

## Supplementary Information for

**Title:** Insula serotonin 2A receptor binding and gene expression contribute to serotonin transporter polymorphism anxious phenotype in primates

**Short title:** Insula serotonin 2A receptor and anxiety

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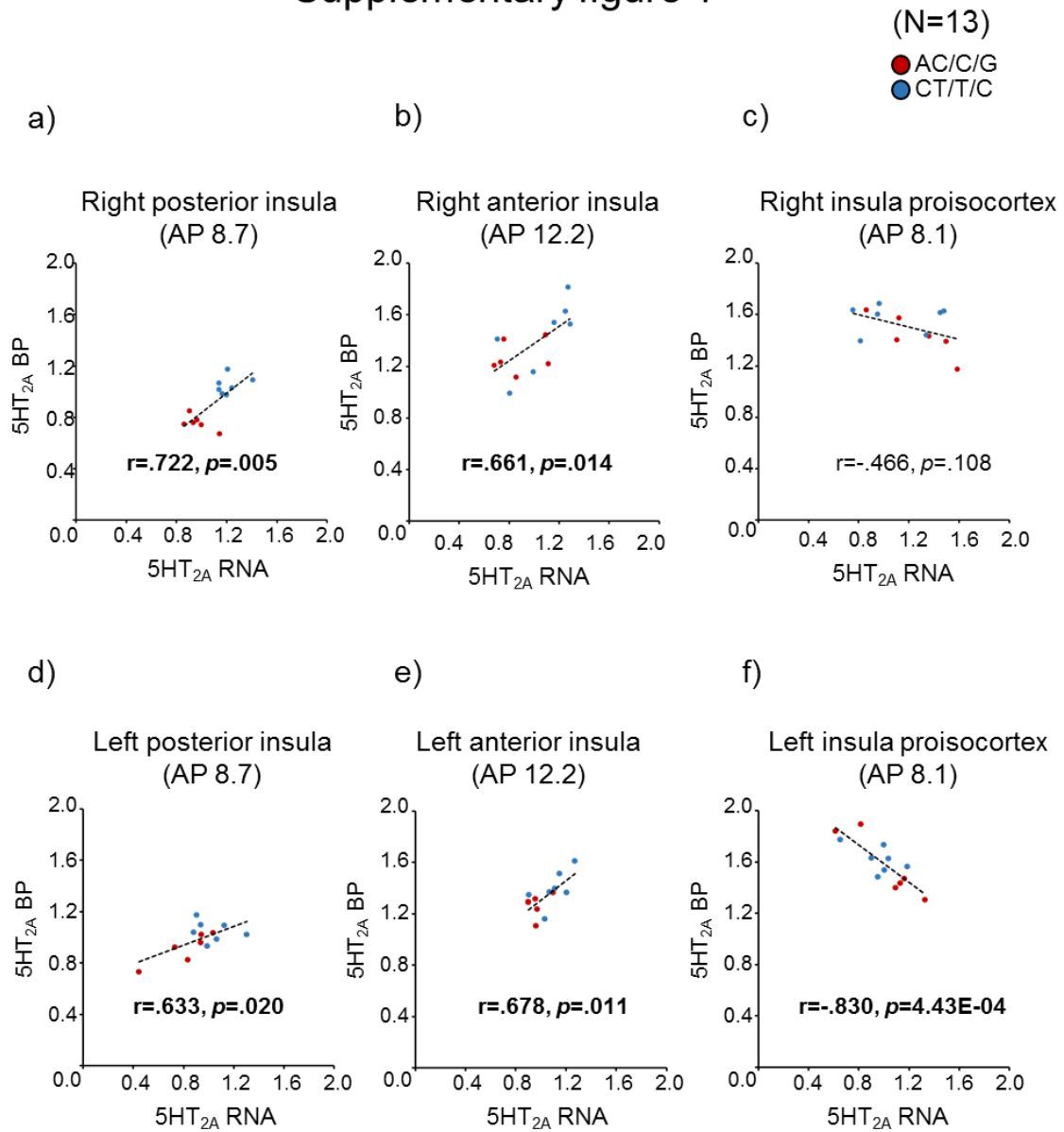
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### **This PDF file includes:**

