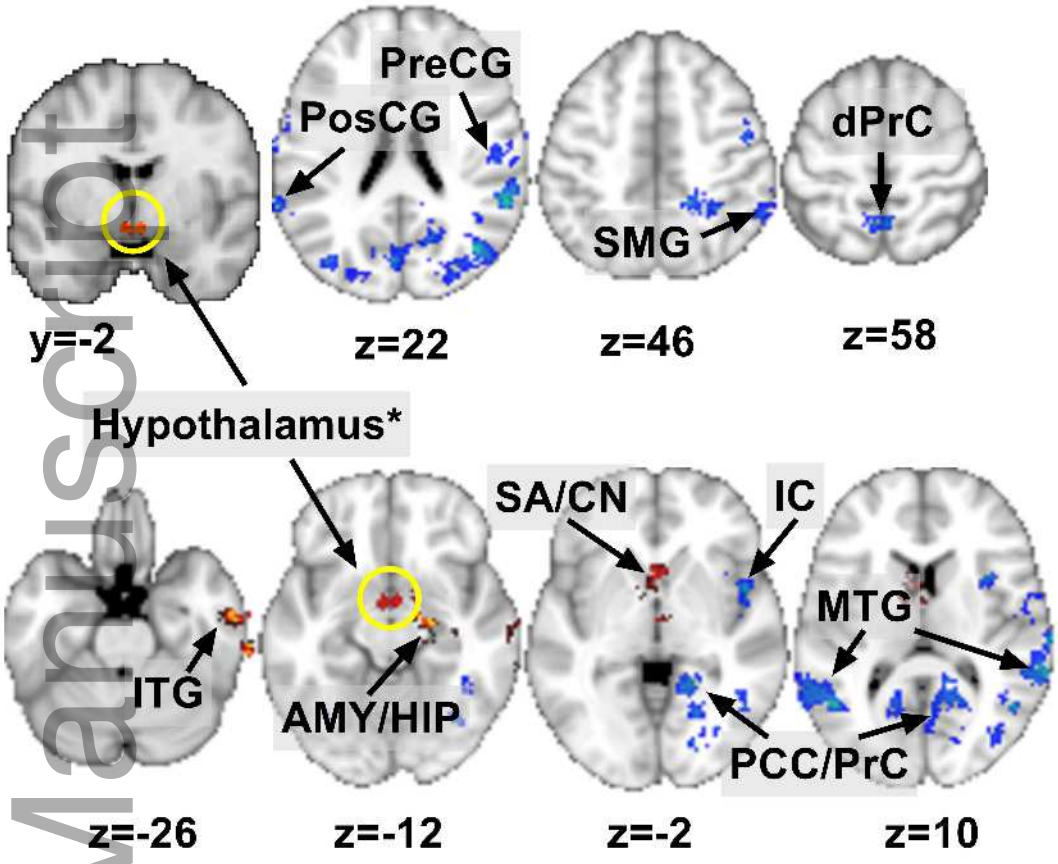

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