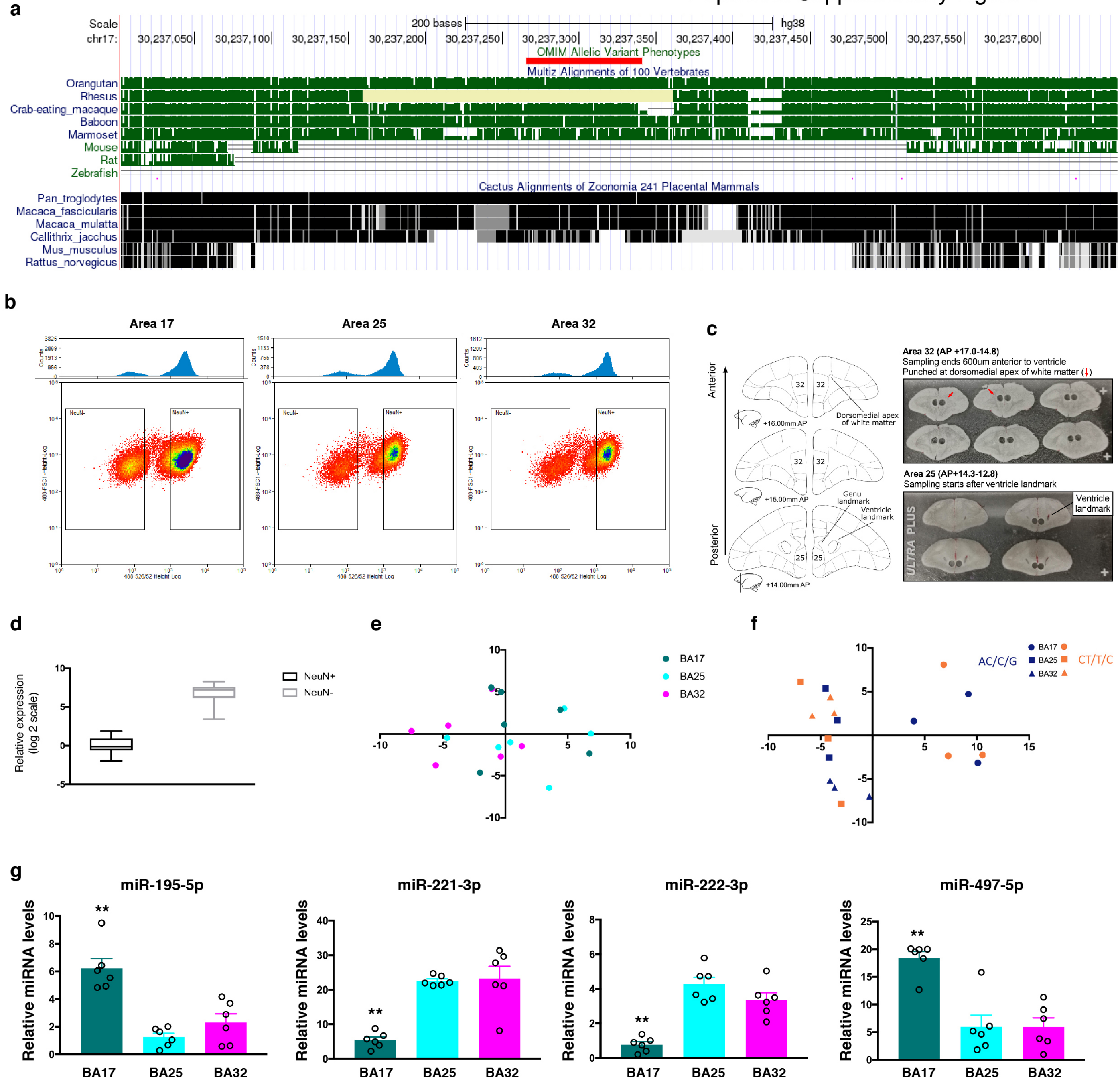
Popa et al Supplementary Figure 1



Supplementary Figure 1.

a) Alignement of the 5-HTTLPR region using two different algorithms (multiZ and Cactus) shows the conserva tion across primates but not in rodents or fish. Red rectangle represents the location of l and s alleles.

b) Representative example of the NeuN staining and windows used for FACS sorting.

c) Schematic representation of the anatomical landmarks used for harvesting tissue from area 25 and 32.