Figs. S1 to S5

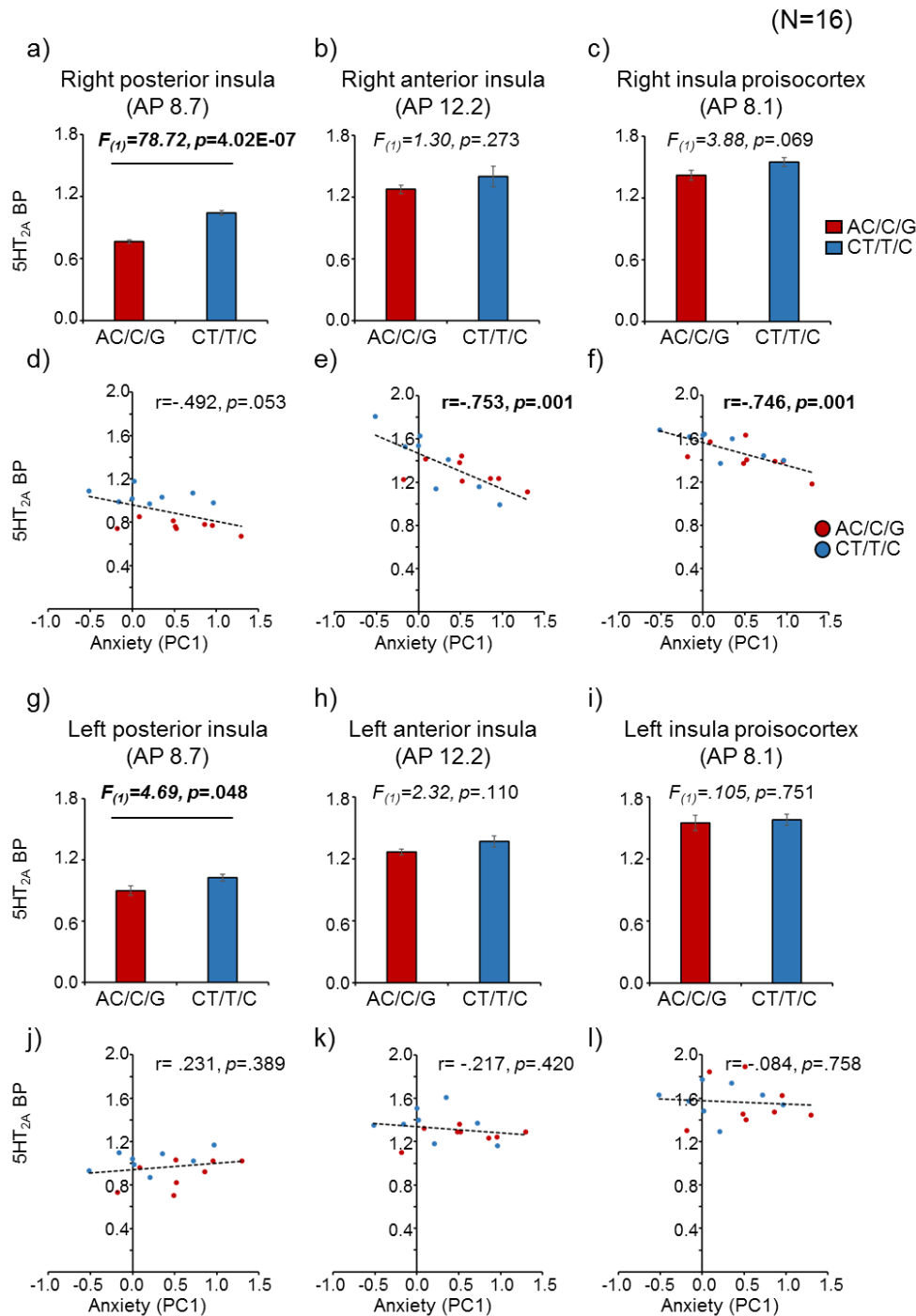
Tables S1 to S3

## Supplementary figure 1



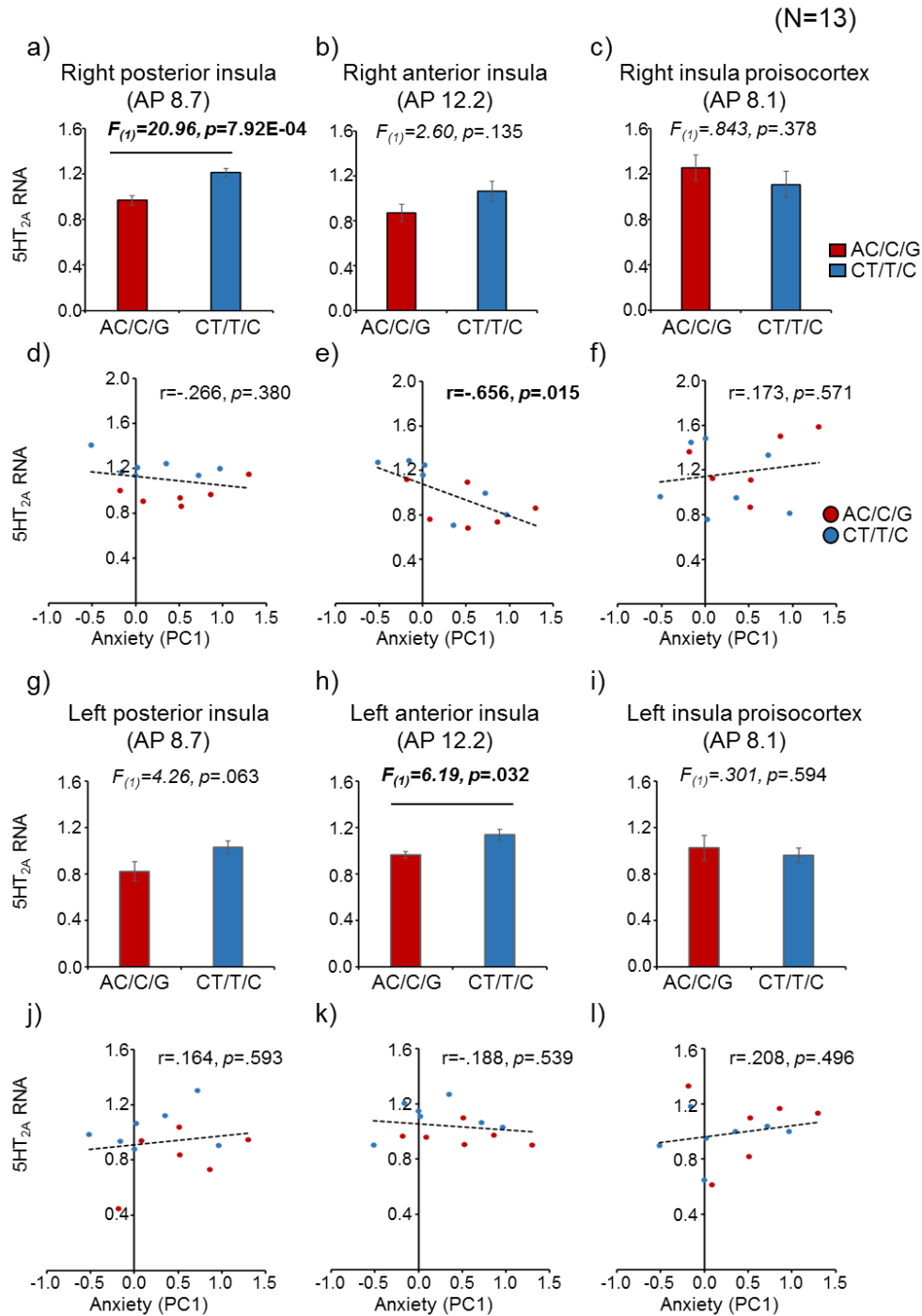
**Supplementary figure 1:** Correlations between 5HT<sub>2A</sub> BP and RNA expression levels for the right **a-c**) and left **d-f**) posterior insula (AP 8.7), anterior insula (AP 12.2) and insula proisocortex (AP 8.1) regions. Pearson correlation (N=13),  $p < .05$  was considered significant, highlighted in bold.

## Supplementary Figure 2



**Supplementary figure 2:** 5HT<sub>2A</sub> BP comparison between *SLC6A4* homozygous genotypes (a-c, right; g-i, left) and correlations with anxiety scores in the human intruder test (d-f, right; j-l, left) for the posterior insula (AP 8.7), anterior insula (AP 12.2) and insula proisocortex (AP 8.1) regions. One-way ANOVA and Pearson correlation, respectively. N=16,  $p<.05$  was considered significant, highlighted in bold.

