The scripts used in this analysis are described in alphabetical order. These definitions are followed by the processing order/diagram of how they were sequentially used with the datasets.

Alphabetic List of Code Used for PAG Analysis				
File	Type	Databaser*	Dataset input(s)	Description
'addglobalmean subjects.m'	MATLAB script	Yes	MAPPAll_Dec2017 masterlist	Add the global signal from the average of all scanning sites to the demeaned by site subject specific connectivity file
'AnalyzeMotionFile.m'	MATLAB script	Yes	MAPPAll_Dec2017 masterlist	Examine the effect of head motion in healthy controls and patients
'cmd.MNI.txt'	AfNI code	No	Corrected connectivity files for MNI-trace	Perform 3dttest++ from AfNI on all healthy control MNI-trace subjects
'cmd.Wei.txt'	AfNI code	No	Corrected connectivity files for MNI-sphere	Perform 3dttest++ from AfNI on all healthy control MNI-sphere subjects
'CodeforExtractingROIs_MNI.txt'	AfNI code	No	1.Clust_mask_MNI+tlrc 2.Corrected healthy control MNI-trace subjects 3.Corrected patient MNI-trace	Extract the MNI-trace corrected connectivity signal from healthy control and patients

			subjects	
'CodeforExtractingROIs_Wei.txt'	AfNI code	No	1.Clust_mask_Wei+tlrc 2.Corrected healthy control MNI-sphere subjects 3.Corrected patient MNI-sphere subjects	Extract the MNI-sphere corrected connectivity signal from healthy control and patients
'demeansubjects.m'	MATLAB script	Yes	MAPPAll_Dec2017 masterlist	Demean the subjects specific connectivity file by site
'DifferenceTRADHANDMNIHAND.m'	MATLAB script	Yes	PAGConnectivityall30altMNI092018.mat	Perform statistics on the difference from MNI-trace to traced-participant and MNI-sphere to traced-participant
'Differences_WeiPreprocess.m'	MATLAB script	Yes	PAGConnectivityall30altMNI092018_OrigDat_Connectivity.mat **OR** PAGConnectivityall30altMNI092018_ValDat_Connectivity.mat	Add to the connectivity .mat file by calculating percent differences and statistics on the MNI-sphere, MNI-trace, and traced-participant connectivity
'GenFig_IQR_OrigVal.m'	MATLAB script	Yes	1.PAGConnectivityall30altMNI092018_OrigDat_Connectivity_Differences.mat	Generate the Power et al. atlas regions that perform better or worse

			2.PAGConnectivityall30altMNI092018_ValDat_Connectivity_Differences.mat 3.PP264PAGxyzlabelcolor.mat	
'GenFig_IQR_Voxelwise.m'	MATLAB script	Yes	1.PAGConnectivity30all09212018_OrigDat_Connectivity_Differences.mat 2.PAGConnectivity30all09212018_ValDat_Connectivity_Differences.mat 3.MaskVoxelCoord.mat	Generate the brain images of voxels that perform better or worse
'GenFig_Visualize_Voxelwise.m'	MATLAB script	Yes	1.OBetter_2mm.nii 2.OWorse_2mm.nii 3.VBetter_2mm.nii 4.VWorse_2mm.nii	Visualize each brain image for better performance, worse performance, and overlapping voxels for each condition
'MotionPlotsandStats.m'	MATLAB script	No	MotionSignals.mat	Visualize healthy control and patient head motion differences
'PlotSlice_withActivity.m'	MATLAB script	Yes	1.MNI152_T1_1mm_brain.nii.gz 2.MNI_HC_in_1.	Visualize cluster activity from AfNI on a MNI brain

			nii.gz **OR** 2.Wei_HC_in_1. nii.gz	
'resampleBetter Worse.txt'	AfNI code	No	OBetter.nii **OR** VBetter.nii **OR** OWorse.nii **OR** VWorse.nii	Use AfNI code to resample images from 1mm to 2mm datasets
'ROIttest.m'	MATLAB script	No	CustoutMNIonM NIgm.txt **OR** CustoutWeionW eigm.txt	Perform statistics on healthy control and patient ROIs
'SelectSubjects. m'	MATLAB script	Yes	SelectForPAG_ DataTable.mat	Select subjects for the test dataset
'SelectSubjectsS et2_redo.m'	MATLAB script	Yes	SelectForPAG_ DataTable.mat	Select subjects for the validation dataset
'SplitDataOrigVa l_Voxelwise.m'	MATLAB script	Yes	1.PAGConnectiv ity30all0921201 8.mat 2.OriginalID.mat	Split output data into original dataset and validation dataset for voxelwise analysis
'Step01_Extract PowerSignalsPA G_MNIalt_2.m'	MATLAB script	Yes	MasterList_PAG Sep2018.mat 1mmMask.txt	Extract ROI signals (including PAG traces) from resting state image

