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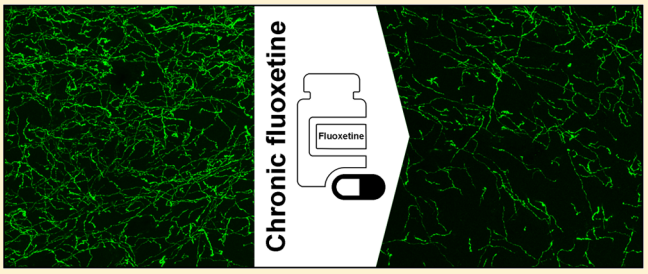
Fluoxetine Induces Morphological Rearrangements of Serotonergic Fibers in the Hippocampus

Serena Nazzi,[†] Giacomo Maddaloni,[†,∥] Marta Pratelli,[†,§] and Massimo Pasqualetti*[,†,‡]

[†]Department of Biology, Unit of Cell and Developmental Biology, University of Pisa, 56127 Pisa, Italy
[‡]Istituto Italiano di Tecnologia, Center for Neuroscience and Cognitive Systems, 38068 Rovereto, Italy

Supporting Information

ABSTRACT: Serotonin (5-HT)-releasing fibers show substantial structural plasticity in response to genetically induced changes in 5-HT content. However, whether 5-HT fibers appear malleable also following clinically relevant variations in 5-HT levels that may occur throughout an individual's life has not been investigated. Here, using confocal imaging and 3D modeling analysis in $Tph2^{GFP}$ knock-in mice, we show that chronic administration of the antidepressant fluoxetine dramatically affects the morphology of 5-HT fibers innervating the dorsal and ventral hippocampus resulting in a reduced density of fibers. Importantly, GFP fluorescence levels appeared unaffected in the somata of both dorsal and median *raphe* 5-HT neurons, arguing against potential fluoxetine-mediated down-regulation of the *Tph2* promoter driving GFP expression in the $Tph2^{GFP}$ mouse model. In keeping with this notion, mice bearing the pan-serotonergic driver *Pet1-Cre* partnered with a Cre-responsive tdTomato allele also showed similar morphological alterations in hippocampal 5-HT circuitry following chronic fluoxetine treatment. Moreover 5-HT fibers innervating the cortex showed proper density and no overt morphological disorganization, indicating that the reported fluoxetine-induced rearrangements were hippocampus specific. On the whole, these data suggest that 5-HT fibers are shaped in response to subtle changes of 5-HT homeostasis and may provide a structural basis by which antidepressants exert their therapeutic effect.



KEYWORDS: *serotonin, serotonergic fibers, fluoxetine, hippocampus, plasticity*

INTRODUCTION

Neurons that synthesize brain serotonin (5-HT) regulate diverse physiological and behavioral functions in health and disease. Such modulatory role stems, at least in part, from an elaborate network of long-range projections that collectively innervate the entire central nervous system.[1]

During postnatal development, 5-HT fibers undergo plastic remodeling as transient increase in innervation followed by fiber pruning has been described in various target districts, including the hippocampus.[2,3] Under nonphysiological circumstances such as physical or chemical lesion to the brain, 5-HT fibers may display morphological rearrangements also during adulthood, suggesting retention of plasticity.[4−7] Intriguingly, 5-HT fibers appear highly malleable also in response to changes in 5-HT homeostasis with 5-HT itself acting as an autocrine signal.[8,9] Indeed, genetic inactivation of tryptophan hydroxylase 2 (*Tph2*) leading to lifelong abrogation of 5-HT synthesis, as well as its selective inactivation during adulthood, result in altered density of 5-HT fibers within various targets, with the hippocampus displaying the highest level of plasticity. In particular, the density of hippocampal 5-HT fibers is dramatically increased in both genetic models.[10] Remarkably, those abnormalities are rescued following pharmacological reestablishment of 5-HT synthesis, highlighting an unexpected degree of adult plasticity in response to variations of 5-HT levels.[9] However, whether serotonergic fibers can be remodeled in response to fluctuating 5-HT levels within the peri-physiological range is so far unexplored.

Selective serotonin reuptake inhibitors (SSRIs), the most prescribed antidepressant class worldwide, act by inhibiting presynaptic serotonin reuptake likely elevating synaptic 5-HT.[11] Here, we explore whether chronic treatment with the SSRI fluoxetine is able to promote structural changes in 5-HT fibers innervating the hippocampus. $Tph2^{GFP}$ knock-in mice were used as a tool to highlight fiber morphology and innervation pattern independently of serotonergic immunoreactivity. We show that chronic fluoxetine exposure dramatically reduces the density of 5-HT axons and promotes intrinsic morphological rearrangements, including shrinkage of axonal diameter. These results further highlight the plasticity of 5-HT axons in response to chronic pharmacologically induced changes in 5-HT homeostasis and provide a previously unreported structural effect of antidepressants on the 5-HT circuitry.