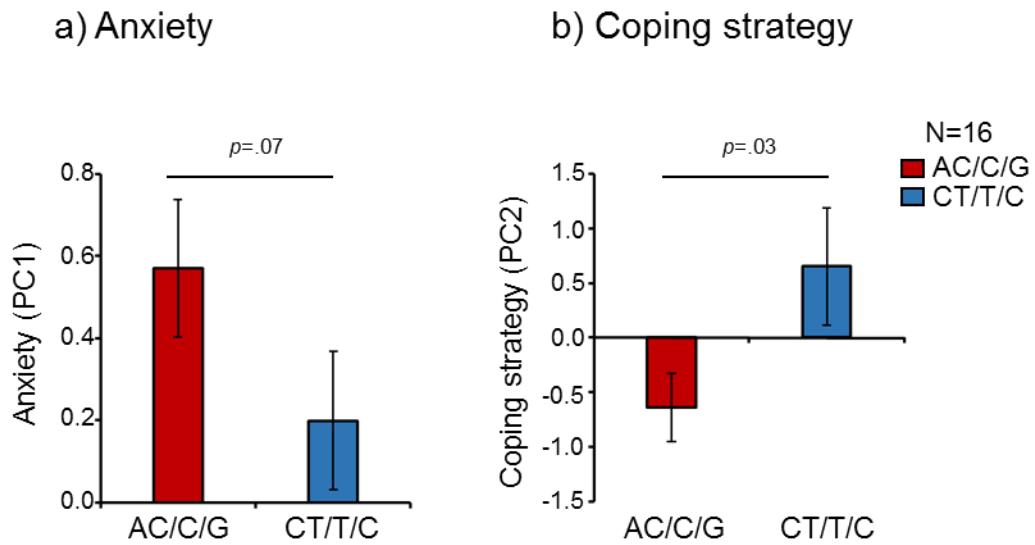
## Supplementary Figure 3



### Supplementary figure 3: 5HT<sub>2A</sub> RNA expression comparison between *SLC6A4*

homozygous genotypes (**a-c**, right; **g-i**, left) and correlations with anxiety scores in the human intruder test (**d-f**, right; **j-l**, left) for the posterior insula (AP 8.7), anterior insula (AP 12.2) and insula prouisocortex (AP 8.1) regions. One-way ANOVA and Pearson correlation, respectively. N=13,  $p<.05$  was considered significant, highlighted in bold.

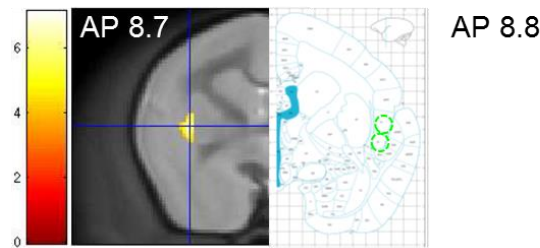
## Supplementary Figure 4



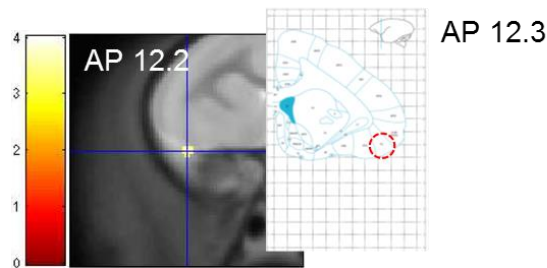
**Supplementary figure 4:** Comparison between marmosets homozygous for each *SLC6A4* haplotypes AC/C/G and CT/T/C, for **a)** anxiety scores (PC1), **b)** coping strategy scores (PC2) measured with the human intruder test, within this study cohort (N=16), 1-tailed t test,  $p < .05$  was considered significant.

## Supplementary Figure 5

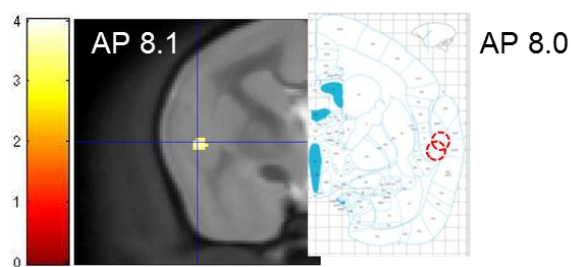
### a) Right posterior insula



### b) Right anterior insula



### c) Right insula proisocortex



**Supplementary figure 5:** Schematics of the location of the punches extracted from frozen 200 $\mu$ m-thick brain sections to use in RNA expression measurements. **a)** right posterior insula, **b)** right anterior insula and **c)** right insula proisocortex. Left panels show the clusters obtained with the voxel-base analysis centered in the peak of significance, with antero-posterior (AP) coordinates from ref. 70. Right panels show the corresponding location in a marmoset brain atlas (70). Depicted in green, punches for the genotype contrast analysis (AC/C/G vs CT/T/C) and in red for the correlational analysis with anxiety scores (PC1). Punches of 1.5 mm were used for a) and c), and 2 mm for b). Punches were taken from 600 $\mu$ m anterior to 600 $\mu$ m posterior to the location of the most significant peak (shown in the pictures).