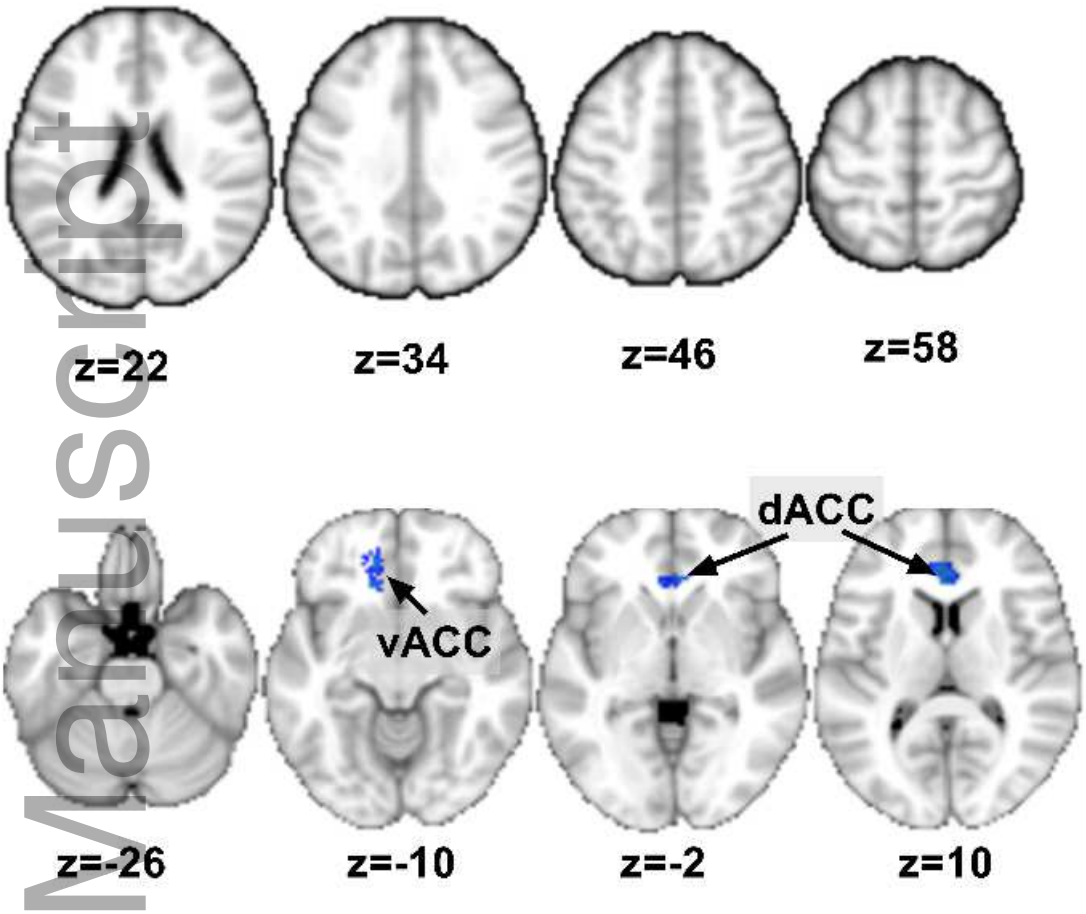

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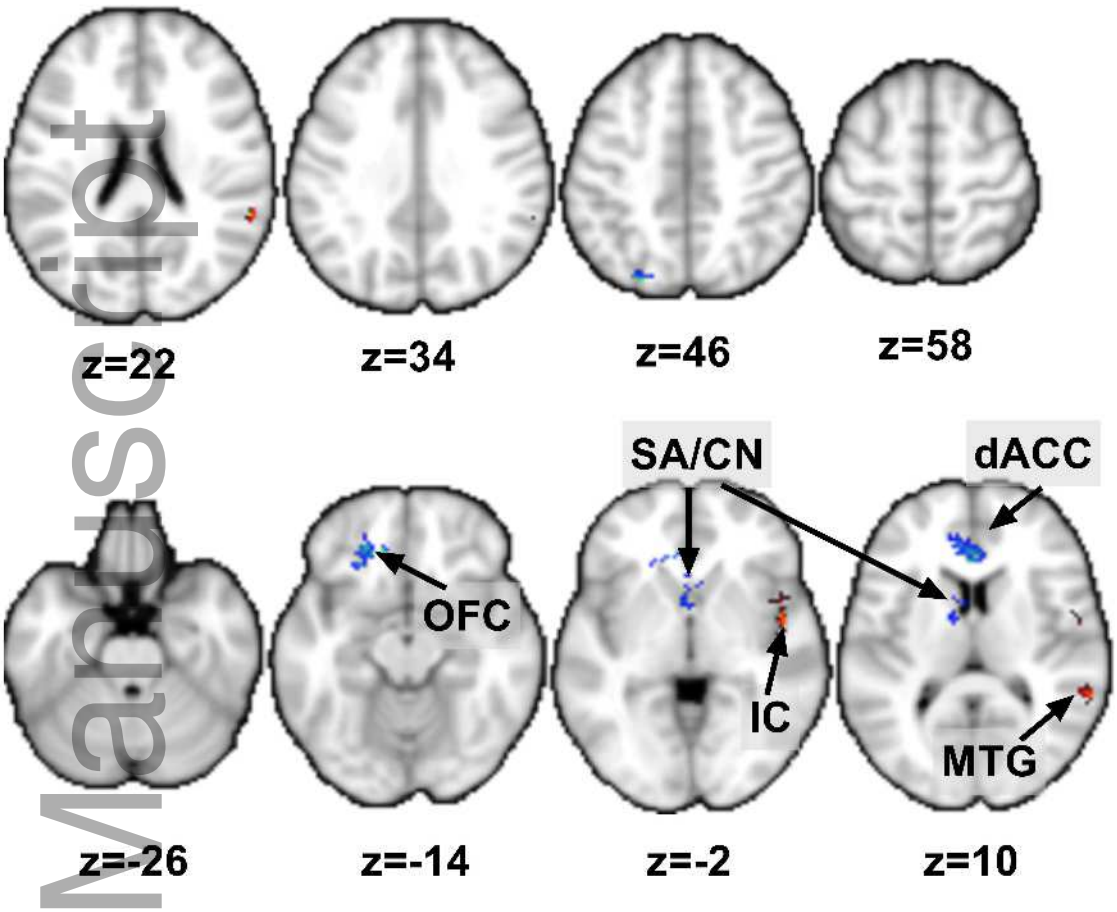

