Serotonin Transporter Binding Potentials in Brain of Juvenile Monkeys One Year After Discontinuation of a Two-Year Treatment With Fluoxetine

SUPPLEMENTARY INFORMATION

Table of Contents

List of acronyms in text

3DRP—3 dimensional reprojection
5HT--serotonin
5HTTLPR--serotonin transporter gene polymorphism
ADHD -attention deficit hyperactivity disorder
ANOVA--analysis of variance
ANS --advanced normalization tools
ASD-autism spectrum disorder
BBA--BioBehavioral Assessment
Beta CIT--Iodine-123-beta-carbomethoxy-3 beta-(4-iodophenyltropane)
BP--binding potential
CNPRC--California National Primate Research Center
DA--dopamine
DASB--3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile
FDR--false detection rate
FSPGR-- fast spoiled gradient echo
GBq--gigabequerel
LL/SL/SS --allele based genotypes for 5HTTLPR; L=long, S=short variant allele
MAO—monoamine oxidase
MAOA---isoform of MAO
MAOB--isoform of MAO
MBq--megabecquerel
MRI--magnetic resonance imaging
NE--norepinephrine
PET--positron emission tomography
ROI--region of interest
SERT-serotonin transporter
SRTM-s-imple reference tissue model
SSRI--selective serotonin reuptake inhibitor
UCDavis--University of California Davis
uVNTR—upstream variable number of tandem repeats

Table S1. Technical details for PET and MRI scans

[^{11}C]DASB synthesis	Cyclotron	Siemens RDS111
	Synthesis software	Tracerlab FXC Pro automated synthesis module (GE)
	Purity	>95%
	Specific activity EOS	48.5 to 273 Ci/µmol
	Specific activity at injection	22.9 to 117 Ci/µmol
PET scanning	Scanner	Siemens Primate 4 (P4) microPET system
	Spatial resolution	1.8 mm
	Duration	90 min
	Ketamine-scan interval	70 min
PET analysis	software	PMOD 3.802
	BP measurement	Logan reference method
MRI scanning	Scanner	GE 1.5T Signa HDx 16.0 scanner
	Coil	HD TRknee PA
	Sequence	T1 3D FSPGR
	FOV	16 cm
	TR	6.8 msec
	TE	3.3 msec
	ET	1 msec
	FA	12
	Slice thickness	1mm
	Slice spacing	-0.5mm
	NEX	4
	RBw	31.25 Hz
MRI ROI mapping	Atlas	Paxinos
	Template alignment	ANTS
	Transformation model	SyN, stepsize 0.10

FSPGR - fast spoiled gradient echo
FOV - field of view
TR - repetition time
TE - echo time
FA - flip angle
NEX - number of excitations
RBw - receive bandwidth
ET - echo-train
ANTS – advanced normalization tools

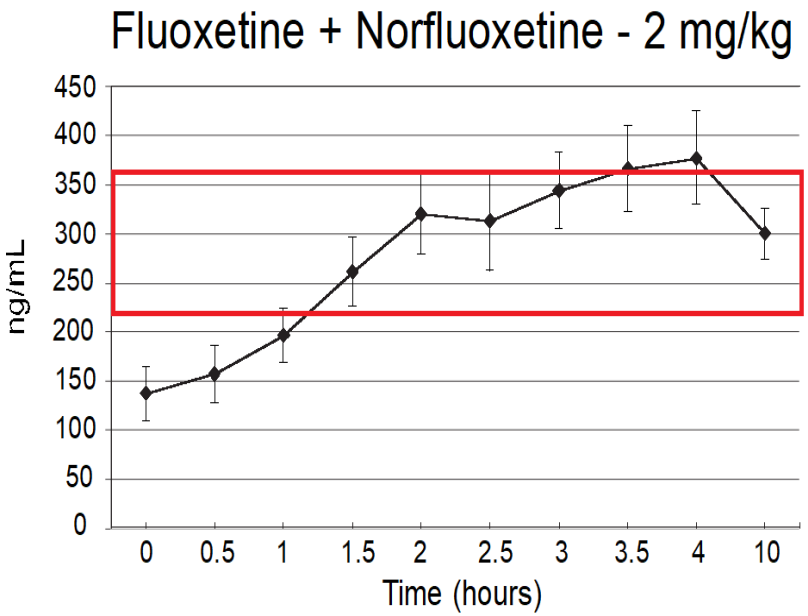

Figure S1. Time course of active agent (fluoxetine + norfluoxetine) after a single oral administration of 2 mg/kg fluoxetine to juvenile male rhesus. Data from a preliminary pharmacokinetic study[1]. Red lines indicate range of active agent in children 6-11 years of age monitored during treatment with fluoxetine[2]. In children, a 2 mg/kg/d dose would be achieved with a 40 mg/day dose of fluoxetine in a 6 y.o. boy or an 80 mg/day dose in an 11 y.o. boy. Plasma levels at the end of dosing in the main study were 273±31 ng/ml. Similar levels of active agent have been reported at this dose in adult rhesus[3].