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RESULTS AND DISCUSSION

Genetic depletion of 5-HT dramatically increases serotonergic fiber density in the hippocampus, which is normalized by

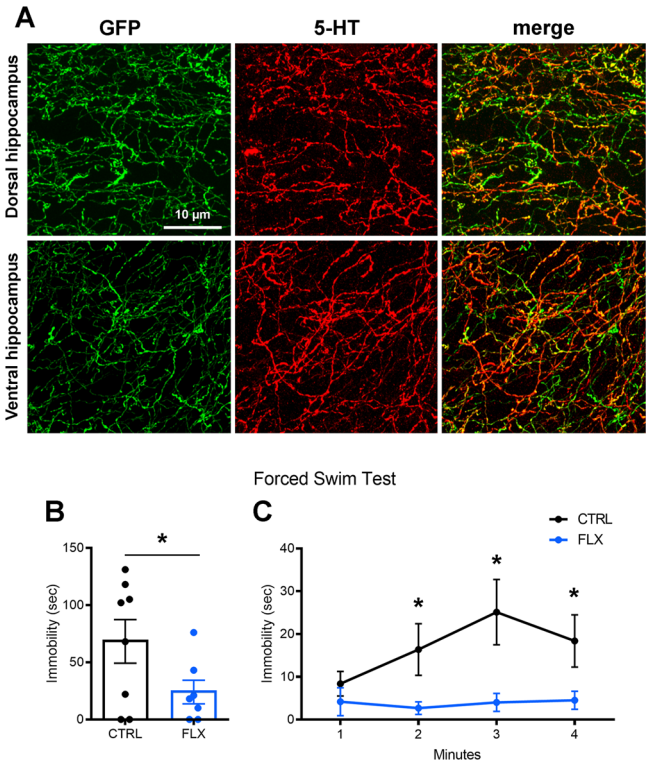

Figure 1. *Tph2*^{GFP} mice as a powerful model to investigate FLX effect on 5-HT circuitry. (A) Representative high magnification confocal images of 5-HT fibers in the dorsal and ventral hippocampus of *Tph2*^{GFP} mice showing GFP and 5-HT colocalization. (B) Immobility time in the FST (CTRL, $n = 8$; FLX, $n = 7$; one-tailed unpaired t test, $t(13) = 0.0361$). (C) Average time spent immobile during each minute of the FST (CTRL, $n = 8$; FLX, $n = 7$; one-tailed unpaired t test, minute 1, $t(12) = 0.9624$, $p = 0.1774$; minute 2, $t(12) = 1.9116$, $p = 0.0397$; minute 3, $t(12) = 2.321$, $p = 0.0193$; minute 4, $t(12) = 1.891$, $p = 0.0415$). *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001. FST, forced swim test; CTRL, control mice; FLX, fluoxetine-treated mice; n indicates biological replicates.

pharmacological rescue of 5-HT signaling,[8,9] suggesting bidirectional plasticity of 5-HT axons in response to changes in 5-HT levels. Whether serotonergic fiber morphology is malleable also in response to clinically relevant fluctuations of 5-HT levels, which may be induced pharmacologically and commonly experienced during an individual's life, is unknown. If so, based on our previous findings, we predicted that presumptive elevations in 5-HT levels would result in a decrease of serotonergic innervation to the hippocampus.[8,9] To test this hypothesis, we chronically treated adult *Tph2*^{GFP} knock-in heterozygous male mice with the antidepressant fluoxetine (FLX).

Tph2^{GFP} mice selectively express GFP in serotonergic cell bodies and fibers allowing an accurate analysis of the serotonergic wiring of the brain independently of serotonergic immunoreactivity (Figure 1A), as well as without interfering with serotonergic development and neurotransmission.[8] Moreover, *Tph2*^{GFP} heterozygous mice show unambiguous colocalization of GFP and SERT immunoreactivity in hippocampal serotonergic axons (Figure S1A) and comparable SERT