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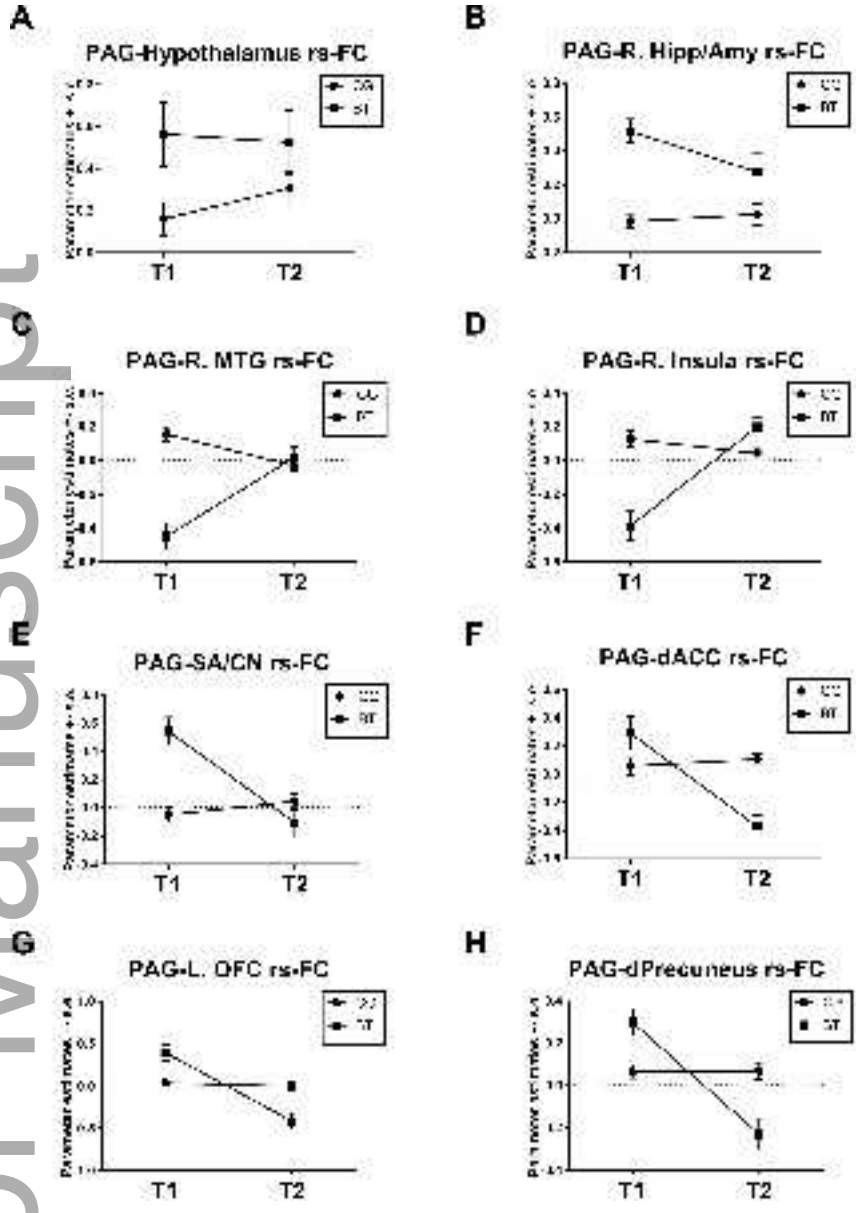

