SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

Lenti-SERT vectors

Real time PCR for SERT mRNA quantification

For quantitative real-time PCR, primer sets for rat SERT were designed to amplify 100-to-200-bp products, using PRIMER3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3). The following primer pairs were used for rat SERT: 5'-TCGCCTCCTACTACAACACC-3' and 5'-AGGAGTTCGTGCAGCTAGTC-3'. Total RNA was extracted from the HEK293T cells using TRIzol reagent (Invitrogen, Basel, Switzerland), including a RNase-free DNase step, according to the manufacturer's protocol. The yield and the integrity of RNA were determined by A_{260} determinations and agarose gel electrophoresis respectively. First strand cDNA was generated from 2 µg of total RNA and Oligo (dT12-18)-primer with the M-MLV reverse transcription kit (Invitrogen) in a total volume of 20 µl. SYBR Green real time-PCR reactions were performed in 96-well plates using 7900HT Fast Real-Time PCR System (Applied Biosystem). 2 µl of each cDNA sample was amplified in triplicate, and every reaction included a negative control (having no template) to eliminate the possibility of contamination. Thermal cycling conditions comprised an initial step

at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15s and 60°C for 1 min. Gene expression levels were quantified by the comparative threshold cycle (*CT*) method (Schmittgen and Livak, 2008) using hypoxanthine phosphoribosyltransferase (HPRT) as an internal control gene (Pernas-Alonso et al., 1999). The fractional number of PCR cycles *CT* required to obtain a given amount of amplified product in the exponential phase of amplification was determined for the gene of interest and for HPRT in each cDNA sample. The relative expression level of the gene of interest was then expressed as $2^{-\Delta CT}$ where ΔCT = *CT* gene of interest - *CT* HPRT.

The rat SERT cDNA (accession number 013034) was kindly provided by Dr. Claire Desnos from the University of Paris "Decartes" (Paris, France).

References

R. Pernas-Alonso, F. Morelli, U. di Porzio, C. Perrone-Capano, Multiplex semi-quantitative reverse transcriptase-PCR of low abundance neuronal mRNAs, Brain Res. Brain Res. Protoc. 4 (1999) 395-406.

T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative C(T) method, Nat. Protoc. 3 (2008) 1101-1108.

Subjects

During 6-weeks habituation to housing conditions, rats (Charles River, Italy) were housed in couples within Makrolon® Type-III cages with sawdust bedding, kept in an air-conditioned room (temperature 21±1°C, relative humidity 60±10%), on a 12-h reversed light-dark cycle (lights off at 8:00). Water was available *ad libitum* whereas food (Altromin-R, A. Rieper S.p.A, Italy) was available *ad libitum* unless otherwise stated.

All procedures were approved by Italian Ministry of Health, and were in agreement with 86/609/EEC and Italian law. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, if available.

Stereotaxic surgery

To receive the bilateral intra-hippocampal inoculation of Lenti-SERT vectors, subjects (N=17) were anesthetized with ketamine (100mg/kg) and xilazine (10mg/kg), administered intraperitoneally, and secured in a stereotaxic frame fitted with atraumatic earbars. Following 90s-long inoculation (1μl of a mix of the three LV-siSERTs or 1μl of heat-inactivated lentiviruses), the Hamilton's needle was left in place for 5 min before the 4 min-long withdrawal, to allow diffusion of the vectors. To obtain the heat-inactivated lentiviruses, inoculated in controls, Lenti-SERT vectors were maintained in a bath at 45-50°C, for 1h, one day before inoculation.

Intolerance to Delay (ID) task for impulsivity

Apparatus

Computer-controlled operant chambers (Coulbourn Instruments, Allentown, PA, USA), were provided with two nose-poking holes, two feeding devices, two feeding magazines where precision pellets (45 mg, F0021, BioServ, Frenchtown, NJ, USA) were dropped, two magazine lights (signalling the length of timeout), two

chamber lights (placed over each nose-poking hole, signalling the length of delay). Nose-poking into holes was detected by a photocell and was recorded by a computer, which also controlled food delivery.

Food restriction schedule

Standard food was removed from home-cages 24h prior to start of the protocol. During training and testing days, the same mild level of food restriction was applied to increase animals' motivation to work for food delivery. Specifically, rats were restricted to 90 ± 3% of their free-feeding body-weight, having access to both precision pellets in the operant chambers and 5 g each of standard food in the home-cage when returned to their home-cage after each daily session (40 min).

SUPPLEMENTARY RESULTS

ID task for impulsivity

Inadequate nose-pokes for the SS reward

The nose-poking in either hole during the course of the delay interval had no scheduled consequences and was considered as an inadequate response. In the ID task, at the beginning of the testing phase, rats generally show significantly more nose-poking at the LL than at the SS hole. Conversely, as the length of the delay increases, rats usually increase the proportion of nose-poking directed to the SS hole (see e.g. Adriani and Laviola, 2003; Adriani et al., 2003).