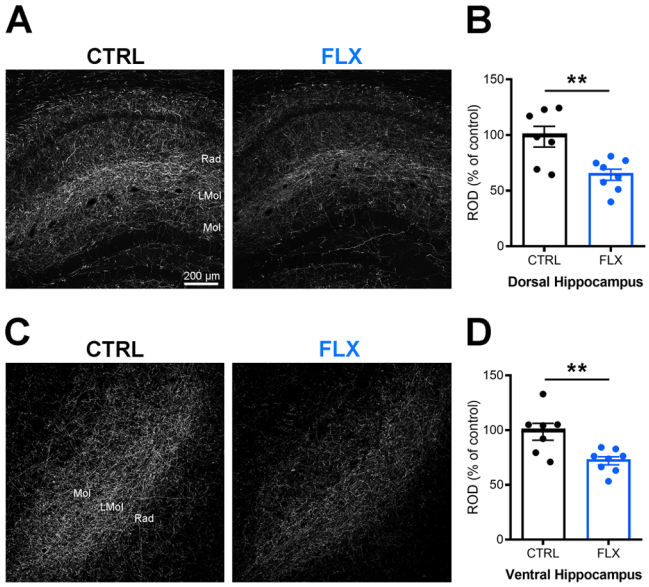

Figure 2. Altered serotonergic innervation to the hippocampus in fluoxetine-administered mice. (A) Representative images of GFP immunofluorescence of serotonergic fibers in the dorsal hippocampus in control animals and FLX-treated animals. (B) Average ROD measurement of the 2 cohorts in the dorsal hippocampus (CTRL, $n = 7$; FLX, $n = 8$; two-tailed unpaired t test, $t(13) = 3.344$, $p = 0.0053$). (C) Representative images of GFP immunofluorescence of serotonergic fibers in the ventral hippocampus in control animals and FLX-treated animals. (D) Average ROD measurement of the 2 cohorts in the ventral hippocampus (CTRL, $n = 7$; FLX, $n = 8$; two-tailed unpaired t test, $t(13) = 3.240$, $p = 0.0064$). CTRL, control mice; FLX, fluoxetine-treated mice; ROD, relative optical density; Mol, stratum moleculare; LMol, stratum lacunosum moleculare; Rad, stratum radiatum; *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001; n indicates biological replicates.

immunoreactivity to what is observed in wild-type littermates (Figure S1B,C), and they appear undistinguishable when tested in the FST (Figure S1D). These data support the use of *Tph2*^{GFP} heterozygous animals as a valuable tool for studying serotonergic axons and the impact that antidepressants may have on their morphology and organization.

Tph2^{GFP} mice were administered FLX in drinking water for 28 days, and water intake was monitored on a daily basis in order to individually adjust fluoxetine concentration to ensure that all subjects were administered 16 (mg/kg)/day of fluoxetine.[12] Oral administration has several advantages: it mimics human FLX intake route, it ensures continuous administration, and it prevents stressful events for the animal as compared to intraperitoneal administration. Chronic administration was selected as morphological changes within serotonergic innervation likely require a certain amount of time to occur. This idea is supported by our previous findings employing conditional *Tph2* knockout mice, in which the appearance of morphological changes in the hippocampal 5-HT fibers was detected 30 days but not 14 days after *Tph2* conditional gene inactivation.[9] That said, we selected treatment dose (16 (mg/kg)/day) and duration (28 days) that have been previously demonstrated to exert antidepressant effects in C57/Bl6 mice,[12] the mouse strain employed in the present study.

At the end of the 4 weeks of treatment, we used the Porsolt forced swim test (FST) to assess the behavioral immobility of FLX-administered mice as a proxy for the efficacy of FLX

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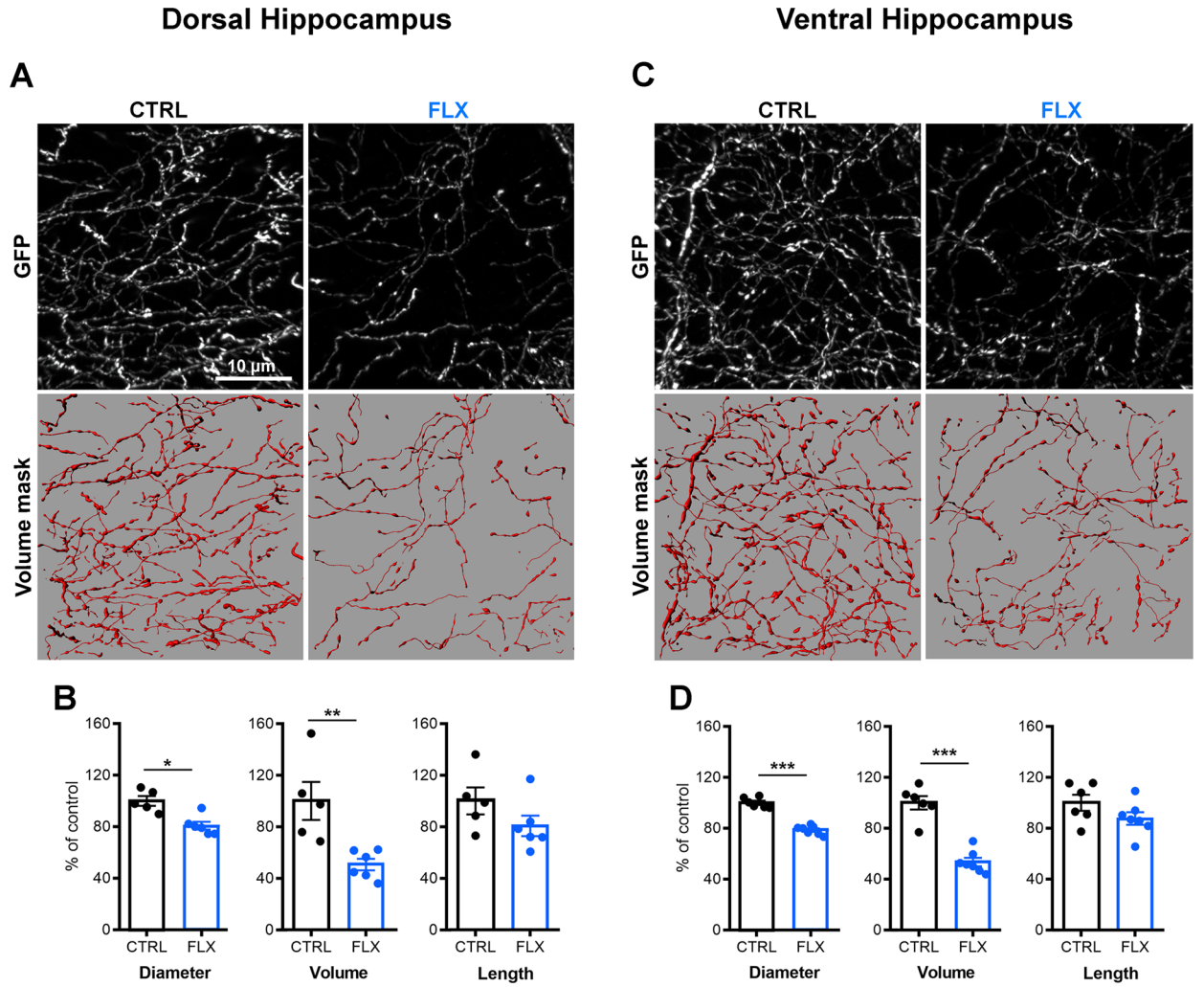