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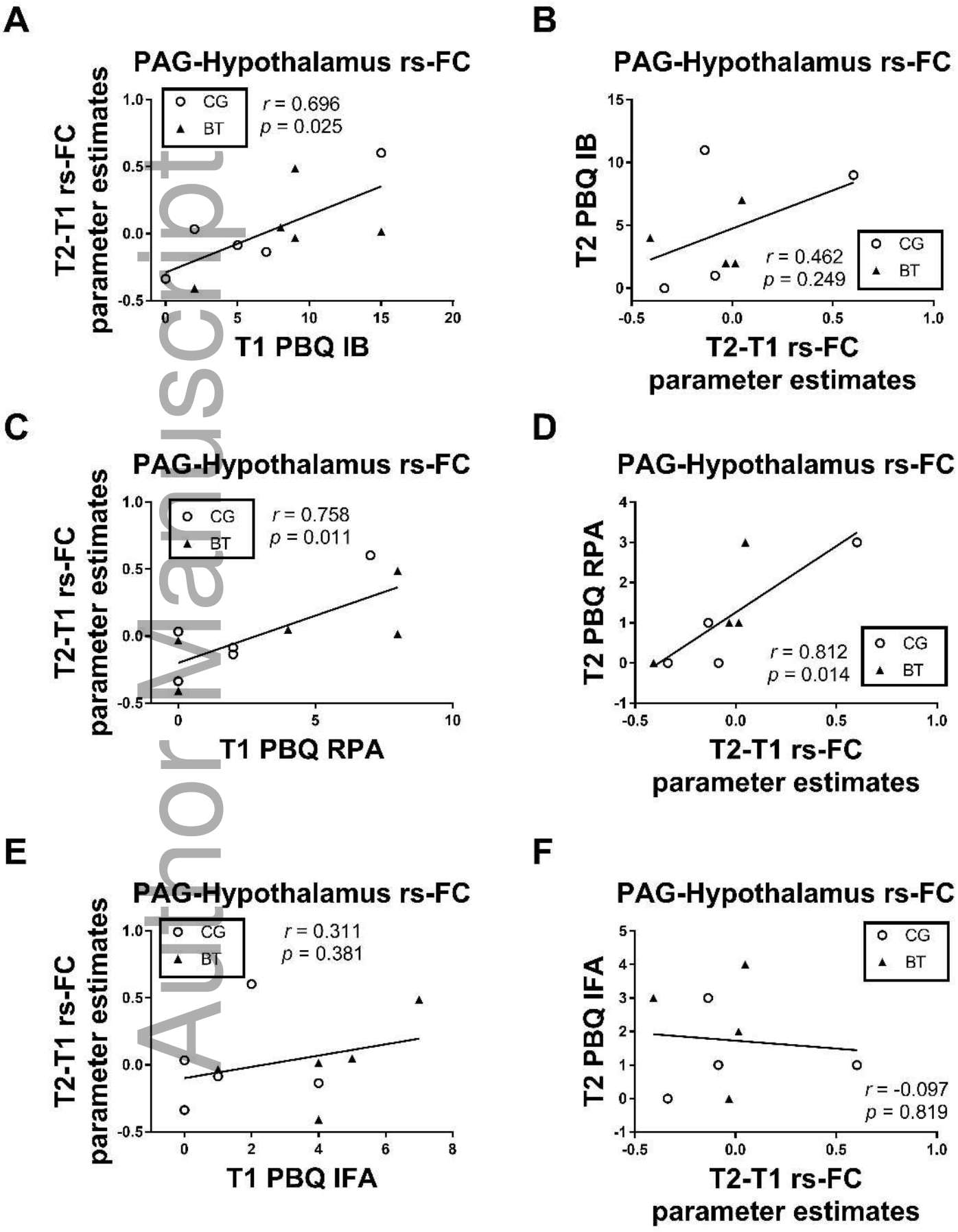

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