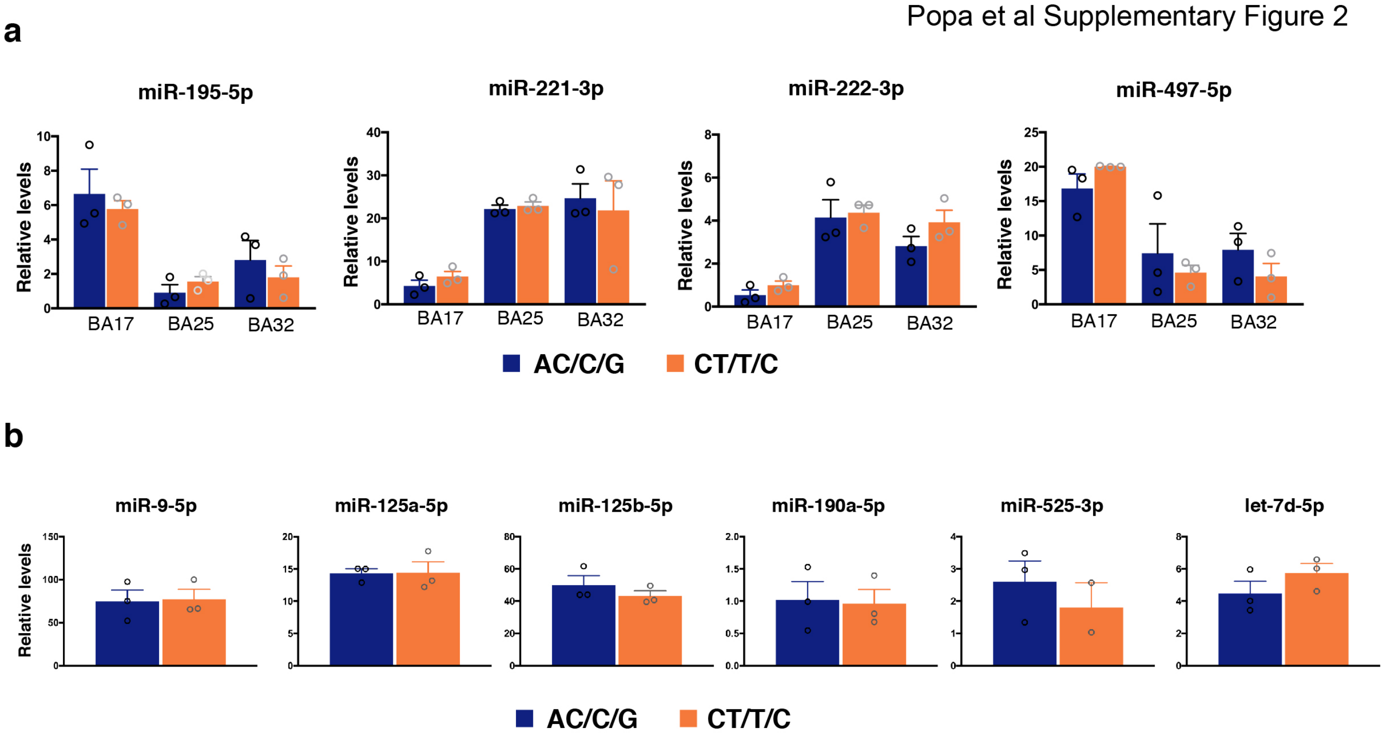
d) Expression of microglial marker Aif1 in NeuN$^+$ and NeuN$^-$ fractions.

