Supplemental Material

Animals

Animal grouping, mechanisms of drug administration, win-loss observations for rank and the distribution of ranks among the pens and treatment groups are shown below in **Figure A and Table A.**

Figure A. Illustration of animal housing and drug treatment. For year 1, the 6 animals that had been previously housed together were not expected to integrate with the animals from the corral. Therefore, they remained together in Pen A. The 14 animals from the corral were split between Pen B and Pen C. Illustration of Year 1 drug administration: [1] all animals were shifted to the bottom section of Pen A and the door was shut. [2] Each animal was individually shifted to the middle section of the pen, where a treat with the proper drug was administered and verified as eaten [3] the animal was then shifted to the top section of Pen A and the door was closed. Steps 1-3 were repeated for each animal until all of the animals were treated and gathered in the top section of Pen A. Then, the doors between sections were opened and the animals dispersed into all sections according to their preference. The entire procedure was repeated for Pens B and C.

For year 2, the remaining 10 animals were moved to a different housing area with indoor and outdoor access. Two of the animals that were originally in Pen A were assaulted when placed with any of the 8 remaining animals and they had to be moved immediately to adjacent caging. In year 2, all of the treatments were accomplished with subcutaneous implants.

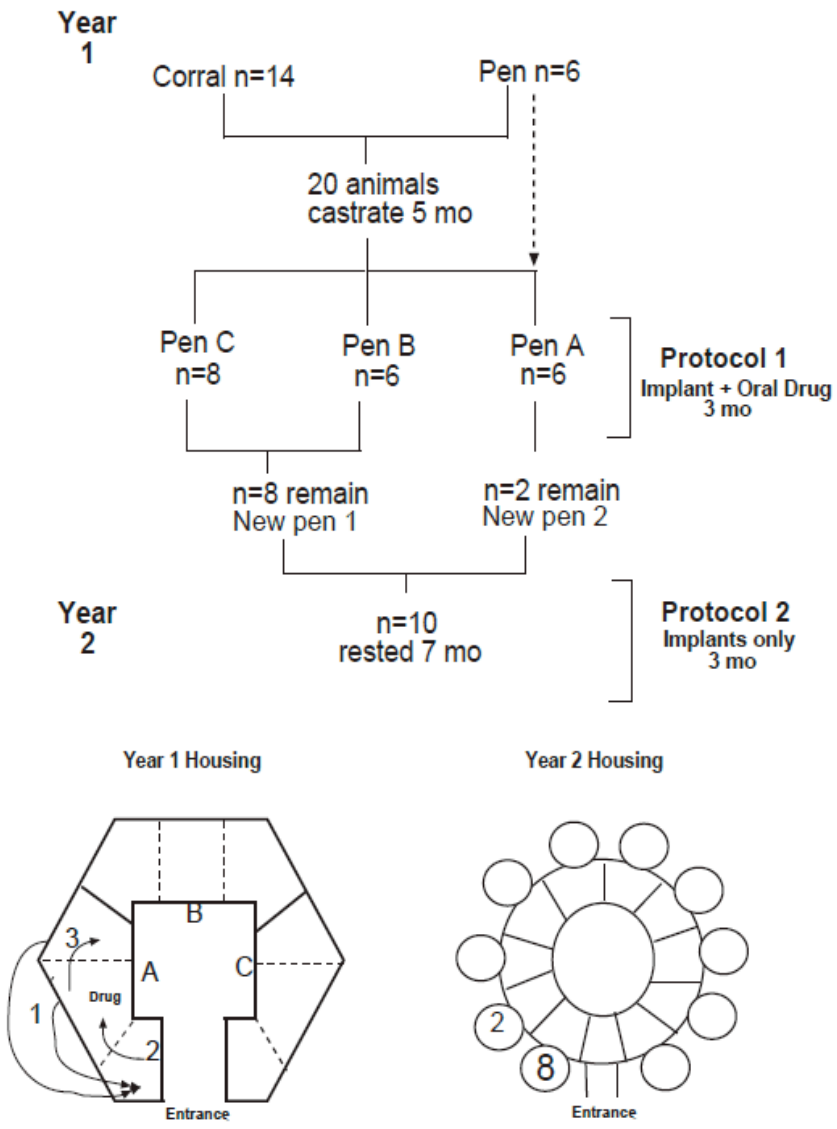


Figure A.

