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Somatostatin-28 modulates prepulse inhibition of the acoustic startle response, reward processes and spontaneous locomotor activity in rats

Svetlana Semenova^{1,✉}, Daniel Hoyer², Mark A. Geyer¹, and Athina Markou¹

¹Department of Psychiatry, University of California San Diego, La Jolla, CA 92093, USA

²Psychiatry/Neuroscience Research, Novartis Institute for Biomedical Research CH 4002 Basel, Switzerland

Abstract

Somatostatins have been shown to be involved in the pathophysiology of motor and affective disorders, as well as psychiatry disorders, including schizophrenia. We hypothesized that in addition to motor function, somatostatin may be involved in somatosensory gating and reward processes that have been shown to be dysregulated in schizophrenia. Accordingly, we evaluated the effects of intracerebroventricular administration of somatostatin-28 on spontaneous locomotor and exploratory behavior measured in a behavioral pattern monitor, sensorimotor gating, prepulse inhibition (PPI) of the acoustic startle reflex, and brain reward function (measured in a discrete trial intracranial self-stimulation procedure) in rats. Somatostatin-28 decreased spontaneous locomotor activity during the first 10 min of a 60 min testing session with no apparent changes in the exploratory activity of rats. The highest somatostatin-28 dose (10 µg/5 µl/side) induced PPI deficits with no effect on the acoustic startle response or startle response habituation. The somatostatin-induced PPI deficit was partially reversed by administration of SRA-880, a selective somatostatin 1 (sst₁) receptor antagonist. Somatostatin-28 also induced elevations in brain reward thresholds, reflecting an anhedonic-like state. SRA-880 had no effect on brain reward function under baseline conditions. Altogether these findings suggest that somatostatin-28 modulates PPI and brain reward function but does not have a robust effect on spontaneous exploratory activity. Thus, increases in somatostatin transmission may represent one of the neurochemical mechanisms underlying anhedonia, one of the negative symptoms of schizophrenia, and sensorimotor gating deficits associated with cognitive impairments in schizophrenia patients.

Keywords

sensorimotor gating; intracranial self-stimulation; reward thresholds; anhedonia; schizophrenia; SRA-880

✉Svetlana, Semenova, Ph.D., Department of Psychiatry, School of Medicine, University of California San Diego, 9500 Gilman Drive, M/C 0603, La Jolla, California 92093-0603, USA; tel: (858) 534-1528; fax: (858) 858-534-9917; ssemenova@ucsd.edu.

Disclosure/conflict of interest

DH is a full-time employee of Novartis. MAG holds an equity interest in San Diego Instruments, Inc.

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1. Introduction

Somatostatins, also known as somatotropin-release inhibiting factors (SRIFs), have been shown to be involved in the pathophysiology of motor and affective disorders, as well as psychiatric disorders, including schizophrenia (Brownstein et al., 1975; Vincent and Johansson, 1983; Rubinow et al., 1985; Rubinow, 1986; Vecsei and Widerlov, 1988a; Vecsei and Klivenyi, 1995; Epelbaum et al., 2009). Somatostatin and somatostatin receptors are widely distributed in the brain, including the cerebral and cingulate cortex, hippocampus, amygdala, and most structures of the limbic system and basal ganglia (Hoyer et al., 1995; Patel, 1999; Viollet et al., 2008) involved in motor, reward, and cognitive function. However, preclinical investigations of the role of endogenous brain somatostatin in reward and pre-cognitive sensory gating functions have been very limited due to the lack of selective non-peptide receptor agonists/antagonists for this peptide, very limited permeability of these peptides across the blood-brain barrier, and their fast degradation.

Somatostatins are peptides that exist in the brain in two main forms: somatostatin-14 (a tetradecapeptide) and somatostatin-28 (an amino-terminally extended octacosapeptide; (Hoyer et al., 1995; Hoyer et al., 2004). Somatostatin inhibits the release of a variety of pituitary hormones and plays a role as a neurotransmitter in the central nervous system (Patel, 1999; Bissette, 2001). Somatostatin (sst) receptors, which belong to the G-protein-coupled receptor family, are divided into five subtypes (sst₁–sst₅) and classified into two groups, SRIF1 (sst₁, sst₄) and SRIF2 (sst₂, sst₃, sst₅) receptors (Hoyer et al., 1995; Patel, 1999; Fehlmann et al., 2000; Olias et al., 2004; Siehler et al., 2008; Viollet et al., 2008).