e) PCA analysis on miRNAs levels in NeuN$^-$ nuclei shows no regional discrimination in this fraction.

f) PCA analysis on levels of 192 miRNAs expressed in NeuN$^+$ nuclei reveals a distribution across regions and genotypers similar to those shown in Fig 1d and 2a.

g) Examples of miRNAs differentially expressed in the visual cortex. miR-221-3p/222-3p levels are significantly lower in BA17 compared to both areas of the vmPFC whereas miR-195-5p/497-5p are enriched in the visual cortex ($p<0{,}001$, 1-way ANOVA, Bonferroni test).

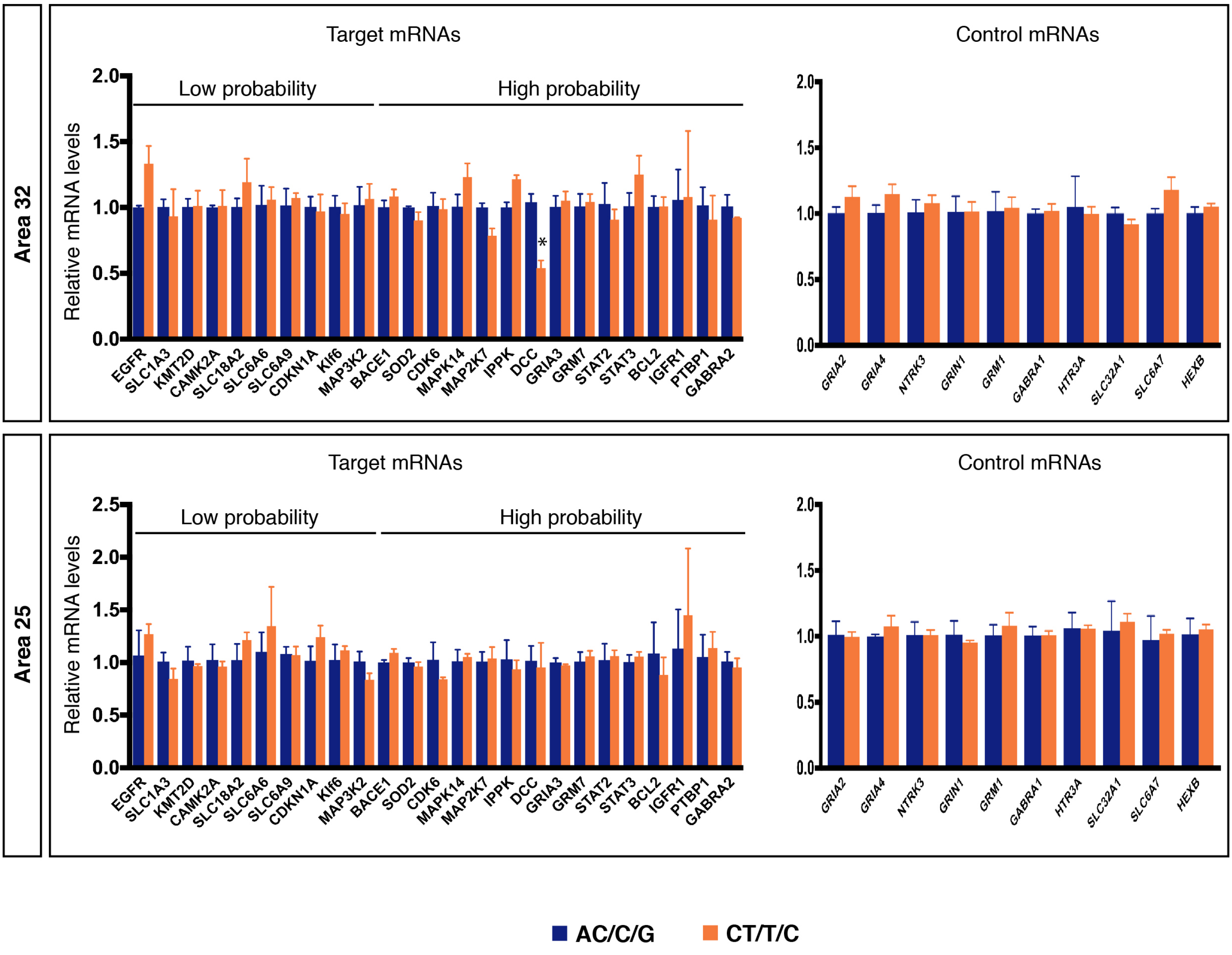
Popa et al Supplementary Figure 2



Supplementary Figure 2.

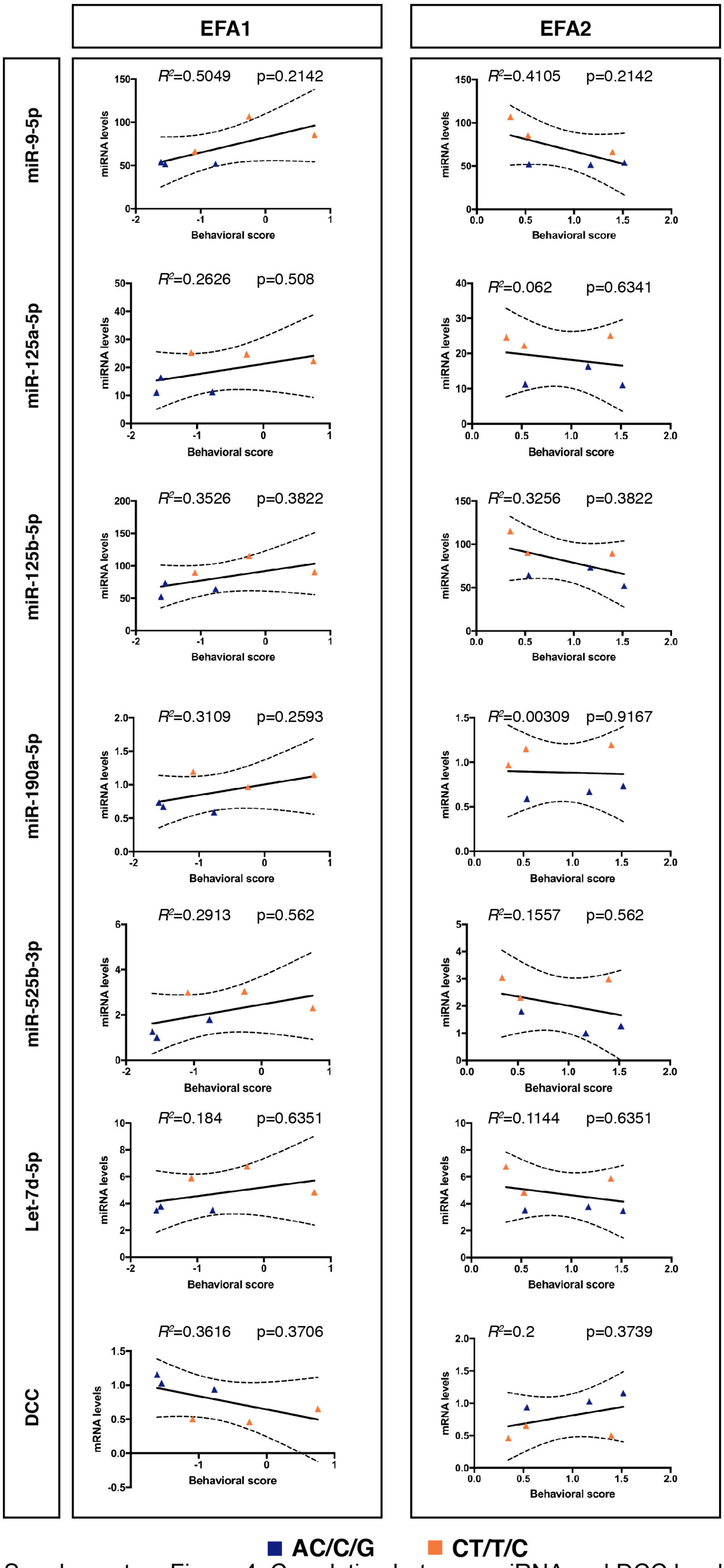
a) SLC6A4 polymorphisms do not affect the levels of miR-195-3p, miR-221-3p, miR-222-3p and miR-497-5p.