Figure 3. Three-dimensional quantitative analysis of axon morphology in the LMol layer of the hippocampus of FLX-administered *Tph2*^{GFP} mice. (A) Representative images of serotonergic innervation in the LMol layer of the dorsal hippocampus and their 3D reconstruction (CTRL, n = 5; FLX, n = 6). (B) Graphs showing the 3 morphological parameters considered: diameter (two-tailed unpaired t test, $t(9)$ = 4.033, p = 0.0030), volume (two-tailed unpaired t test, $t(9)$ = 3.479, p = 0.0069), and length (two-tailed unpaired t test, $t(9)$ = 1.507, p = 0.1662) of the fibers in the LMol layer of the dorsal hippocampus. (C) Representative images of serotonergic innervation in the LMol layer of the ventral hippocampus and their 3D reconstruction (CTRL, n = 6; FLX, n = 7). (D) Graphs showing the 3 morphological parameters considered: diameter (two-tailed unpaired t test, $t(11)$ = 10.23, p = < 0.0001), volume (two-tailed unpaired t test, $t(11)$ = 7.712, p < 0.0001), and length (two-tailed unpaired t test, $t(11)$ = 1.560, p = 0.1470) of the fibers in the LMol layer of the ventral hippocampus. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001. CTRL, control mice; FLX, fluoxetine-treated mice; n indicates biological replicates.

treatment. FLX-administered mice (FLX) showed reduced immobility time as compared to controls (CTRL; Figure 1B,C). When analyzed in separate 1 min time bins, FLX-administered mice showed significantly reduced immobility in the last 3 min of the test, validating the efficacy of the treatment (Figure 1C).

We next investigated whether FLX administration impacts serotonergic circuitry, focusing on 5-HT fibers innervating the hippocampus. This brain region represents an important area to investigate for two reasons. First, hippocampal 5-HT fibers appeared particularly susceptible to changes in 5-HT levels induced via genetic manipulations.[8,9] Second, antidepressants, including fluoxetine, exert their therapeutic effect by promoting plastic changes in cellular and functional properties of the hippocampus itself, including adult neurogenesis.[13,14]

Remarkably, the density of serotonergic fibers appeared clearly reduced in the hippocampus of FLX-administered mice as compared to control (Figure 2A,C). This change appeared evident in both the dorsal (Figure 2A) and ventral (Figure 2C) hippocampus, and it was corroborated by relative optical density (ROD) measurements (Figure 2B,D). These data suggest that FLX treatment may reduce the density of serotonergic axons in the hippocampus.

To investigate the nature of these changes, we performed a 3D reconstruction and morphological analysis of GFP+ serotonergic fibers present in the stratum lacunosum moleculare (LMol) of the dorsal and ventral hippocampus. Results revealed that FLX treatment dramatically induced shrinkage of serotonergic fibers, as shown by a clear reduction in fiber mean diameter as compared to controls in both hippocampal areas (Figure 3A,B dorsal hippocampus; Figure 3C,D ventral hippocampus). Moreover, fibers displayed a trend, albeit nonsignificant, toward a reduction in their total length in FLX-administered as compared to control mice (Figure 3B,D). In line, the mean volume of 5-HT fibers in the analyzed volume, which depends on fiber thickness and length,

