RNAi-mediated Serotonin Transporter Suppression Rapidly Increases Serotonergic Neurotransmission and Hippocampal Neurogenesis

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Introduction

The present study was performed to evaluate the selectively of partial SERT suppression in a mouse model following short-term SERT-siRNA treatment as previously reported for 5-HT$_{1A}$ autoreceptor [1,2]. We addressed whether SERT-siRNA accelerates the onset of adaptive changes compared to classical fluoxetine [3]. We demonstrated the relevance of post-transcriptional SERT regulation as a target for rapid-action antidepressants, thus bringing RNAi closer to the clinic as a potential therapy for depression.

Summary and Conclusions

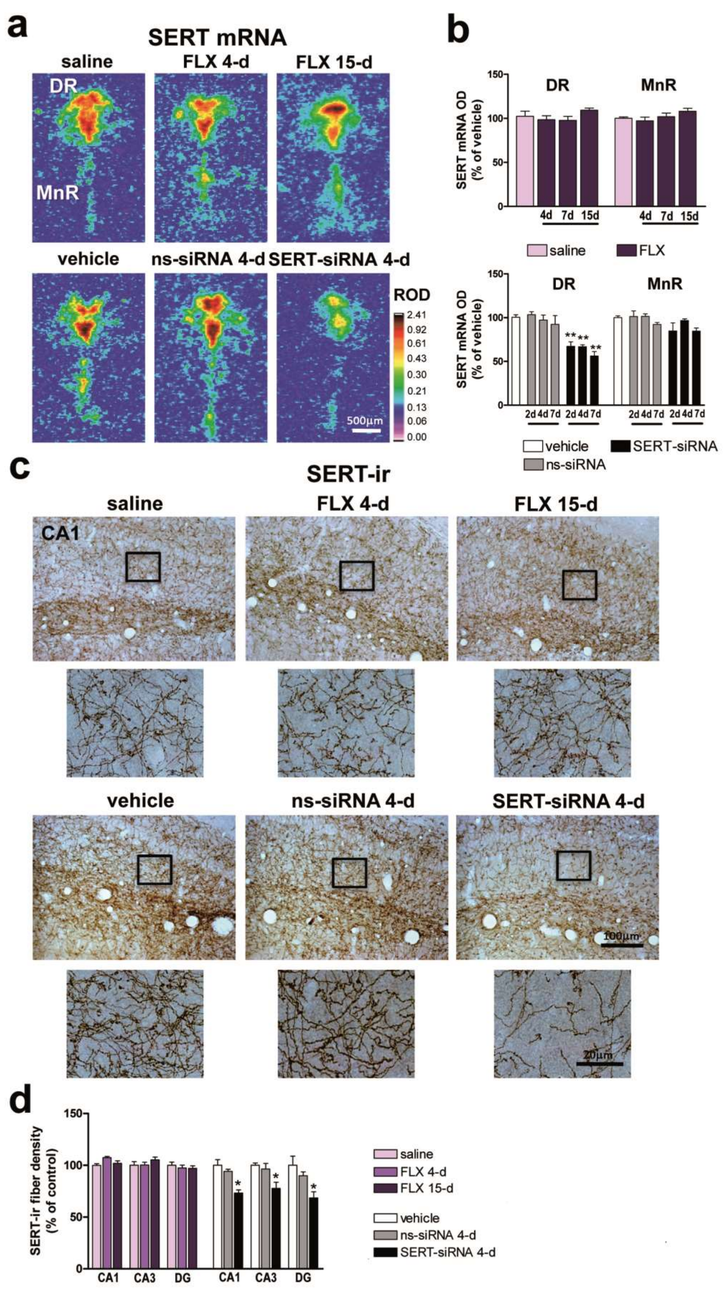
Our results indicate that partial RNAi-based reduction of SERT expression in mouse DR has dramatic effects on serotonergic neurotransmission. Short-term SERT-siRNA treatment evoked a number of changes in molecular, cellular, neurochemical and behavioral variables predictive of antidepressant activity, such as:

- **down-regulated 5-HT$_{1A}$-autoreceptor function,**
- **increased extracellular 5-HT levels in forebrain,**
- **accelerated neural proliferation and neurogenesis in DG,**
- **increased plasticity-associated gene expression and,**
- **reversion of the behavioral dysfunctions induced by corticosterone.**

These changes occurred much earlier than those evoked by fluoxetine treatment. These findings highlight relevance of post-transcriptional SERT regulation as a new therapeutic approaches to develop fast-acting antidepressants.

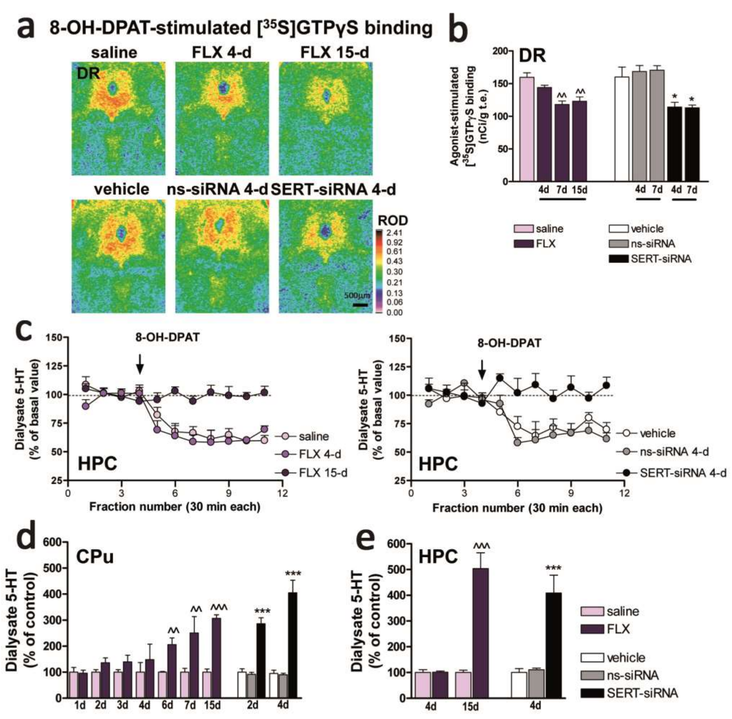
Results

1- Different Regulation of SERT mRNA and Protein Levels after SERT-siRNA or Fluoxetine Treatment



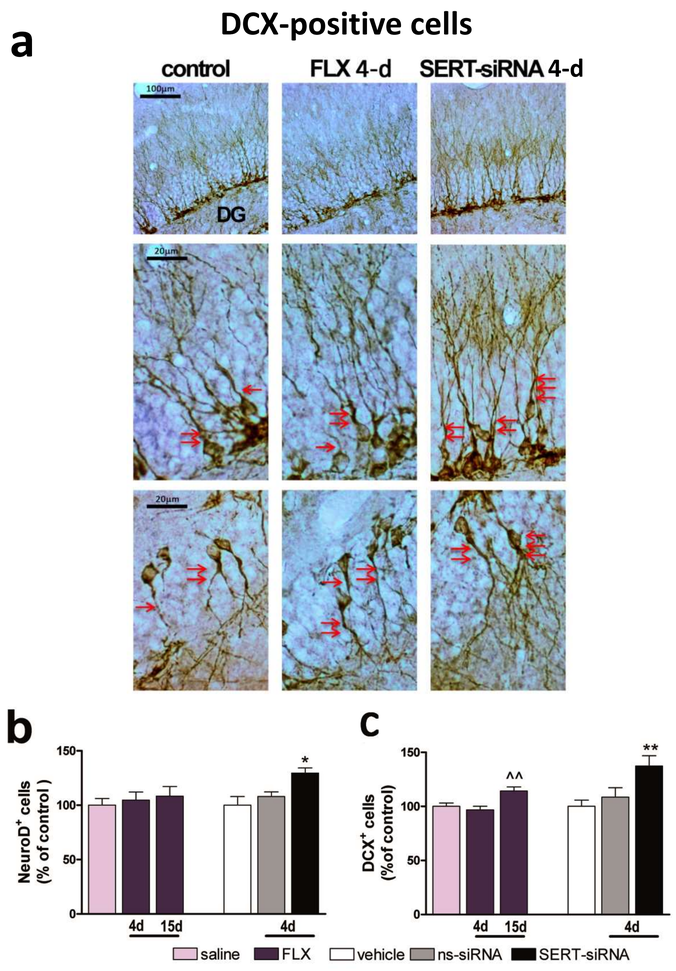
*a. Representative coronal brain sections showing SERT mRNA expression in raphe nuclei from vehicle, fluoxetine, ns-siRNA and SERT-siRNA-treated mice. b. Densitometric quantification of SERT mRNA levels in dorsal and median raphe nuclei (DR and MnR, respectively). ** P < 0.01 compared with vehicle and ns-siRNA groups c. Representative images of SERT-immunoreativity (SERT-ir) axons in the hippocampal CA1 region. d. Quantitative analysis of SERT-ir fiber density in different hippocampal subfields. *P < 0.05 versus vehicle and ns-siRNA-treated mice.*

2- Short-term SERT-siRNA Treatment, but not Fluoxetine, Induces Fast Desensitization of 5-HT$_{1A}$ Autoreceptor and Increases Forebrain Serotonin Levels



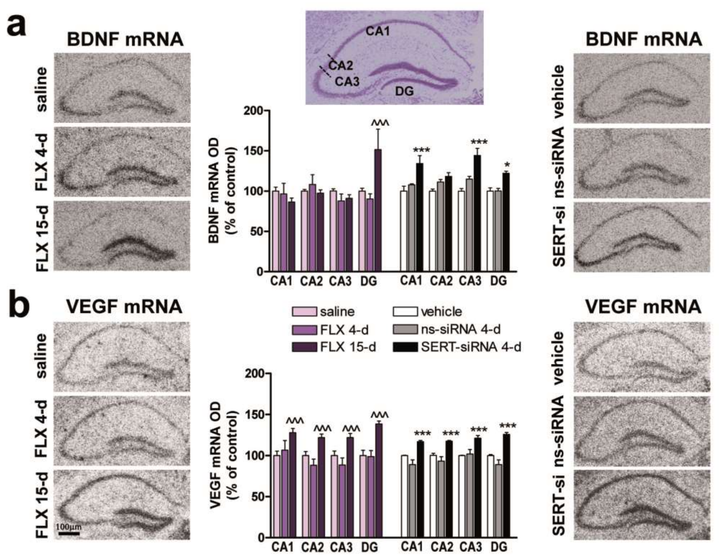
*a. Autoradiograms of coronal midbrain sections of mice showing 5-HT$_{1A}$R 8-OH-DPAT agonist-stimulated [35S]GTPγS binding b. Quantification of 8-OH-DPAT-stimulated [35S]GTPγS binding levels in DR. ^^P < 0.01 vs. saline; *P < 0.05 vs. vehicle and ns-siRNA groups c. 5-HT$_{1A}$ agonist 8-OH-DPAT (0.5 mg/kg, i.p.) decreased extracellular 5-HT levels in the ventral hippocampus (HPC) of saline and fluoxetine-treated mice (4-days), but not after 15-days. Similarly, 8-OH-DPAT had no effect on HPC 5-HT release in SERT-siRNA-treated mice (4-doses), at difference to respective controls. d-e. Time course of extracellular serotonin levels in forebrain areas following SERT-siRNA or fluoxetine treatment. ^^P < 0.01, ^^^P < 0.001 vs. saline i.p.; ***P < 0.001 vs. vehicle and ns-siRNA groups.*

3- SERT Silencing Rapidly Increases the Number of Neuro-D and DCX-positive Cells in Hippocampus



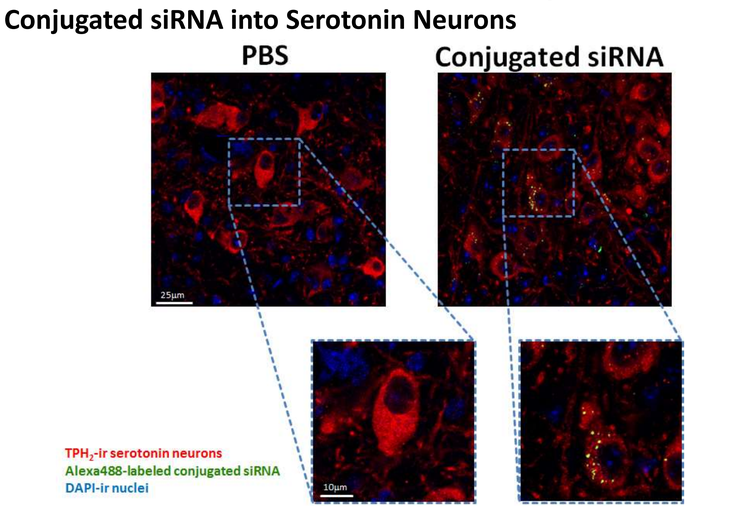
*a. Immunohistochemical images showing DCX-expressing cells, bearing a complex dendritic morphology in the mouse dentate gyrus (DG). Note that mice treated with SERT-siRNA showed an increased number of DCX-positive cells with secondary and tertiary branches compared to control and FLX-treated mice. Arrows indicate DCX-positive cells in DG at different stages of maturation: → = immature cells that lack dendrites or have short dendrites that lack branches; →→ = differentiating cells which dendrites that have secondary branches; →→→ = neurons with dendrites that have tertiary branches b. Quantitative analysis of DG NeuroD+ cells. *P < 0.05 vs. vehicle and ns-siRNA-treated mice c. Quantitative analysis of DG DCX+ cells. ^^P < 0.01 vs. saline; **P < 0.01 vs. vehicle and ns-siRNA-treated mice.*

4- SERT Silencing Accelerates Hippocampal Plasticity-Associated Gene Expression

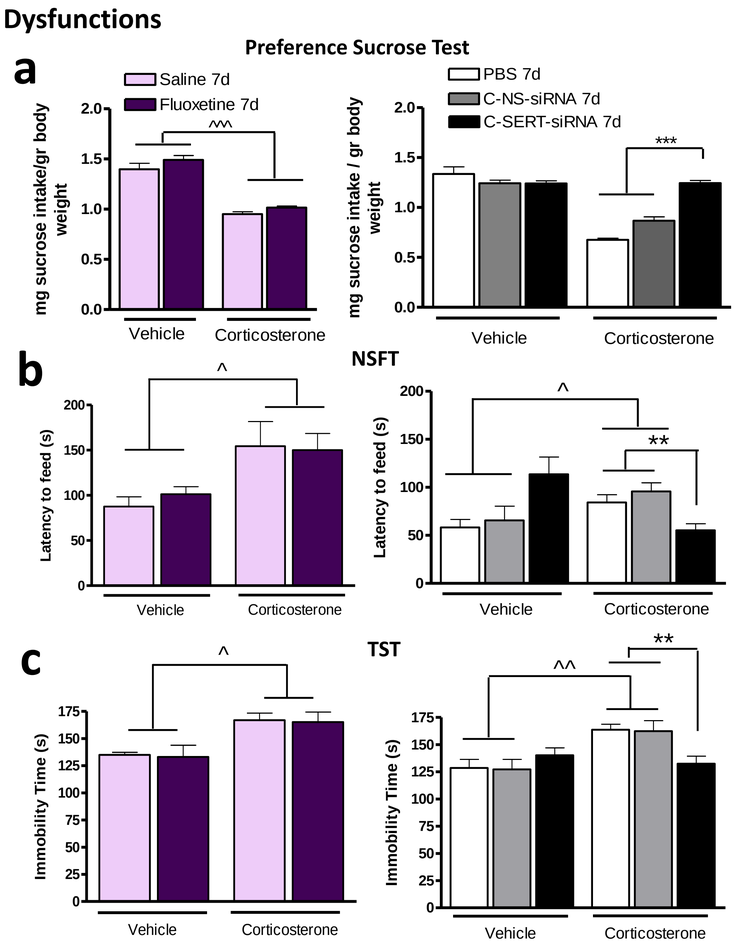


*a-b. Representative autoradiograms of hippocampal sections from vehicle, fluoxetine, ns-SIRNA and SERT-siRNA-treated mice are shown for BDNF and VEGF mRNA expression. Levels of mRNA in the hippocampal subfield are shown in the bar graphs. ^^^P < 0.001 vs. vehicle; *P < 0.05, ***P < 0.001 vs. vehicle and ns-siRNA groups.*

5- Co-Immunolocalization of Intranasally Administered Conjugated siRNA into Serotonin Neurons



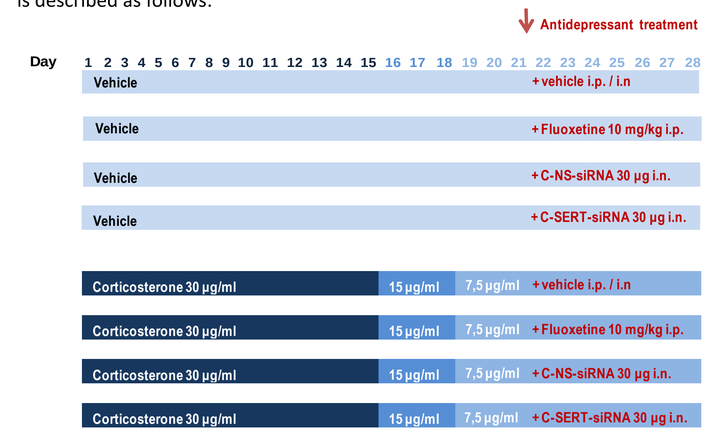
6- Short-term Intranasally Administered C-SERT-siRNA, but not Fluoxetine, Reverses Corticosterone-induced Behavioral Dysfunctions



*a-c. Effects of 7 days of antidepressant treatment (fluoxetine or C-SERT-siRNA), started after 3 weeks of corticosterone (30,15 and 7.5 µg/ml/day) on anxiety- and depression-like behaviors in the preference sucrose test (a), novelty suppressed feeding test (b) and tail suspension test (c). ^P < 0.05, ^^P < 0.01, ^^^P < 0.001 vs. vehicle group; ** P < 0.01, *** P < 0.001 vs. PBS and C-NS-siRNA-treated mice that received corticosterone.*

Methods

- **Animals.** Adult male C57BL/6J mice of 8-15 weeks of age were used.
- **Treatments.** ***Unmodified siRNAs:*** Mice received 10 µg/1µl/day into dorsal raphe nucleus (DR) of: a) a SERT-targeting siRNA (SERT-siRNA) (nt: 1230-1250, GenBank accession NM_010484)[4], or b) an unrelated siRNA duplex with no homology to mouse (nonsense siRNA - ns-siRNA). Controls groups were infused with aCSF. ***Conjugated siRNAs***: mice were intranasally administered (30µg/10µl/day) with: a) conjugated SERT-targeting siRNA (C-SERT-siRNA), or b) conjugated NS-siRNA. Control groups received PBS by intranasal route. ***Fluoxetine-SSRI:*** Mice were daily injected intraperitoneally (i.p.) with fluoxetine 10 or 20 mg/kg or vehicle, respectively.
- **Depression mouse model induced by oral corticosterone** [5,6]. The treatment protocol is described as follows:



- **BrdU administration** [7]
- ***In situ* hybridization** [1]
- **5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS autoradiography** [8]
- **Immunohistochemistry and immunofluorescence**
- **Microdialysis procedures** [9]
- **Tail suspension test, novelty suppressed feeding test, sucrose intake and open field**
- **Statistical analysis** was performed using one-, two-, or three-ANOVA following Neuman-Keuls multiple comparison test. Statistical significance has been set at the 95% confidence level.

References

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