Regarding the present data, as the delay length increased, all animals showed a progressive rise in the frequency of inadequate SS nose-pokes (per trial), as expected (group: $F_{(1,14)}$=0.03, P=0.8628; session: $F_{(7,98)}$=22.96, P<0.0001). However, the two profiles of inadequate responding were not completely overlapped (session x group: $F_{(7,98)}$=2.31, P=0.0320). Post hoc comparisons evidenced a significant difference on the last delay (90s), with the Lenti-SERT animals performing a higher frequency of inadequate nose-pokes in comparison to controls (see Table 1). This result suggests that, while waiting for delivery of the large reinforcer, during the last 90s delay, Lenti-SERT rats failed to rest and demanded the immediate reinforcer more often. We may conclude that Lenti-SERT subjects developed an increase of motor impulsivity. We have indeed proposed (see e.g. Adriani and Laviola, 2003; Adriani et al., 2003) that a sustained expression of nose-

poking behaviour during the length of the delay, when it is not reinforced anymore, may be considered as an index of restlessness.

This increase in motor impulsivity and concomitant decrease in cognitive impulsivity is not surprising and did not occur simultaneously in Lenti-SERT rats. While the latter was specifically found at the 30s-delay (a relatively crucial value, being delay equal to the length of TO; see Zoratto et al., 2012), the former only emerged on longer delay. Similar dissociations have been already reported in studies aimed at investigating the relation among different behavioural measures of impulsivity (see e.g. Winstanley et al., 2004). It has become clear that the constructs of impulsivity describe a wide range of behaviours, including perseveration of a non-rewarded response, intolerance to delay of reward, inability to withhold a response, etc. (see e.g. Winstanley, 2011). Studies in both human and rats have shown that serotonergic manipulations have contrasting effects on different measures of impulsivity. For example, 5,7-DHT lesions (which massively deplete forebrain 5-HT levels) increased impulsive action in the five-choice serial reaction time task (5CSRT), had null or mixed effects on impulsive action in the stop-signal task (SST), and did not alter impulsive choice in the ID task (for a review see Winstanley, 2011).

Total number of trials per session

During each daily session (40 min), rats could express their choice (by nosepoking in one of the two holes) at their own individually variable rate. Control and Lenti-SERT rats did not differ in the amount of trials performed during each session ($F_{(1,14)} = 1.17$, $P = 0.2979$). Specifically, control and Lenti-SERT rats did on average 45.85 ± 2.05 and 42.56 ± 1.50 trials per session respectively.

Response time

No differences were found between control and Lenti-SERT rats in response time ($F_{(1,14)} = 0.001$, $P = 0.9750$). Specifically, response time in control and Lenti-SERT rats was on average 14.31 ± 2.20 and 14.19 ± 1.06 respectively.

References

W. Adriani, G. Laviola, Elevated levels of impulsivity and reduced place conditioning with d-amphetamine: two behavioral features of adolescence in mice, Behav. Neurosci. 117 (2003) 695-703.

W. Adriani, A. Caprioli, O. Granstrem, M. Carli, G. Laviola, The spontaneously hypertensive-rat as an animal model of ADHD: evidence for impulsive and non-impulsive subpopulations, Neurosci. Biobehav. Rev. 27 (2003) 639-651.

C.A. Winstanley, J.W. Dalley, D.E. Theobald, T.W. Robbins, Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behaviour, Neuropsychopharmacology 29 (2004) 1331-43.

C.A. Winstanley, The utility of rat models of impulsivity in developing pharmacotherapies for impulse control disorders, Br. J. Pharmacol. 164 (2011) 1301-21.

F. Zoratto, G. Laviola, W. Adriani, Choice with delayed or uncertain reinforcers in rats: influence of timeout duration and session length, Synapse 66 (2012) 792-806.

Table 1. Inadequate nose-pokes for the SS reward.

	0	**7**	**15**	**30**	**45**	**60**	**75**	**90**
Control	0.336 ± 0.123	0.417 ± 0.087	0.452 ± 0.131	0.791 ± 0.205	1.205 ± 0.352	1.556 ± 0.324	1.395 ± 0.349	1.352 ± 0.152
Lenti-SERT	0.328 ± 0.072	0.363 ± 0.076	0.372 ± 0.082	0.648 ± 0.127	0.788 ± 0.189	1.332 ± 0.223	1.856 ± 0.173	2.031 ± 0.200*****

Mean (± SEM) number of inadequate nose-pokes (i.e. performed during the TO interval), which were recorded but were without any consequences, shown by Lenti-SERT (n=10) and control (n=6) animals. On the last delay (delay 90 s), Lenti-SERT rats performed a higher frequency of inadequate nosepokes in comparison to controls.
***** $P < 0.05$ in post hoc test.