b) Expression of miR-9-5p, miR-125a-5p, miR-125b-5p, miR-190a, miR-525-3p and let-7d-5p in BA17.



Supplementary Figure 3.
Expression of 25 target mRNAs identified by network analysis in area 32 (top) and 25 (bottom). Only DCC was altered in area 32. No significant differences were found in any of the tested transcripts in area 25. As a control, 10 reference genes were also quantified in both areas ($p<0,05$, 2-way ANOVA, Tukey test).

Popa et al Supplementary Figure 4



Supplementary Figure 4. Correlation between miRNA and DCC levels in area 32 and behavioral response in the snake test. Two EFA factors are considered in this analysis as previously reported (Quah et al. 2020).p values are adjusted for multiple comparison using the Holm-Sidak correction.

a

Human	GAAGGCGCUUCCCUUUAGAGCG
Chimp	GAAGGCGCUUCCCUUUAGAGCG
Gorilla	GAAGGCGCUUCCCUUUAGAGCG
Orangutan	GAAGGCGCAUCCCUUUAGAGCG
Macaque	GAAGGCGCAUCCCUUUGGAGCG
Baboon	GAAGGCGCAUCCCUUUGGAGCG
Marmoset	GAAAGTGCUUCCCUUUAGAGTG
Squirrel monkey	GAACTTGCUUCCCUUUAGAGCG

b

miR-190a-5p binding site

4150

Human	AUACCACACCCAUAUCAGCAAAUGAAUAUUACU
Marmoset	AUACCACAUCCAUAUCAGCAAAUGAAUAUUGCU
Rat	GUGCAGUGCCCAUAUGAGCAAAUGCGAGUAACU

Supplementary Figure 5. a) Alignment of miR-525-3p in different primate species. b) Alignment of the 3' UTR DCC mRNA in humans, marmoset and rat to illustrate conservation/divergence of binding sites for miR-190a-5p.

Supplementary Materials

Human Intruder Test

The human intruder test has been set up in marmosets to assess anxiety-like behaviours by measuring responses to an uncertain threat (the human intruder) [1]. Briefly, during testing the marmosets are restricted to a separate quadrant within their own home cage. An unfamiliar person wearing a realistic latex human mask stands 40 cm in front of the cage keeping eye contact with the marmoset for 2 mins. The marmoset behaviours are recorded with a HD video camera and a shotgun condenser microphone. A wide range of behavioural repertoire is scored off line by an experimenter blind to the genotype/treatment. The behaviours measured includes:

To measure approach/avoidance behaviour, the test quadrant was divided into three zones (front, middle, back) respect to human intruder.

Time spent at the front: percentage of time spent at the front (approach).

Time spent at the back: Percentage of time spent at the back of the cage (avoidance).

To measure flight behavior, the test quadrant was divided into five zones, from bottom to top of the cage (top of the nest box, high, middle, low, floor).

Average height: Average height in cm weighted by the proportion of time spent at each zone, respect to the floor of the cage.

Locomotion: Percentage time spent changing locations within the home-cage defined as changing position of all four limbs.

Head and body bobs: number of rapid and repetitive side-to-side movements of the upper body while sitting and staring at the object of interest.

Tse-egg calls: vocalizations consisting of a single utterance of tse followed by a single or a series of egg calls (vigilance) (Bezerra and Souto, 2008).