'Step01_Extract PowerSignalsPA G_Voxelwise_Gr oupMask.m'	MATLAB script	Yes	MasterList_PAG Sep2018.mat 1mmMask.txt	Extract voxel signals (including PAG traces) from resting state image
'Step01_Voxelwi seCorrelation_H and_nomask.m'	MATLAB script	Yes	MAPPAll_Dec20 17 masterlist	Create voxelwise correlation using AfNI to traced-participan t
'Step01_Voxelwi seCorrelation_M NI_nomask.m'	MATLAB script	Yes	MAPPAll_Dec20 17 masterlist	Create voxelwise correlation using AfNI to MNI-trace
'Step01_Voxelwi seCorrelation_W ei_nomask.m'	MATLAB script	Yes	MAPPAll_Dec20 17 masterlist	Create voxelwise correlation using AfNI to MNI-sphere
'Step02_Concat enate_SNRrejec tPAG_AFNI_2.m '	MATLAB script	Yes	MasterList_PAG Sep2018.mat	Concatenate subjects into a .mat file for further analysis and perform signal to noise calculations
'Step02_Concat enate_SNRrejec tPAG_AFNI_Vox elwise.m'	MATLAB script	Yes	MasterList_PAG Sep2018.mat	Concatenate subjects into a .mat file for further analysis
'transformTrace d2SubjectPAG_ AFNItransform. m'	MATLAB script	Yes	MasterList_PAG Sep2018.mat	Transform PAG participant-trace in the original dataset to subject standard space

'transformTraced2SubjectPAG_AFNItransform_3.m'	MATLAB script	Yes	MasterList_PAGSep2018.mat	Transform PAG participant-trace in the validation dataset to subject standard space
'WeiPreprocess_to_Connectivity_2.m'	MATLAB script	Yes	PAGConnectivityall30altMNI092018_OrigDat.mat **OR** PAGConnectivityall30altMNI092018_ValDat.mat	Create connectivity .mat file for Power et al. atlas ROIs
'WeiPreprocess_to_Connectivity_2_Voxelwise.m'	MATLAB script	Yes	PAGConnectivity30all09212018_OrigDat.mat **OR** PAGConnectivity30all09212018_ValDat.mat	Create connectivity .mat file for voxelwise analysis

*Databaser: a GUI interface created in the AMPL lab which allows a given function to process the input of multiple datasets.

Dataset descriptions (in order of appearance on the alphabetic list for code used):

MAPPAll_Dec2017: masterlist of all MAPP I subject directories (318 subjects total)
Corrected connectivity files for MNI-trace: subject files (healthy controls and patient) for the corrected z-score correlation from the MNI-trace to every other voxel
Corrected connectivity files for MNI-sphere: subject files (healthy controls and patient) for the corrected z-score correlation from the MNI-sphere to every other voxel
Clust_mask_MNI+tlrc: healthy control group mask generated in AfNI's GUI of the significant ROIs correlated to the MNI-trace
Corrected healthy control MNI-trace subjects: only healthy control subject files for the corrected z-score correlation from the MNI-trace to every other voxel
Corrected patient MNI-trace: only patient subject files for the corrected z-score correlation from the MNI-trace to every other voxel
Clust_mask_Wei+tlrc: healthy control group mask generated in AfNI's GUI of the significant ROIs correlated to the MNI-sphere

Corrected healthy control MNI-sphere subjects: only healthy control files for the corrected z-score correlation from the MNI-sphere to every other voxel

Corrected patient MNI-sphere subjects: only patient subject files for the corrected z-score correlation from the MNI-sphere to every other voxel

PAGConnectivityall30altMNI092018.mat: dataset containing extracted Power et al. atlas signals for 30 healthy controls from MAPP I