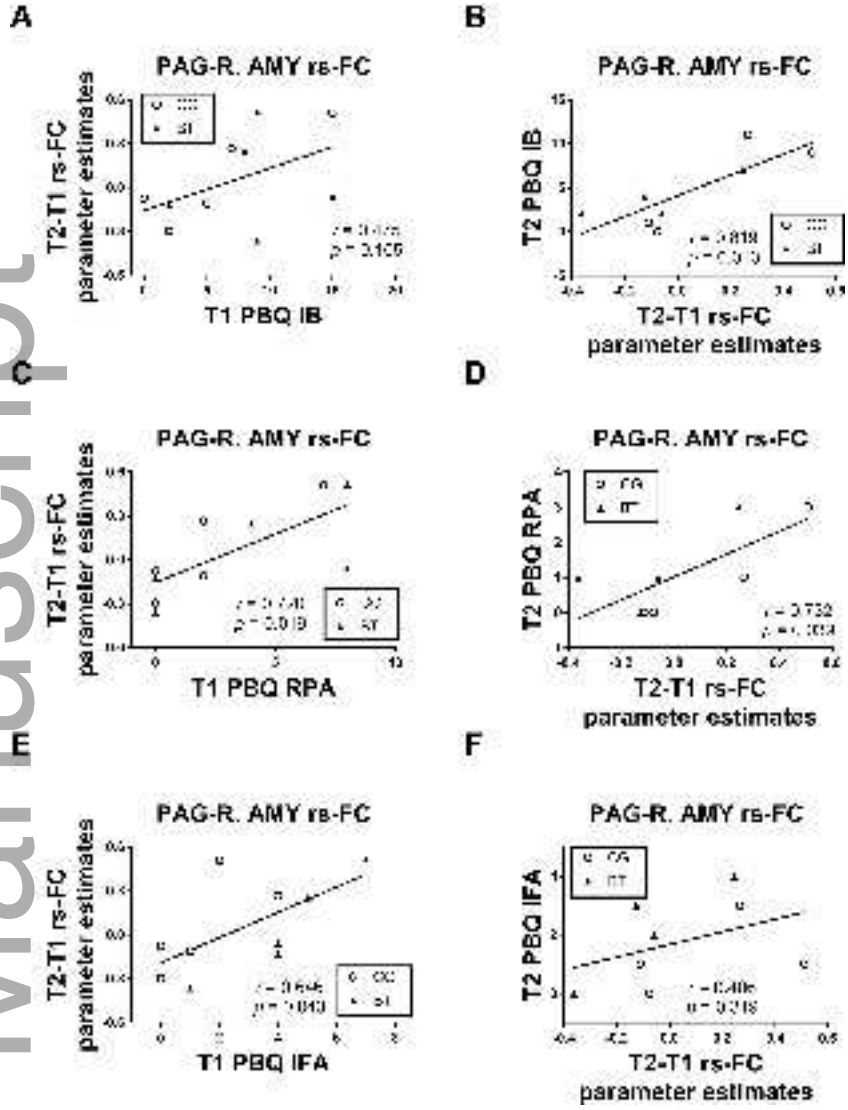

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