Table A. Rank, treatments and distribution of animals in the pens in year 1. Wins and losses from encounters were obtained from focal observations during the treatment period. Animals were trained to shift location, *one at a time*, to receive food treats with drug inserted.

Dominance results			
Pen A	ID	wins	losses
	24056	25	0
	24045	8	6
	22725	7	8
	23305	1	17
	25477	0	1
	25475	0	9
Pen B	ID	wins	losses
	24663	32	4
	24048	14	1
	24038	4	12
	24624	2	15
	24633	1	13
	24024	0	8
Pen C	ID	wins	losses
	23332	25	0
	25447	15	7
	23319	2	10
	21986	2	5
	24055	2	4
	25455	1	3
	23276	0	13
	25459	0	5

ID	Pen	Dominance	Takes Treats	Implant	Drug
22725	A	Mid	Yes	T	Dutersteride
23305	A	Mid	Yes	T	Letrazole
24045	A	Dom	No	Empty	0
24056	A	Dom	Yes	T	Dutersteride
25475	A	Sub	No	T	0
25477	A	Sub	Yes	T	Letrazole
24024	B	Sub	No	T	0
24038	B	Mid	Yes	T	0
24048	B	Dom	Yes	T	Dutersteride
24624	B	Sub	Yes	Empty	0
24633	B	Mid	Yes	Empty	0
24663	B	Dom	Yes	T	Letrazole
21986	C	Mid	Yes	Empty	0
23276	C	Mid	Yes	T	Dutersteride
23319	C	Mid	Yes	T	0
23332	C	Dom	Yes	T	0
24055	C	Mid	Yes	T	Letrazole
25447	C	Dom	Yes	Empty	0
25455	C	Sub	Yes	T	Letrazole
25459	C	Sub	Yes	T	Dutersteride

RT- PCR examination of gene expression

We sought preliminary evidence that the pivotal genes necessary for the responses of the animals in this study were expressed in the dorsal raphe. Dorsal raphe blocks were obtained from an intact male Japanese macaque. RNA was extracted with Trizol and purified using the Qiagen RNeasy mini kit (Velencia, CA). Complementary DNA (cDNA) synthesis was performed using Oligo-dT 15 primer (Invitrogen, Carlsbad, CA) and M-MLV reverse transcriptase (100 U/µg of RNA, Invitrogen) at 42°C for 1 hr. All PCR reactions were performed in a PTC-200 thermal cycler (MJ Research, Watertown, MA) in a total volume of 50 µl per reaction containing synthesized cDNA from 80 ng of the purified RNA, 1 X PCR buffer, 200 mM each of dNTPs (Invitrogen, Carlsbad, CA), 0.1 mM each of sense and anti-sense primers and 1 U of Platinum Taq DNA polymerase (Invitrogen). Thirty-nine cycles of amplification were performed for 30 sec, and at 72°C for 45 sec. At the end of the amplification cycles, each reaction was additionally incubated at 72°C for 10 min. The PCR products were separated on agarose gels and photographed for examination.

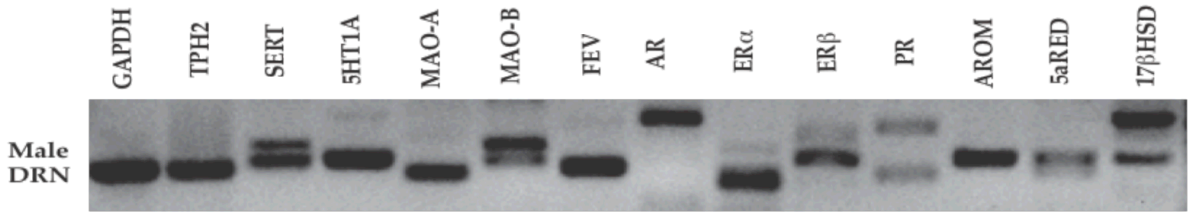


Figure B.

The dorsal raphe region of the male Japanese macaque exhibited gene expression for the serotonin-related genes, TPH2, SERT, 5HT1A autoreceptor, MAO-A, MAO-B and Fev (fifth Ewing variant), the serotonin master gene (Hendricks, Francis, Fyodorov, & Deneris, 1999). The dorsal raphe region also exhibits gene expression for AR, ERα, ERβ and PR (progestin receptor). The PR amplicon was expected at 250 bp corresponding to the bottom band. The higher band at 500 bp is a dimerization artifact. Finally, gene expression for 3 critical enzymes in steroid metabolism was detected: aromatase, 5α reductase and 17β hydroxysteroid dehydrogenase. The results are illustrated in **Figure B**. The primers for RT-PCR, based on human or monkey sequences, were obtained from Invitrogen Life Technologies and are shown below in **Table B.**

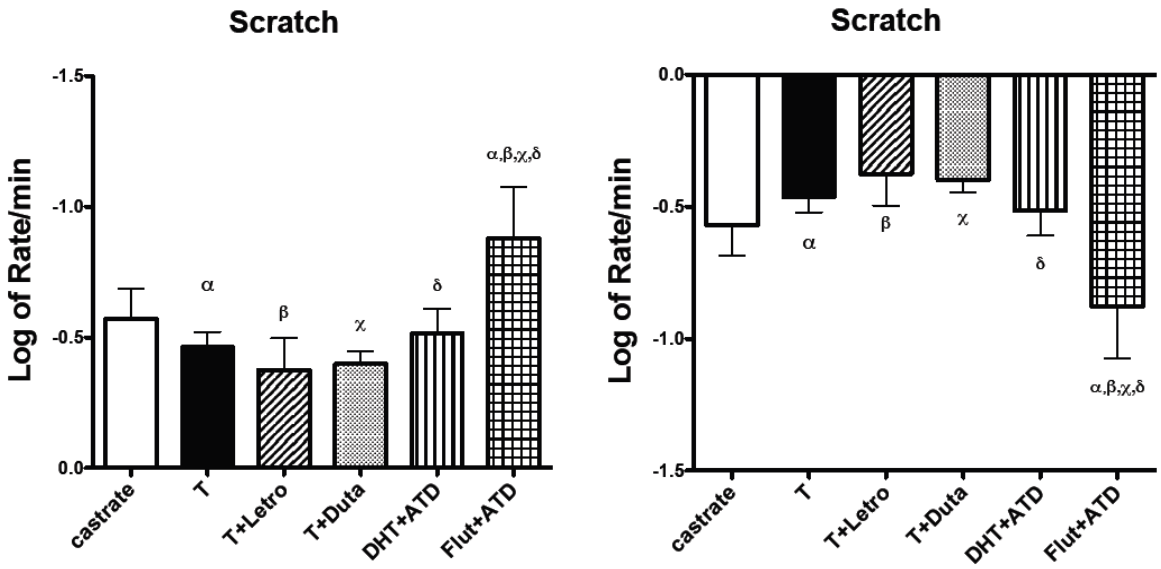
In summary, the pivotal genes needed for the fenfluamine results are apparent in this male dorsal raphe, preliminarily suggesting that macaque males likely express these genes in the dorsal raphe region.

Table B.	Primer sequences used in this study.		
	Accession ID	**Forward**	**Reverse**
mRNA			
Coding regions			
GAPDH	BC029340	F_CAT TGC CCT CAA CGA CCA C	R_CCC CTC CCC TCT TCA AGG
TPH2	AY_098914	F _CTGACACTGAGTACGTCGTGGA	R _TCCCAGTGACAGGAAGAG
SERT	EF126285	F_CAGAGGTGGTGTGGGTGACAG	R_GACAAATCCCGAAACGAAGCT
5HT1A	XM_001083407	F_ACCGGGTCCCTTTGAGACC	R_ACGTCCAGGGCGATAAACAG
MAO-A	XM_001096840	F_CCTCCTGGGATCATGACTCAA	R_AGGTTCCTCTCCCAGAAGGTG
MAO-B	XM_001096953	F_TGTGTGTCACTGCAGAGACCC	R_TTCATGCCCAGAGTAGGAGGA
Fev	XM_001095962	F_CAGAAAGGCAGCGGACAGAT	R_CCTGGAAGTCGAAGCGGTAG
AR	NM_001032911	F_AAGGCCAGTTGTATGGACCG	R_TTCCGAAGACGGCAAGATG
ERa	XM_001097228	F_ACCAACCAGTGCACCATTGA	R_GGCCGGGCTGTTCTTCTTA
ERb	XM_001101433	F_TGCGCTGTCTGCAGTGATTT	R_TGCCACTCCTCTTGGCCTT
PR-B	XM_001095317	F_AGGGCCTGCAGCAGGTCTA	R_TGCGGATTTTATCAACGATGC
Aromatase	NM_031226	F_ATTACAGCTCTCGATTCGGCA	R_GCACTTTCGTCCAAAGGGATC
5aReduct	XM_001083276	F_TCGGTGCTTAATTTACCCATTTC	R_TTGGCTGCAGTTACGTATTCAA
17bHSD	NM_001114958	F_CACAGGAGACGGTGCAGCT	R_CGACAACCACTGGACACCAG