PAGConnectivityall30altMNI092018_OrigDat_Connectivity.mat: dataset containing connectivity measures using the ROIs from the Power et al. atlas for PAG traces in the original set of 15 healthy controls

PAGConnectivityall30altMNI092018_ValDat_Connectivity.mat: dataset containing connectivity measures using the ROIs from the Power et al. atlas for all traces in the validation set of 15 healthy controls

PAGConnectivityall30altMNI092018_OrigDat_Connectivity_Differences.mat: dataset containing connectivity measures using the ROIs from the Power et al. atlas and statistics for PAG traces in the original set of 15 healthy controls

PAGConnectivityall30altMNI092018_ValDat_Connectivity_Differences.mat: dataset containing connectivity measures and statistics for PAG traces in the validation set of 15 healthy controls

PP264PAGxyzlabelcolor.mat: ROI coordinates and color code from the Power et al. atlas

PAGConnectivity30all09212018_OrigDat_Connectivity_Differences.mat: dataset containing connectivity measures from all voxels for PAG traces in the original set of 15 healthy controls

PAGConnectivity30all09212018_ValDat_Connectivity_Differences.mat: dataset containing connectivity measures from all voxels for PAG traces in the validation set of 15 healthy controls

MaskVoxelCoord.mat: coordinates for all voxels within the brain area given by a group mask generated in AfNI

OBetter_2mm.nii: NIfTI image file in 2mm space for voxels in the original set whose connectivity difference from the MNI-trace to the traced-participant perform "better" than the MNI-sphere to the traced-participant

OWorse_2mm.nii: NIfTI image file in 2mm space for voxels in the original set whose connectivity difference from the MNI-trace to the traced-participant perform "worse" than the MNI-sphere to the traced-participant

VBetter_2mm.nii: NIfTI image file in 2mm space for voxels in the validation set whose connectivity difference from the MNI-trace to the traced-participant perform "better" than the MNI-sphere to the traced-participant

VWorse_2mm.nii: NIfTI image file in 2mm space for voxels in the validation set whose connectivity difference from the MNI-trace to the traced-participant perform "worse" than the MNI-sphere to the traced-participant

MotionSignals.mat: dataset containing variables related to the head motion between healthy controls and patients

MNI152_T1_1mm_brain.nii.gz: skull stripped MNI152 standard-space T1-weighted average structural template NIfTI image in 1mm space

MNI_HC_in_1.nii.gz: AfNI clusterized NIfTI image file of MNI-trace connectivity in healthy controls transformed into 1mm space for visualization purposes

Wei_HC_in_1.nii.gz: AfNI clusterized NIfTI image file of MNI-sphere connectivity in healthy controls transformed into 1mm space for visualization purposes

OBetter.nii: NIfTI image file in 1mm space for voxels in the original set whose connectivity difference from the MNI-trace to the traced-participant perform "better" than the MNI-sphere to the traced-participant

VBetter.nii: NIfTI image file in 1mm space for voxels in the validation set whose connectivity difference from the MNI-trace to the traced-participant perform "better" than the MNI-sphere to the traced-participant

OWorse.nii: NIfTI image file in 1mm space for voxels in the original set whose connectivity difference from the MNI-trace to the traced-participant perform "worse" than the MNI-sphere to the traced-participant

VWorse.nii: NIfTI image file in 1mm space for voxels in the validation set whose connectivity difference from the MNI-trace to the traced-participant perform "worse" than the MNI-sphere to the traced-participant

CustoutMNIonMNIgm.txt: output text file containing AfNI clusterized healthy control ROI extracted signals of healthy controls and patients from the MNI-trace connectivity

CustoutWeionWeigm.txt: output text file containing AfNI clusterized healthy control ROI extracted signals of healthy controls and patients from the MNI-sphere connectivity

PAGConnectivity30all09212018.mat: dataset containing extracted voxel signals for 30 healthy controls from MAPP I

SelectForPAG_DataTable.mat: dataset of MAPP I healthy controls and patients

OriginalID.mat: list of the original set of MAPP I healthy controls (15 out of the 30 total)

MasterList_PAGSep2018.mat: masterlist of only healthy control original and validation MAPP I subject directories (30 subjects total)