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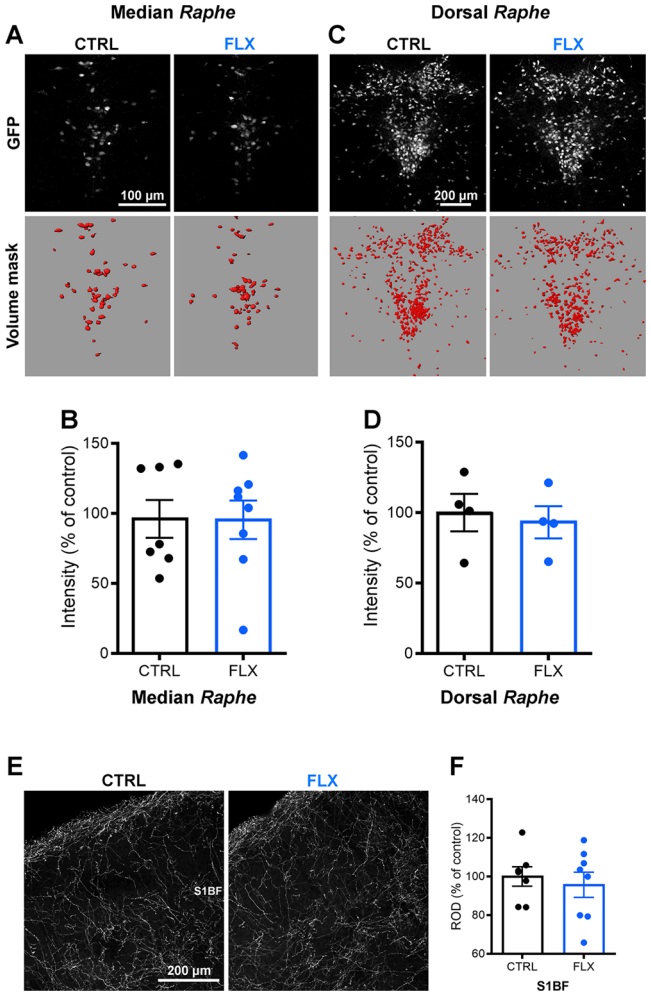

Figure 4. FLX treatment does not alter the expression of endogenous GFP and does not change the serotonergic innervation of the somatosensory cortex. (A) Representative images of the endogenous GFP signal in the cell bodies of the serotonergic neurons in the median *raphe* and volume mask used to delimit the regions of interest in which the signal was measured. (B) Average fluorescence intensity for the 2 cohorts (CTRL, $n = 7$; FLX, $n = 7$; two-tailed unpaired t test, $t(13) = 0.03298$, $p = 0.9742$). (C) Representative images of the endogenous GFP signal in the cell bodies of the serotonergic neurons in the dorsal *raphe* and volume mask used to delimit the regions of interest in which the signal was measured. (D) Average fluorescence intensity for the 2 cohorts (CTRL, $n = 4$; FLX, $n = 4$; two-tailed unpaired t test, $t(6) = 0.0396$, $p = 0.7075$). (E) Representative images of serotonergic innervation in the S1BF. (F) Average ROD measurement of the 2 cohorts (CTRL, $n = 7$, FLX; $n = 8$; two-tailed unpaired t test, $t(13) = 0.5076$, $p = 0.6203$). *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001. CTRL, control mice; FLX, fluoxetine-treated mice; S1BF, somatosensory cortex barrel field; ROD, relative optical density; n indicates biological replicates.

was strongly reduced in FLX-administered mice (Figure 3B, D), suggesting the presence of fewer axons with smaller diameter.

An underlying assumption of this experiment is that FLX does not impact the transcriptional control of the endogenous *Tph2* locus within serotonergic neurons, as this would affect GFP expression. To rule out the possibility that the observed changes in the density of GFP were due to a FLX-induced down-regulation of GFP expression, we measured the intensity of endogenous live GFP within the soma of serotonergic neurons of the median and dorsal *raphe* nuclei, which are known to project to the hippocampus.[15] Fluorescence intensity measurements performed within single serotonergic cells failed to reveal significant alterations in the expression of GFP between fluoxetine-administered animals and control mice in both median and dorsal *raphe* nuclei (Figure 4A–D). Moreover, the number of GFP+ cells did not differ between FLX-administered mice and control mice, in both *raphe* nuclei (Figure S2). As an additional control, in the same animals, we analyzed serotonergic innervation to the primary somatosensory cortex barrel field (S1BF), a region where no alterations in serotonergic fiber densities were detected following either constitutive or adult-induced genetic disruption of brain 5-HT synthesis.[8,9] We did not observe differences in patterns of serotonergic fiber innervation between FLX-administered and control mice, as further confirmed by ROD measurements that showed comparable fiber densities (Figure 4E,F).

As a further control, trans-heterozygous mice with Cre recombinase expression under the control of the pan-serotonergic driver *Pet1* (*Pet1-Cre*)[16] and expressing the Ai65(RCFL-tdT)Δ-FRT (in short *Pet1-Cre/tdTom* mice) were also chronically treated with FLX (i.e., 28 days, 16 (mg/kg)/day). In keeping with what we observed in $Tph2^{GFP}$ animals, FLX-treated *Pet1-Cre/tdTom* mice also showed reduced volume, diameter, and length of 5-HT axons in both the dorsal and ventral hippocampus (Figure 5A,B dorsal hippocampus; Figure 5C,D ventral hippocampus), as compared to controls. As tdTomato expression in these animals is under the control of the endogenous Gt(ROSA)26Sor promoter/enhancer regions, this experiment provides further evidence that chronic FLX treatment potently induces morphological rearrangements of serotonin fibers in the hippocampus.

Overall, we showed that chronic FLX treatment reduced hippocampal 5-HT axon diameter and density. No changes in GFP expression were detected in median and dorsal *raphe* 5-HT neuron cell bodies nor in 5-HT fibers innervating the cortex, arguing against potential fluoxetine-mediated down-regulation of GFP expression and identifying a region-specific phenomenon. Supporting this notion, we detected similar structural changes in FLX-treated transgenic animals in which the transcriptional regulation of the reporter tdTomato was under the control of the endogenous Gt(ROSA)26Sor promoter/enhancer regions, thus further strengthening our findings. As predicted, hippocampal 5-HT axons can undergo bidirectional structural rearrangements in response to extracellular 5-HT level fluctuations. The present results, along with our previous findings employing genetic manipulations,[8,9] likely suggest the presence of an autoregulatory mechanism according to which certain 5-HT neurons innervating specific brain targets are able to sense extracellular 5-HT levels and to adjust the density of their fibers, thus tuning the amount of neurotransmitter releasing sites, accordingly. This has, among others, two important implications. First, the region specificity of the observed effects argues in favor of divergent subcellular "sensing" machinery among different 5-HT neurons, which may add novel features to the increasingly emerging transcriptional and functional diversity of 5-HT neurons.[17−21] Moreover, albeit lacking functional measures, our data challenge the simplistic view according to which SSRIs boost serotonergic tone. Rather, they promote plastic events even within the serotonergic circuitry that are only beginning to be understood. Finally, whether such "sensing" mechanism is altered in

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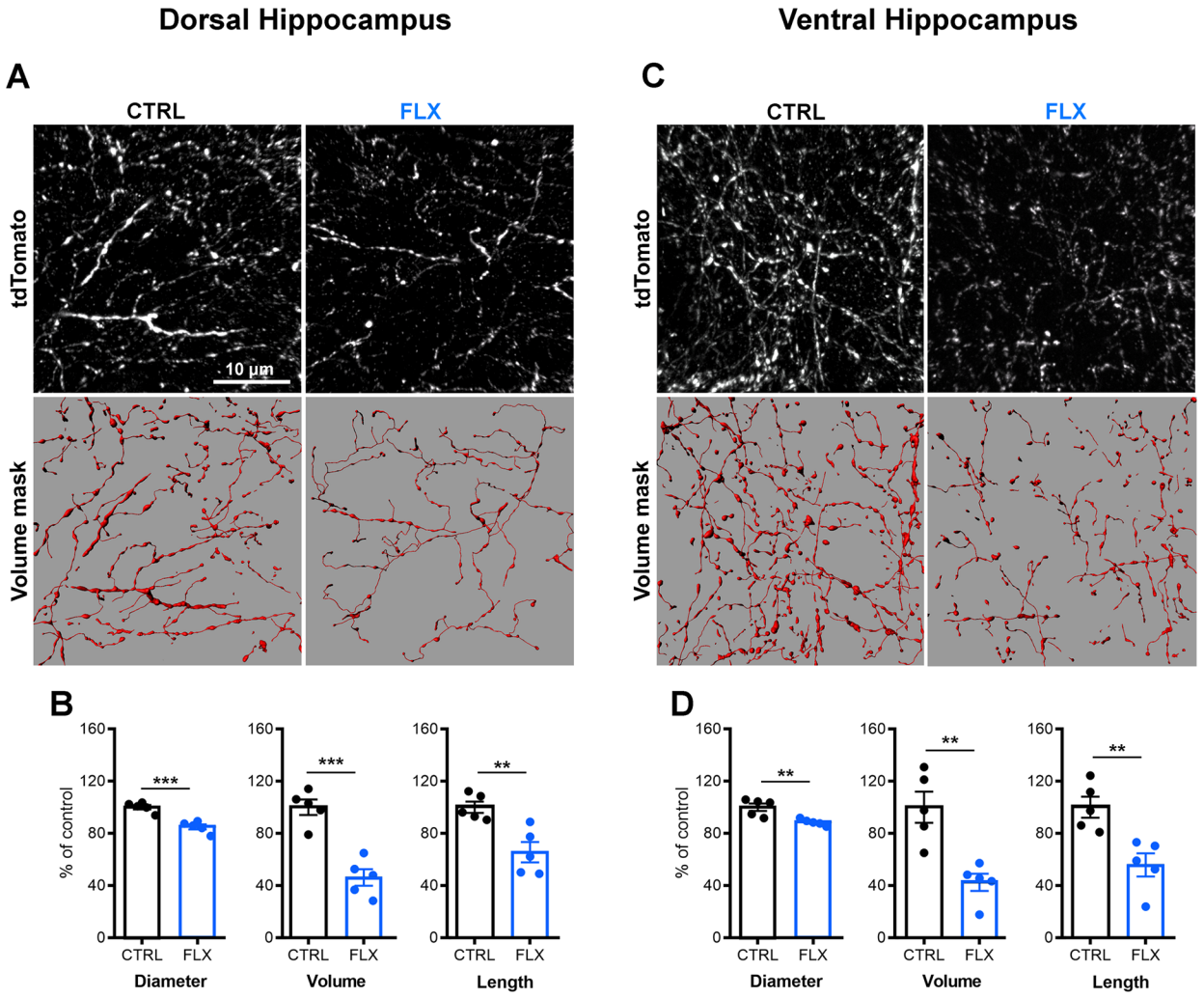

Figure 5. Three-dimensional quantitative analysis of axon morphology in the LMol layer of the hippocampus of FLX-administered *Pet1-Cre/tdTom* mice. (A) Representative images of serotonergic innervation in the LMol layer of the dorsal hippocampus and their 3D reconstruction (CTRL, n = 5; FLX, n = 5). (B) Graphs showing the 3 morphological parameters considered: diameter (two-tailed unpaired t test, $t(8) = 5.865$, $p = 0.0004$), volume (two-tailed unpaired t test, $t(8) = 6.324$, $p = 0.0002$), and length (two-tailed unpaired t test, $t(8) = 3.877$, $p = 0.0047$) of the fibers in the LMol layer of the dorsal hippocampus. (C) Representative images of serotonergic innervation in the LMol layer of the ventral hippocampus and their 3D reconstruction (CTRL, n = 5; FLX, n = 5). (D) Graphs showing the 3 morphological parameters considered: diameter (two-tailed unpaired t test, $t(8) = 3.757$, $p = 0.0056$), volume (two-tailed unpaired t test, $t(8) = 4.234$, $p = 0.0029$), and length (two-tailed unpaired t test, $t(8) = 3.711$, $p = 0.0059$) of the fibers in the LMol layer of the ventral hippocampus. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001. CTRL, control mice; FLX, fluoxetine-treated mice; n indicates biological replicates.

disease involving 5-HT signaling constitutes an intriguing area of research.

In conclusion, this work further highlights the plasticity of 5-HT axons in response to peri-physiological and commonly experienced changes in 5-HT homeostasis and provides a previously unreported structural effect of antidepressants on the 5-HT circuitry.

METHODS

Animals and Pharmacological Treatment. Control and FLX-administered mice were individually housed in conventional plexiglass cages in standard conditions with a 12h light/dark cycle at constant temperature of 21−23 °C with food and water available *ad libitum*. FLX-administered mice were treated with fluoxetine (Sigma-Aldrich) dissolved in drinking water at dose of 16 (mg/kg)/day for 28 days.[12] Individual housing was necessary in order to monitor water intake and to adjust FLX concentration in drinking water on a daily basis. No effect of FLX on water consumption was observed (not shown). In the first experiment, the mice used were $Tph2^{GFP}$ heterozygous adult males[8] divided into 2 cohorts: untreated (CTRL) and subjected to pharmacological treatment with fluoxetine. The second experiment was conducted using trans-heterozygous male mice bearing the pan-serotonergic driver *Pet1-Cre*[16] and the Ai65(RCFL-tdT)Δ-FRT (referred in the main text as *Pet1-Cre/tdTom*). Ai65(RCFL-tdT)Δ-FRT mice were obtained from the Ai65(RCFL-tdT) line bearing a FRT-stop-FRT and a LoxP-stop-LoxP double cassette in front of tdTomato (JAX Stock no. 021875) that had been previously mated to a FLP-deleter mouse line. Likewise, *Pet1-Cre/tdTom* trans-heterozygous animals were divided into 2 cohorts: untreated (CTRL) and subjected to pharmacological treatment with FLX (16 (mg/kg)/day) for 28 days. All animals used in each experiment were on a C57BL/6 genetic background. Experimental protocols were conducted in accordance with the Ethics Committee of the University of Pisa and approved by the Veterinary Department of the Italian Ministry of Health.

Behavioral Analysis. To address the antidepressant efficacy of the FLX treatment while minimizing stress on the experimental subjects, 1-day forced swim test protocol[14,22] has been performed on the same animals used for morphological studies. Briefly, animals were

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filmed for 6 min, and then the immobility time was manually scored starting from the second minute, considering the initial minutes as adaptation. Immobility was considered as absence of any active movement of the paws.

Immunohistochemistry. Animals were anesthetized (Avertin, ip 20 μL/g) and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The extracted brains were postfixed overnight at 4 °C with 4% PFA and cut on the coronal plane with a vibratome (Leica Microsystems) with a thickness of 50 μm. Immunohistochemistry was performed on the sections using a rabbit anti-GFP primary antibody (1:2000, ThermoFisher Scientific) incubated overnight at 4 °C, followed by a secondary antibody Rhodamine Red-X anti-rabbit (1:500, ThermoFisher Scientific) incubated overnight at 4 °C.

The immunohistochemistry for the visualization of GFP and 5HT was performed using rabbit anti-5-HT (1:500, Sigma-Aldrich) and chicken anti-GFP (1:1000, Abcam) primary antibodies incubated overnight at 4 °C followed by secondary antibodies Alexa Fluor 594 anti-rabbit (1:500, ThermoFisher Scientific) and FITC conjugates anti-chicken (1:500, ThermoFisher Scientific) incubated overnight at 4 °C.

The immunohistochemistry against tdTomato was performed using a rabbit anti-RFP primary antibody (1:500, Abcam) incubated overnight at 4 °C, followed by a secondary antibody Oregon green 488 anti-rabbit (1:500, ThermoFisher Scientific) incubated overnight at 4 °C.

The immunohistochemistry against SERT was performed using a rabbit anti-serotonin transporter primary antibody (1:500, Millipore) incubated for 2 overnights at 4 °C, followed by a secondary antibody, Alexa Fluor 594 anti-rabbit (1:500, ThermoFisher Scientific), incubated overnight at 4 °C. For the quantification of endogenous GFP fluorescence in the dorsal and median *raphe* nuclei, coronal brain sections containing the region of interest were mounted and imaged without any immunohistochemical detection.

Image Acquisition and ROD (Relative Optic Density) Measurement. Image acquisition was performed using a Nikon A1 confocal microscope, with a 10× objective for hippocampus and somatosensory cortex. Series of 5 optical plans in *Z* were acquired at 1024 × 1024 pixel resolution with a *z*-step of 1 μm. A 20× objective was used for the acquisition of the median and dorsal *raphe* nuclei. The images were acquired with a single stack at 1024 × 1024 pixel resolution. The software ImageJ was used for the quantification of fluorescence intensity (optical density, OD) in circumscribed regions of interest: the 3 layers of the hippocampus (radiatum, lacunosum moleculare, and molecular) and the primary somatosensory cortex (barrel field, S1BF). The relative optic density (ROD) value was calculated by subtracting the background OD value.

Endogenous GFP Measurement and Cell Counting. For the quantification of endogenous GFP fluorescence and the number of GFP+ cells in the dorsal and median *raphe* nuclei, 50 μm thick coronal brain sections were collected every other section, were selected at a comparable anatomical level between FLX-treated and control mice, and were mounted and imaged without any immunohistochemical detection.

Four sections containing the median *raphe* nucleus and seven sections containing the dorsal *raphe* nucleus were used. The intensity of endogenous GFP in the serotonergic neuron cell bodies of the median and dorsal *raphe* nuclei was calculated using IMARIS software (Bitplane), which allows production of a mask over the cell bodies. The number of GFP+ cells present in those images was determined using ImageJ plug-in Cell Counter.

Image Acquisition and 3D Reconstructions. Three-dimensional reconstruction of serotonergic fibers was performed in the lacunosum moleculare layer (LMol) of the dorsal and ventral hippocampus according to Maddaloni and co-workers.[3] Using the Nikon A1 confocal microscope, two images were acquired for each animal with a 60× objective. For each image, *Z*-series of 69 stacks were acquired at 1024 × 1024 pixel resolution, with a pixel size of 0.21 μm and a *z*-step of 0.15 μm. The parameters were set according to data reported in previous analyses.[3] The fiber reconstruction was performed using the IMARIS software (Bitplane), which allows production of a semiautomatic reconstruction of the filaments and then manual correction to eliminate the "false" filaments added by the program. For each acquisition, 3 blocks were reconstructed, 300 pixels × 300 pixels × 69 stacks (xyz = 63 × 63 × 10 μm^3). The software measures for each reconstruction a series of fiber morphological parameters: length, volume, and mean diameter.

Statistical Tests. The two-tailed Student's *t* test statistical test was used, and the value of significance, *p*-value, was set at 0.05.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.8b00655.

Data supporting the validity of the model and verifying that FLX treatment does not affect the number of GFP+ neurons (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*M.Pa. Mailing address: University of Pisa, Department of Biology, Unit of Cell and Developmental Biology, ss 12 Abetone e Brennero, 56127 Pisa, Italy. E-mail: massimo.pasqualetti@unipi.it.

ORCID

Massimo Pasqualetti: 0000-0002-0844-8139

Present Addresses

[‖]G.M.: Harvard Medical School, Department of Genetics, Harvard University, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.

[§]M.Pr.: Neurobiology Section, Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093, USA.

Author Contributions

S.N. and G.M. contributed equally to this work. G.M., M.Pr., and M.Pa. designed research; S.N. and G.M performed research and analyzed data; G.M., M.Pr., and M.Pa. wrote the paper.

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Notes

The authors declare no competing financial interest.

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