[1]Golub MS, Hogrefe CE. Fluoxetine: juvenile pharmacokinetics in a nonhuman primate model. Psychopharmacology (Berl). 2014 Oct;231(20):4041-7.

[2]Wilens TE, Cohen L, Biederman J, Abrams A, Neft D, Faird N, Sinha V. Fluoxetine pharmacokinetics in pediatric patients. J Clin Psychopharmacol 2002 22:568–575.

[3]Fontenot MB, Musso MW, McFatter RM, Anderson GM. Dose-finding study of fluoxetine and venlafaxine for the treatment of self-injurious and stereotypic behavior in rhesus macaques (Macaca mulatta). J Am Assoc Lab Anim Sci. 2009 48(2):176-84.

Table S2. Fluoxetine interactions with MAOA genotype during dosing.

Domain	**Fluoxetine*MAOA genotype interaction**	**Reference**
Activity & Sleep	Fluoxetine increase in sleep fragmentation was greater in the high-MAOA subjects.	(1)
Social Interaction	In the fluoxetine group, more behavior invitations and initiations were seen in the high-MAOA subjects than the low-MAOA subjects.	(2)
Emotional Response to Pictures	Fluoxetine decreased emotional response in the low-MAOA group.	(3)
Short Term Memory test	Fluoxetine decreased the number of trial initiations in the high-MAOA group.	(4)

1. Golub MS, Hogrefe CE (2016): Sleep disturbance as detected by actigraphy in pre-pubertal juvenile monkeys receiving therapeutic doses of fluoxetine*. Neurotoxicol Teratol*, 55: 1-7.

2. Golub MS, Hogrefe CE, Bulleri AM (2016): Peer social interaction is facilitated in juvenile rhesus monkeys treated with fluoxetine*. Neuropharmacology*, 105: 553-560.

3. Golub MS, Hogrefe CE, Bulleri AM (2016): Regulation of emotional response in juvenile monkeys treated with fluoxetine: MAOA interactions*. Eur Neuropsychopharmacol*, 26(12): 1920-1929.

4. Golub MS, Hackett EP, Hogrefe CE, Leranth C, Elsworth JD, Roth RH (2017): Cognitive performance of juvenile monkeys after chronic fluoxetine treatment*. Dev Cogn Neurosci*, 26: 52-61.

Picture-elicited Emotional Response protocol

(from Golub MS, Hogrefe CE, Bulleri AM (2016): Regulation of emotional response in juvenile monkeys treated with fluoxetine: MAOA interactions*. Eur Neuropsychopharmacol*, 26(12): 1920-1929.)

Emotional responsiveness to pictures with varying affective content was assessed one year after the conclusion of dosing (four years of age). A series of eight pictures were presented on a monitor via a PowerPoint slide show. Animals were transferred to a familiar test cage with a clear plexiglass front and placed approximately 40 cm away from the video monitor in a darkened room. A video camera and light placed above the monitor recorded the session for later coding of behavior. Each slide was presented for 30 s followed by a 1 min interslide interval of a black screen. Behavior was coded during the slide presentations. The eight slides were: a plain light green colored slide; fruit (apple slice and half peeled banana); a snake; a cage (identical to the home cage); an adult male monkey with an open mouth stare; a mother and infant monkey; two monkeys grooming; and a technician dressed in protective clothing wearing leather gauntlet/hand catching gloves.

Videos were scored with The Observer (Noldus Information Technology, Wageningen, The Netherlands) using an ethogram that with a number of expressive behaviors including facial expressions, vocalizations and simple behaviors known to reflect emotional response in rhesus. All videos were scored blind by the same observer (AMB), with an average intra-observer reliability of 89%.

Pictures

Picture #	*Code*	*Definition*
1	p1	Two monkeys grooming
2	p2	Aggressive monkey in tree
3	p3	Green control blank
4	p4	Banana and apple
5	p5	Snake
6	p6	Mother with nursing infant
7	p7	Cage

Ethogram used to score videotapes

Behavior	*Code*	*Definition*
Fear Grimace	fg	A wide “smile” or grin with the teeth showing
Crouch	cr	Chest is near the ground, limbs are flexed, head is below the shoulders
Lipsmack	lp	Rapid movement of pursed lips, usually accompanied by a “kissing” or smacking sound
Threat	th	One or more of the following: open mouth stare, head bob, ear flap, cage shake, lunge
Grunt	gt	Soft, guttural, bubbly vocalization, generally affiliative in nature
Bark	br	Gruff, low-pitched vocalization
Scream	sm	Loud, high-pitched shrieking distress vocalization
Coo	co	Medium pitched, clear call
Yawn	ya	Self explanatory
Scratch	sc	Self explanatory
Convulsive jerk	cj	Sudden whole body shaking or convulsive contractions
Self clasp	cl	Placing a hand or foot on another limb or fur
Cage shake	cs	Shaking cage or moving/displacing the test box
Other	ot	Any special situation that should be recorded
Tooth grind	tg	Loud gnashing of teeth
Rump present	rp	Animal stands still, lifts tail and presents rump to observer or other animal
Self groom	gr	Animal grooming self
Self inspect	si	Animal touching genitals