1mmMask.txt

PAGConnectivityall30altMNI092018_OrigDat.mat: dataset containing extracted Power et al. atlas signals for 15 healthy controls in the original set from MAPP I

PAGConnectivityall30altMNI092018_ValDat.mat: dataset containing extracted Power et al. atlas signals for 15 healthy controls in the validation set from MAPP I

PAGConnectivity30all09212018_OrigDat.mat: dataset containing extracted voxel signals for 30 healthy controls in the original set from MAPP I

PAGConnectivity30all09212018_ValDat.mat: dataset containing extracted vosel signals for 15 healthy controls in the validation set from MAPP I

Analysis processing order:

The following steps were implemented after the preprocessing of resting state fMRI images through a Freesurfer-Afni pipeline as described in Drysdale and colleagues (CITE).

1) Extracted time series signals using ROIs defined by Power et al, 2011 from resting state images and saved into dataset **PAGConnectivityall30altMNI092018.mat on** 30 subjects total (15 original dataset, 15 validation dataset from the masterlist **MasterList_PAGSep2018.mat**). In addition to the 264 ROIs, the dataset includes time series from four PAG traces: MNI-sphere (3mm sphere around coordinates: [-4 -26 -14] and [4 -26 -14]), MNI-trace, and traced-participant (267 total ROIs). Note that in our final analysis, our MNI-sphere refers to coordinates [4 -26 -14].

   a) Subject specific PAG traces were transformed using **transformTraced2SubjectPAG_AFNItransform.m** for the original dataset and transform **transformTraced2SubjectPAG_AFNItransform_3.m** for the validation dataset. This delineation was done prior to the creation of this dataset and is explained further in our Methods section.

b) The voxel signals were extracted from resting state images preprocessed file and residuals file using **Step01_ExtractPowerSignalsPAG_MNIalt_2.m**. These signals are then stored in each subject file along with descriptor information. In our analysis the residuals file was used.

c) The signals stored in each subject were averaged and an option to exclude voxels with signal to noise ratio (SNR) less than 100 (however, this was not used in our analysis) using **Step02_Concatenate_SNRrejectPAG_AFNI_2.m**.

d) **PAGConnectivityall30altMNI092018.mat** is the final dataset with all 30 subjects containing averaged signals for for each ROI (267 in total).

e) **DifferenceTRADHANDMNIHAND.m** was used to calculate statistical differences from MNI-sphere to traced-participant and MNI-trace to traced-participant (Figure 1)

2) **PAGConnectivityall30altMNI092018.mat** was then converted into a new dataset with connectivity values to each of the four PAG traces (Figure 2).

a) First the data from **PAGConnectivityall30altMNI092018.mat** is split into the test and validation dataset, each containing 15 subjects using **SplitDataOrigVal_Voxelwise.m** (used also for the voxelwise data analysis as explained in Step 4).

b) **WeiPreprocess_to_Connectivity_2.m** is the code that creates these connectivity values whose output is either **PAGConnectivityall30altMNI092018_OrigDat_Connectivity.mat** or **PAGConnectivityall30altMNI092018_ValDat_Connectivity.mat**

c) Percent differences and basic statistics were performed using these connectivity datasets using **Differences_WeiPreprocess.m** and whose results were added to to the connectivity output and saved as **PAGConnectivityall30altMNI092018_OrigDat_Connectivity_Differences.mat** or **PAGConnectivityall30altMNI092018_ValDat_Connectivity_Differences.mat**

3) Differences in connectivity was also accounted for by comparing the MNI-sphere to the traced-participant and the MNI-trace to the traced-participant for each subject (Figure 2 and Figure 3).

a) Quantification of this comparison was calculated using the interquartile range and median across subjects and ROIs from the Power et al., 2011 atlas are plotted throughout the brain in **GenFig_IQR_OrigVal.m**.

b) Differences between the MNI-trace to the traced-participant performed "better" when the interquartile range was smaller and the median was closer to zero compared to differences between the MNI-sphere to the traced-participant.

c) Differences between the MNI-trace to the traced-participant performed "worse" when the interquartile range was larger and the median was farther from zero compared to differences between the MNI-sphere to the traced-participant.

d) The images produced show ROIs that perform "better" or "worse" in the original dataset, the validation dataset, and the overlap of the two datasets.