**Table S1**

<b>Table S1: Variables measured in human intruder test</b>		
<b>Measures</b>	<b>AC/C/G</b>	<b>CT/T/C</b>
Average distance (cm)	88.85±3.25	84.05±3.47
Locomotion (%) (*)	3.98±0.67	8.21±1.65
Bobbing (*)	65.13±7.59	28.25±7.17
Jumps	1±0.46	1±0.57
Tsik calls	1.38±0.82	8.38±3.88
Egg calls (*)	23.75±5.21	12.25±3.04
Tsik-Egg calls	14±6.64	20.75±7.65
Tse-like calls	5.13±3.05	9.88±4.66
PC1 (†)	0.57±0.17	0.20±0.17
PC2 (*)	-0.64±0.31	0.65±0.54

Mean±SEM, AC/C/G vs CT/T/C, 1-tailed t test (\*)  $p < .05$ ; (†)  $p = .07$

Table S2

Measures	AC/C/G				CT/T/C					
	V average	V 1	M100907 0.1mg/kg	M100907 0.3 mg/kg	V 2	V average	V 1	M100907 0.1mg/kg	M100907 0.3 mg/kg	V 2
<b>Average distance*</b>	67.08±2.52	69.31±4.57	68.93±7.39	78.59±5.56	65.43±2.17	64.77±1.48	66.94±3.37	64.11±3.08	62.82±3.18	62.59±2.37
Locomotion (%)	4.19±1.15	3.39±1.09	4.10±1.46	3.19±0.87	5.22±1.91	6.47±0.67	5.30±1.17	6.06±0.95	6.18±0.89	7.64±1.29
Bobbing	34.17±8.71	38.00±8.68	29.17±8.60	29.67±10.78	31.40±11.40	11.71±6.02	19.00±9.99	14.43±6.97	8.71±3.26	4.43±2.17
Jumps	4.33±1.49	4.00±1.75	4.00±1.29	3.67±1.26	4.60±2.34	6.07±1.20	5.71±1.21	6.71±1.52	5.86±2.10	6.43±2.29
Tsik calls	2.08±1.04	2.33±1.17	6.50±3.13	1.50±0.67	2.00±1.14	12.79±3.06	12.14±3.40	17.00±5.94	9.86±2.34	13.43±3.99
Egg calls	7.50±3.86	7.83±3.23	8.17±3.65	11.00±8.66	7.40±5.69	3.93±1.64	5.00±1.75	6.29±3.31	6.29±3.41	2.86±1.62
Tsik-Egg calls	18.08±6.11	20.17±7.14	20.83±8.77	19.67±10.41	13.00±6.24	11.93±4.44	17.14±6.11	11.71±6.17	6.00±2.84	6.71±3.67
Tse-like calls	5.83±1.80	7.17±1.99	6.67±2.14	11.00±3.79	2.80±1.16	8.50±2.83	9.43±4.85	13.29±5.41	16.43±4.41	7.57±2.19
PC1	0.04±0.33	0.27±0.43	0.15±0.38	0.58±0.41	-0.25±0.36	-0.56±0.12	-0.23±0.31	-0.44±0.23	-0.52±0.29	-0.89±0.26
PC2	-0.47±0.44	-0.44±0.39	-0.11±0.60	-0.65±0.77	-0.60±0.61	0.39±0.25	0.37±0.31	0.49±0.53	-0.02±0.21	0.41±0.26

\* AC/C/G group, V average vs M100907 0.3 mg/kg, paired t-test p<.05

V1 and V2 first and last vehicle administration, V average=(V1 + V2)/2



**Table S3**

<b>Table S3: Sequences of primers used in the real timePCR</b>		
<b>Gene symbol</b>	<b>Gene name</b>	<b>Sequence (5'→3')</b>
<i>HTR2A</i>	Serotonin 2A receptor	F: GCAGAATGCCACCAACTATT R: GGAATATGCGCCCATAGAGA
<i>ACTB</i>	β-Actin	F: AGCAGTCGGTTGGAGCGAGCAT R: TGGCTTTTGGGAGGGCAAGGGA
<i>TBP</i>	TATA box binding protein	F: GCCCGAAATGCCGAATATAA R: TTCTTCACTCTTGGCTCCTGTG
<i>GAPDH</i>	Glyceraldehyde-3-phosphate	F: TAAGACCCCCTGGACCATCAGCC R: GGGGCAATTCGGTGTGGTGA
<i>SDHA</i>	Succinate dehydrogenase complex, subunit A	F: TGGGAACAAGAGGGCATCTG R: CCACCACGGCATCAAATTCATG