An overall anxiety score was calculated based on an Exploratory Factor Analysis (EFA) with a principal axis factoring extraction method performed on human intruder data obtained from 171 animals [2]. The main contributors to this factor's score representing anxiety-like behavior included time spent at the front and back, average height, locomotion, head and body bobs and tse-egg calls. Those animals with the highest score spent the majority of their time towards the back of the cage, high up, remaining relatively still, and making head and body bobs and calls.

Snake Test

The model snake test was designed to evaluate innate fear response towards a predator. Since the model snake is placed directly within the homecage, it represents far higher spatial threat imminence compared to the intruder in the human intruder test.

The procedure of the model snake test is based on the methods in [2, 3]. Before the testing session begins, wireless cameras and a microphone are placed to record the animal's behavior from a top-down view and a frontal view. During a test session, the animal is separated from their cagemate and restricted to the upper right quadrant of their home cage. Test session is divided into four 5-min phases: a separation phase, where only the camera and microphone were present; a pre-snake phase, where an empty box without the model snake (a 27 cm tall rubber model of a rearing cobra) is placed in the test quadrant; a snake phase, where the empty box from the previous phase is replaced with a box containing the model snake (a sliding door is removed to expose the model snake once the box is in position); and a post-snake phase, where the empty box from the pre-snake phase is re-introduced into the test quadrant. Parameters quantified during the snake model test are summarized in Suppl. Table 2.

Similar to the human intruder test, EFA analysis on 171 animals resulted in two main components that best defined the response of the animals to the snake model [2].

REFERENCES

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2. Quah SKL, Cockcroft GJ, McIver L, Santangelo AM, Roberts AC. Avoidant Coping Style to High Imminence Threat Is Linked to Higher Anxiety-Like Behavior. *Front Behav Neurosci* 2020; **14**: 34. 32218725
3. Shiba Y, Santangelo AM, Braesicke K, Agustín-Pavón C, Cockcroft G, Haggard M, Roberts AC. Individual differences in behavioral and cardiovascular reactivity to emotive stimuli and their relationship to cognitive flexibility in a primate model of trait anxiety. *Front Behav Neurosci* 2014; **8**: 137. 24795587

Supplementary Table 1. Subjects used in this study

Subject	Genotype	HI-test age,y	ST age, y	Age at death, y
Jetsam	CT/T/C	1.71	2.01	5.66
Sebastian	CT/T/C	1.58	1.64	5.24
Bob	CT/T/C	2.44	2.53	6.13
Bakerloo	AC/C/G	2.43	2.51	6,11
Merry	AC/C/G	2.43	2.76	6.39
Axel	AC/C/G	2.86	3.21	6,83

Supplementary Table 2. Primers used for sequencing

Primer name	Primer sequence
SLC6A4 repeat region forward	**CAGACAACCGTGTTCA TCTG**
SLC6A4 repeat region reverse	**GATTCTAGTGCCACCTAGAC**
Sequencing primer 1	**AGCAGCACCTAACCCTCCTA**
Sequencing primer 2	**TCCCCACTAGGCATTGCTAC**

Supplementary Table 3. Behavioral parameters evaluated in the human intruder and snake test.

Human Intruder Test	Snake Test
Average height	Tsik-egg calls
Head and body bobs	Tsik calls
Time spent (back)	Stare count
Tse-egg calls	Stare duration
Locomotion	Locomotion
Time spent (front)	Average distance
Egg calls	Head-cock
Tsik calls	Egg calls
Tsik-egg calls	