4) Similar steps (1-3) were taken for the extracted time series signals for each voxel in the brain image. The following code and datasets replace the same order of analysis as above with some exceptions (Figure 3).

   a) The signal from each of the PAG traces was averaged using the entire traces, this was correlated to each of the voxels in the brain as a voxelwise connectivity measure. The signal from each of these traces is not altered and uses the same code as above.

   b) Furthermore, "better" or "worse" conditions are defined per voxel rather than per ROI. These voxels are generated in **GenFig_IQR_Voxelwise.m** whose images are output as OBetter.nii, OWorse.nii, VBetter.nii, and VWorse.nii**.** These outputs were resampled using AFNI code **resampleBetterWorse.txt** and visualized using **GenFig_Visualize_Voxelwise.m**. The images produced show voxels that perform "better" or "worse" in the original dataset, the validation dataset, and the overlap of the two datasets.

   c) **PAGConnectivity30all09212018.mat** replaces **PAGConnectivityall30altMNI092018.mat; Step01_ExtractPowerSignalsPAG_Voxelwise_GroupMask.m** replaces **Step01_ExtractPowerSignalsPAG_MNIalt_2.m; Step02_Concatenate_SNRrejectPAG_AFNI_Voxelwise.m** replaces **Step02_Concatenate_SNRrejectPAG_AFNI_2.m;** The same code is used for **SplitDataOrigVal_Voxelwise.m; WeiPreprocess_to_Connectivity_2_Voxelwise.m** replaces **WeiPreprocess_to_Connectivity_2.m; PAGConnectivity30all09212018_OrigDat_Connectivity.mat** replaces **PAGConnectivityall30altMNI092018_OrigDat_Connectivity.mat; PAGConnectivity30all09212018_ValDat_Connectivity.mat** replaces **PAGConnectivityall30altMNI092018_ValDat_Connectivity.mat;** The same code is used for **Differences_WeiPreprocess.m ; PAGConnectivity30all09212018_OrigDat_Connectivity_Differences.mat** replaces **PAGConnectivityall30altMNI092018_OrigDat_Connectivity_Differences.mat; PAGConnectivity30all09212018_ValDat_Connectivity_Differences.mat** replaces **PAGConnectivityall30altMNI092018_ValDat_Connectivity_Differences.mat; GenFig_IQR_Voxelwise.m** replaces **GenFig_IQR_OrigVal.m**.

5) Differences between MNI-trace and MNI-sphere across healthy controls and patients (across all applicable 318 MAPP I subjects from the master list **MAPPAII_Dec2017**) was calculated using programs and code found in AFNI.

   a) **Step01_VoxelwiseCorrelation_MNI_nomask.m** and **Step01_VoxelwiseCorrelation_Wei_nomask.m** were used to calculate the standardized (z-score) voxelwise connectivity measures in each subject to the MNI-trace and MNI-sphere. **Step01_VoxelwiseCorrelation_Hand_nomask.m**

was also used to calculate the connectivity measures in traced-participant and used for additional analysis not included in this paper.

b) To control for site effects, the standardized voxelwise connectivity measures were demeaned using **demeansubjects.m** and a global mean was added using **addglobalmeansubjects.m** across subjects, with corrected connectivity measures for each subject

c) **cmd.MNI.txt** and **cmd.Wei.txt** are used to create average connectivity measures in healthy controls for the MNI-trace and MNI-sphere across subjects.

d) ROIs from the healthy control connectivity to the MNI-trace and MNI-sphere (calculated using AFNI cluster thresholding in 3dttest++ -Clustsim, with a p-value=1e-5, cluster threshold of 6 voxels, and α=0.01) are then used to extract signals from both healthy controls and patients using **CodeforExtractingROIs_MNI.txt** and **CodeforExtractingROIs_Wei.txt** for MNI-trace and MNI-sphere respectively.

e) **ROIttest.m** is used to examine the statistical difference between the average output signal in healthy controls and patients for MNI-trace and MNI-sphere, separately.

f) **PlotSlice_withActivity.m** was used to visualize the healthy control ROIs on the brain

6) **AnalyzeMotionFile.m** was used to analyze head motion in healthy controls and patients. Statistics are performed on the output from this file and results are visualized in **MotionPlotsandStats.m**.

Diagram: