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Impulsivity and home-cage activity are decreased by lentivirus-mediated silencing of serotonin transporter in the rat hippocampus

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HIGHLIGHTS

- Lenti-SERT vectors were designed to suppress SERT gene expression *in vivo*.
- We evaluated a rat model of ADHD through hippocampal inoculation of Lenti-SERT.
- Lenti-SERT rats exhibited less pronounced peaks of circadian activity than controls.
- Lenti-SERT rats displayed a transient decrease in cognitive impulsivity.
- Such phenotype is consistent both with 5-HT manipulations and hippocampal lesions.

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ABSTRACT

Brain serotonin (5-HT) systems modulate emotional, motivational and cognitive processes. Mutations in the serotonin transporter (SERT) gene have been associated with susceptibility towards the development of several psychiatric disorders, both in humans and animal models. Present approach exploited a bilateral intra-hippocampus stereotaxic inoculation of lentiviruses, for enduring *in vivo* silencing of SERT. Control rats were bilaterally inoculated with heat-inactivated lentiviruses. These Lenti-SERT vectors were intended to eventually manipulate the neurotransmitter reuptake at synaptic level, thus enhancing tonic 5-HT transmission. We investigated whether such manipulation could induce behavioural alterations relevant to the modelling of ADHD, in particular symptoms of hyperactivity and impulsivity. Wistar rats were monitored for spontaneous home-cage locomotor activity and studied for impulsivity (Intolerance-to-Delay task). Results show that rats inoculated with Lenti-SERT vectors exhibited less pronounced circadian peaks of activity than controls. Moreover, Lenti-SERT compared to control rats exhibited a transient increase in choice for a delayed-larger reward over an immediate-small reward. This suggests that enhanced hippocampal serotonergic transmission produced a profile of restfulness and a decrease in cognitive impulsivity. This phenotype is consistent with available data both on 5-HT manipulations and hippocampal lesions. In conclusion, present findings may possibly disclose novel avenues towards the development of innovative therapeutical approaches for behavioural symptoms relevant to ADHD.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a heterogeneous syndrome, affecting 2–5% of infants and adolescents as well as about 2% of adults worldwide [59]. Together with inattention and motor hyperactivity, one key symptom is impulsivity, a multifaceted concept implicated in many disorders characterized by inappropriate inhibitory control [16]. It has been suggested that

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impulsivity is not a unitary construct, but describes a range of behaviours and encompasses a variety of related phenomena that may differ in their biological basis [12,60].

The serotonergic system is well known for modulation of emotional, cognitive and motivational processes [11]. Dysfunctions in this system play a crucial role in many psychiatric disorders, including affective and impulse-control disorders [39,50]. By mediating the interplay between limbic and cognitive loops, forebrain serotonin (5-HT) has a key role in the top-down inhibitory control over behavioural initiation and execution, which is important for withholding of instinctive reactions and for an appropriate feedback regulation of behaviour [11,22]. As such, it is central to psychomotor control over subcortical processing of reward and reinforcement [47].

Permanent *in vivo* interference with gene and protein expression and function is nowadays possible using lentiviral vectors [42]. We have recently used this approach exploiting brain inoculation of lentiviruses targeting the dopamine transporter (DAT): its overexpression in rats' nucleus accumbens leads to an impulsive and risk-prone phenotype [1,2]. Similarly, we prepared a novel vector designed to suppress the serotonin transporter (SERT) gene expression. Indeed, mutations in the SERT gene influence the rate of 5-HT reuptake and have been associated with susceptibility towards the development of several psychiatric disorders [10,53]. Specifically, a common polymorphism (5-HTTLPR), localised in the promoter of the SERT-encoding gene, gives rise to two major allelic variants [31] that significantly change the probability of being affected by ADHD [18,40,54]. In particular, the hippocampus was selected as the inoculation site, since its lesions are known to cause hyperactivity and impulsive choice in rats (e.g. [9,27,46]).

Experimental studies with different serotonergic manipulations have demonstrated an inverse relationship between 5-HT levels and impulsivity, with a reduction in the neurotransmitter causing an increase in impulsivity and vice versa (e.g. [8,55]). However, in humans, an increase in impulsivity appears to be associated with the short (s) allelic variant of 5-HTTLPR, leading to reduced SERT gene transcription [41,51,57]. Thus, we aimed to determine whether a partial silencing of the SERT-encoding gene within the hippocampus could induce alterations relevant to the modelling of ADHD, in particular symptoms of hyperactivity and impulsivity. Specifically, for measuring behavioural impulsivity, the Intolerance-to-Delay (ID) task was used, in which impulsive subjects are detected by their intolerance to periods of forced waiting before the delivery of a large reward [17]. Indeed, delay-discounting paradigms in general are among the most successfully utilised tools for measurement of impulsive choice [60].

2. Materials and methods

2.1. Lenti-SERT vectors

2.1.1. Construction of pTK431-SERT-siRNAs

To silence SERT expression *in vivo*, three targets were designed according to the SERT mRNA sequence. The following targets were selected, based on Hannon's design criterion: 1st target: bp64–29, 2nd target: bp2667–2629, 3rd target: bp1805–1829. To each oligo, a *XhoI* restriction site was added at 3′ and a U6-3′-specific 10mer at 5′. Using the pSilencer 1.0-U6 (Ambion, UK) as a template and a U6 promoter-specific forward primer containing *BamHI* restriction site (5′-CGC CGC CGC GGA TCC CGC TCT AGA ACT AGT GC-3′), each siRNA target was added to the mouse U6 promoter by PCR, using the following program: 120 s at 94 °C (initial denaturation) followed by 35 cycles (45 s at 94 °C, 45 s at 64 °C and 45 s at 72 °C) in 4% dimethyl sulfoxide (Sigma, Switzerland). The PCR product was digested with *BamHI* and *XhoI*, cloned into similar sites into pTK431, and sequenced to verify the integrity of each construct.

2.1.2. Lentivirus production

The vector plasmids (pTK431-U6-siSERT1, pTK431-U6-siSERT2, pTK431-U6-siSERT3 and pTK433-GFP), together with the packaging construct plasmid p891 and the envelope plasmid PMD2-G, were co-transfected into HEK293T cells to produce the viral particles [6,7]. Once harvested and concentrated, these viral particles were tested *in vitro*. The experiment of infection and transfection was run with 3, 10 and 25 μl of each lentivirus stock (LV-siSERT1, LV-siSERT2, LV-siSERT3) and 20 μl of a mix of the three siLVs. HEK293T cells were plated and infected with LV-siSERTs and then transfected with a plasmid that expresses SERT. The siLVs were added with Polybrene (Sigma, Switzerland, at 10 μg/ml final concentration) to the cells. After 7 h, cells were transfected with pcDNA3-rat-SERT (2 μg/well). The next day, cells were subjected to total RNA isolation, reverse transcription and real-time PCR, to quantify SERT expression/silencing (see Supplementary Data).

2.2. Subjects

Seventeen adult male Wistar rats (400 g; for housing conditions, see Supplementary Data) were randomly assigned to experimental groups: one group received bilateral inoculation of Lenti-SERT vectors (1 μl of a mix of the three LV-siSERTs) intended to abolish the genetic expression of SERT. Inoculations were made bilaterally at coordinates AP − 3.3, ML ± 2.2, DV − 4.0 from bregma [45]. The other group (controls) received a bilateral inoculation of heat-inactivated lentiviruses (1 μl) at the same coordinates (see Supplementary Data). After surgery, rats were single-housed and left undisturbed for at least one month prior to behavioural experiments.

2.3. Circadian cycle

Rats were continuously monitored for spontaneous home-cage locomotor activity [3,4] by means of an automatic device equipped with small passive infrared sensors placed on a standard rack over the top of each home-cage (ActiviScope system; TechnoSmart, Rome, Italy). These sensors (20 Hz) detected any movement of rats: scores were automatically divided into 60-min intervals. The spontaneous home-cage activity was continuously measured in Lenti-SERT and control subjects, starting from day 50 after inoculation and over 15 days. From this period, we extracted the central 5-days interval, from which a mean day was calculated.

2.4. ID task for impulsivity

Two months later, rats were daily tested (between 10:30 and 15:30) exploiting classical Skinner-boxes (for apparatus and food-restriction schedule, see Supplementary Data). Nose-poking in one hole (termed "Small & Soon", SS) resulted in the immediate delivery of one pellet in one magazine, whereas nose-poking in the other hole (termed "Large & Late", LL) resulted in the delivery of five pellets in the other magazine. After nose-poking and before food delivery, the chamber light corresponding to the nose-poked hole was switched on for 1 s. Following food delivery, the corresponding magazine light was turned on for 30 s, to signal the length of time-out (TO) during which additional nose-poking was recorded but had no scheduled consequences (i.e. inadequate, see Supplementary Data) [49,50]. The testing phase was preceded by three training sessions at delay 0 s, which allowed subjects to reach a significant preference for LL. During the testing phase (eight daily sessions, 40 min each), a delay was inserted between nose-poking in the LL hole and delivery of the 5-pellet reward. The chamber light over the