From Santangelo et al. 2016

Supplementary Table 4. List of miRNAs used for PCA analysis

let-7a-5p
let-7b-3p
let-7b-5p
let-7c-5p
let-7d-5p
let-7f-5p
let-7g-5p
let-7i-5p
miR-1-3p
miR-100-5p
miR-101-3p
miR-103a-3p
miR-107
miR-124-3p
miR-1248
miR-1255a
miR-125a-5p
miR-125b-5p
miR-1260a
miR-128-3p
miR-129-1-3p
miR-133b
miR-144-3p
miR-148a-3p
miR-153-3p
miR-15a-5p
miR-16-5p
miR-17-5p
miR-181a-5p
miR-181b-5p
miR-181c-5p
miR-185-5p
miR-190a-5p
miR-191-5p
miR-195-5p
miR-196a-5p
miR-197-3p
miR-199a-5p
miR-200a-3p
miR-200c-3p
miR-204-5p
miR-21-3p
miR-2110
miR-214-3p
miR-219a-5p
miR-22-3p
miR-24-3p
miR-26a-5p
miR-27b-3p
miR-29a-3p
miR-29b-3p
miR-29c-3p
miR-302b-3p
miR-302d-3p
miR-30b-5p
miR-30c-2-3p
miR-30d-5p
miR-320a
miR-323b-5p
miR-325
miR-338-3p
miR-339-3p
miR-342-3p
miR-361-3p
miR-361-5p
miR-374a-5p
miR-374b-5p
miR-376a-3p
miR-378a-3p
miR-380-3p
miR-423-5p
miR-448
miR-452-3p
miR-483-5p
miR-485-5p
miR-490-3p
miR-497-5p
miR-501-3p
miR-501-5p
miR-502-3p
miR-518f-3p
miR-524-3p
miR-525-3p
miR-548a-3p
miR-548e-3p
miR-548j-5p
miR-551a
miR-562
miR-576-3p
miR-593-3p
miR-606
miR-628-3p
miR-633
miR-645
miR-653-5p
miR-744-5p
miR-765
miR-9-3p
miR-9-5p
miR-92a-3p
miR-93-5p
miR-939-5p
miR-99b-5p

Statistical summary

Figure	Type of test	Multiple comparison	p value
Fig 1b/Suppl Fig 1d NeuN+/NeuN-	2-way ANOVA	No	p< 0.0001
Fig 2b AC/C/G vs CT/T/C	1-way ANOVA	Bonferroni	miR-9-5p **p=0.0475** miR-125a-5p **p=0.0013** miR-125b-5p **p=0.0196** miR-190a-5p **p=0.0032** miR-525-3p **p=0.0019** let-7d-5p **p=0.0208**
Fig 3b AC/C/G vs CT/T/C	2-way ANOVA	Tukey	Area 25 p=0.9857 Area 32 **p=0.0407**
Fig 4 Area 32	Linear regression	Holm-Sidak	miR-9-5p p=0.1609 miR-125a-5p p=0.0807 miR-125b-5p p=0.0544 miR-190a-5p p=0.2593 miR-525-3p **p=0.0024** let-7d-5p **p=0.0067** DCC **p=0.0078**
Fig 4 Area 25	Linear regression	Holm-Sidak	miR-9-5p p=0.3120 miR-125a-5p p=0.9690 miR-125b-5p p=0.7190 miR-190a-5p p=0.9690 miR-525-3p p=0.4831 let-7d-5p p=0.7976 DCC p=0.9918
Suppl. Fig 1g Area 17 vs Area 25	1-way ANOVA	Bonferroni	miR-195-5p **p<0.0001** miR-221-3p **p=0.0001** miR-222-3p **p<0.0001** miR-497-5p **p=0.0003**
Suppl. Fig 1g Area 17 vs Area 32	1-way ANOVA	Bonferroni	miR-195-5p **p=0.0006** miR-221-3p **p<0.0001** miR-222-3p **p=0.0002** miR-497-5p **p=0.0003**
Suppl. Fig 2a Area 17 AC/C/G vs CT/T/C	1-way ANOVA	Bonferroni	miR-195-5p p=0.8560 miR-221-3p p=0.9547 miR-222-3p p=0.8834 miR-497-5p p=0.7379
Suppl. Fig 2a Area 25 AC/C/G vs CT/T/C	1-way ANOVA	Bonferroni	miR-195-5p p=0.9379 miR-221-3p p=0.9982 miR-222-3p p=0.9854 miR-497-5p p=0.7991
Suppl. Fig 2a Area 32 AC/C/G vs CT/T/C	1-way ANOVA	Bonferroni	miR-195-5p p=0.7997 miR-221-3p p=0.9085 miR-222-3p p=0.3471 miR-497-5p p=0.6097
Suppl. Fig 2b Area 17 AC/C/G vs CT/T/C	1-way ANOVA	Bonferroni	miR-9-5p p>0.9999 miR-125a-5p p>0.9999 miR-125b-5p p>0.9999 miR-190a-5p p>0.9999 miR-525-3p p>0.9999 let-7d-5p p>0.9999