Log Transformation of Scratch Data

The rate/min of the scratching in each group was log transformed and analyzed with ANOVA ($p = 0.06$, near significant difference). The ANOVA would not compute posthoc analysis with this probability. Therefore, the groups were examined with a multiple 'one sample' t test, which was allowed. A one-sample t test compares the mean of each group against a hypothetical mean that was set equal to the mean log of the Flut+ATD group. The P value answers this question: If the data were sampled from a Gaussian population with a mean equal to the hypothetical value entered, what is the chance of randomly selecting N data points and finding a mean as far (or further) from the hypothetical value as observed? If the P value is small (< 0.05), then it is unlikely that the discrepancy observed between sample mean and hypothetical mean is due to a coincidence arising from random sampling. The results are shown below (**Figure C**). The raw data indicates that scratching in Flut+ATD group was lower than the androgen-treated groups. With the log transformation, the scale increases in the *negative* direction. **In summary,** the Flut+ATD group had significantly less scratching than the groups treated with T or DHT (androgens).

Figure C.



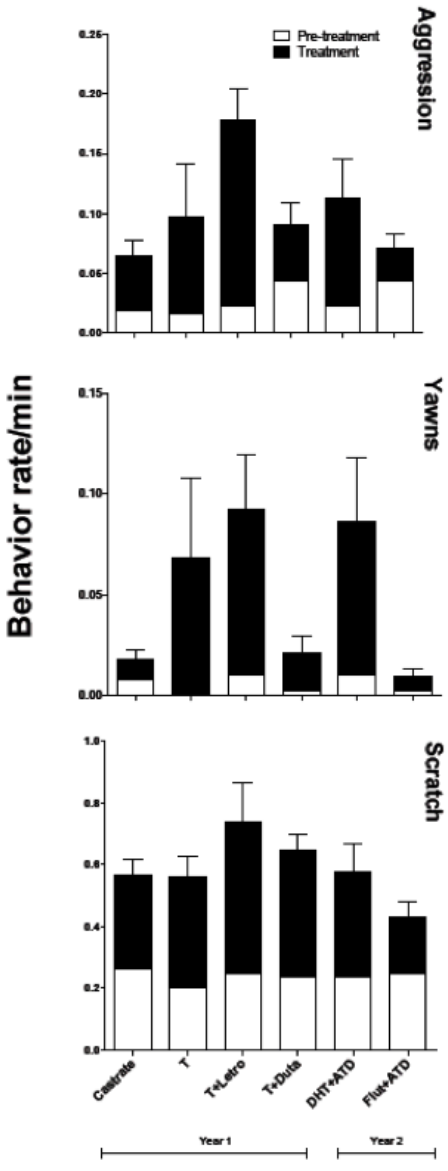


Figure D.

Comparison of Pre-treatment and Post-treatment behavior

Illustration of pre- and post-treatment behaviors in the male macaques. Two-way ANOVA found a significant difference in pre- and post-treatment of aggression ($p = 0.0004$), yawning ($p=0.0001$) and scratching ($p=0.005$).

Literature Cited

Hendricks, T., Francis, N., Fyodorov, D., & Deneris, E. S. (1999). The ETS domain factor Pet-1 is an early and precise marker of central serotonin neurons and interacts with a conserved element in serotonergic genes. *J Neurosci, 19*(23), 10348-10356.