Suppl. Fig 3 Area 25 AC/C/G vs CT/T/C	2-way ANOVA	Tukey	EGFR p=0.7039 SLC1A3 p=0.8228 KMT2D p=0.9918 CAMK2Ap=0.9878 SLC618A2 p=0.7336 SLC6A6 p=0.5684 SLC6A9 p=0.9999 CDKN1 p=0.6346 SLC6A6 p=0.5684 KLF6 p=0.9601 MAP3K2 p=0.7949 BACE1 p=0.9620 SOD2 p=0.9969 CDK6 p=0.7595 MAPK14 p=0.9967 MAP2K7 p=0.9983 IPPK p=0.9572 DCC p=0.9857 GRIA3 p=0.9987 GRM7 p=0.9928 STAT2 p=0.9967 STAT3 p=0.9925 BCL2 p=0.6950 IGFR1 p=0.3373 PTBP1 p=0.9676 GABRA2 p=0.9904 GRIA2 p=0.9999 GRIA4 p=0.9789 NTRK3 p>0.9999 GRIN1 p=0.9899 GABRA1 p>0.9999 HTR3A p>0.9999 SLC32A1 p=0.9836 SLC6A7 p=0.9942 HEXB p=0.9974
Suppl. Fig 3 Area 32 AC/C/G vs CT/T/C	2-way ANOVA	Tukey	EGFR p=0.2937 SLC1A3 p=0.9828 KMT2D p>0.9999 CAMK2Ap=0.9999 SLC618A2 p=0.7540 SLC6A6 p=0.9966 SLC6A9 p=0.9908 CDKN1 p=0.9974 SLC6A6 p=0.9966 KLF6 p=0.9913 MAP3K2 p=0.9947 BACE1 p=0.9719 SOD2 p=0.9536 CDK6 p=0.9993 MAPK14 p=0.6375 MAP2K7 p=0.6603 IPPK p=0.6721 DCC **p=0.0407** GRIA3 p=0.9947 GRM7 p=0.9980 STAT2 p=0.9200 STAT3 p=0.5778

			BCL2 p>0.9999 IGFR1 p=0.9994 PTBP1 p=0.9376 GABRA2 p=0.9668 GRIA2 p=0.9102 GRIA4 p=0.8701 NTRK3 p=0.9828 GRIN1 p>0.9999 GABRA1 p=0.9995 HTR3A p=0.9936 SLC32A1 p=0.9716 SLC6A7 p=0.7708 HEXB p=0.9935
Suppl. Fig 4 Area 32 EFA1	Linear regression	Holm-Sidak	miR-9-5p p=0.2142 miR-125a-5p p=0.5080 miR-125b-5p p=0.3822 miR-190a-5p p=0.2593 miR-525-3p p=0.562 let-7d-5p p=0.6351 DCC p=0.3706
Suppl. Fig 4 Area 32 EFA2	Linear regression	Holm-Sidak	miR-9-5p p=0.2142 miR-125a-5p p=0.6341 miR-125b-5p p=0.3822 miR-190a-5p p=0.9167 miR-525-3p p=0.562 let-7d-5p p=0.6351 DCC p=0.3739