Somatostatin receptors in the striatum and other basal ganglia nuclei are involved in regulation of motor behavior. The striatum and the nucleus accumbens, two terminal areas of dopaminergic neurons, express both sst₁ and sst₂ receptors (Hoyer et al., 1995; Fehlmann et al., 2000). In the nucleus accumbens, the sst₁ receptor is a somatostatin autoreceptor (Vasilaki et al., 2004; Thermos et al., 2006), whereas the sst₂ receptor appears to be responsible for the actions of somatostatin on dopamine release and dopamine-mediated motor behaviors (Raynor et al., 1993; Thermos et al., 1996; Hathway et al., 1999; Viollet et al., 2000; Allen et al., 2003; Ikeda et al., 2009). Somatostatin can modulate locomotor activity by activating sst₁, sst₂ and sst₄ receptors in the ventral pallidum/substantia innominata (Marazioti et al., 2005; Marazioti et al., 2008), and sst₂ and sst₄ receptors in the striatum (Santis et al., 2009). Although the effects of somatostatin on motor behavior have been extensively investigated, the reported findings in literature are not always in agreement. Specifically, the direction of the effects of somatostatins on locomotor activity depended on the dose, brain site of administration and somatostatin peptide (e.g., somatostatin-28, somatostatin-14) used, as well as the locomotor activity tests used (e.g., locomotor activity chamber, open field) (Vecsei et al., 1984; Vecsei and Widerlov, 1988b, 1990; Raynor et al., 1993; Tashev et al., 2001; Marazioti et al., 2005; Marazioti et al., 2008). Surprisingly little is known regarding the effects of somatostatins on quantitative measures of spontaneous locomotor and investigatory behavior. Therefore, one of the aims of the present studies was to assess the effects of intracerebroventricular (i.c.v.) administration of somatostatin-28 on the spatial and temporal sequences of locomotor movements, investigatory holepokes, and rearings of rats using a behavioral pattern monitor allowing for sophisticated multivariate assessments of locomotor behavior (Geyer et al., 1986).

Disturbances in dopaminergic function may contribute to cognitive impairment and deficits in sensory gating observed in schizophrenia patients (Howes and Kapur, 2009). Prepulse inhibition (PPI) of the acoustic startle response (i.e., a reduction of a startle response elicited by an intense acoustic stimulus when it is immediately preceded by a stimulus of lower intensity) has been used as a measure of the loss of sensorimotor gating in patients with

schizophrenia (Swerdlow and Geyer, 1998; Braff et al., 2001; Geyer et al., 2001). In rodents, PPI deficits have been induced with the administration of the direct and indirect dopamine receptor agonists apomorphine and amphetamine, respectively (Mansbach et al., 1988; Geyer et al., 2002), to model aspects of positive schizophrenia symptoms related to dysregulation of the dopaminergic system (Howes and Kapur, 2009). Administration of cysteamine, a sulfhydryl agent that decreases somatostatin levels (Sagar et al., 1982), impaired the development of the acoustic startle response in rats, indicating that somatostatin may influence the maturation of sensorimotor information processing (Kungel et al., 1996). Systemic administration of cysteamine (Feifel and Minor, 1997) or central administration of octreotide, an sst2/sst5 receptor selective octapeptide that pharmacologically mimics natural somatostatin, in the pontine reticular formation had no effect on baseline startle amplitude (Fendt et al., 1996). Interestingly, however, cysteamine reversed amphetamine-induced deficits in PPI, while having no effect on PPI when administered alone (Feifel and Minor, 1997), suggesting that somatostatin may play a role in the positive symptoms of schizophrenia. However, the effects of somatostatins on startle response and PPI have not been investigated.

In addition to cognitive function and sensorimotor gating, corticolimbic brain circuits involved in reward processes (Spanagel and Weiss, 1999; Koob and Volkow, 2010). Dysfunction in corticolimbic circuits may underlie reward deficits and anhedonia, one of the negative symptoms of schizophrenia (American Psychiatric Association, 1994). In animals, the intracranial self-stimulation (ICSS) procedure allows for the assessment of brain reward function (Markou and Koob, 1992). Elevations in thresholds are interpreted as a decrease in the reward value of the stimulation and are an operational measure of anhedonia (Markou and Kenny, 2002; Semenova and Markou, 2003; Paterson and Markou, 2007). Early studies reported that i.c.v. administration of somatostatin decreased self-stimulation rates measured in the rate-frequency self-stimulation procedure (Vecsei et al., 1982, 1983; Balazs et al., 1988), implicating somatostatins in brain reward function. Importantly, the discrete trial ICSS procedure used in this study allows the assessment of reward thresholds that are independent of response rates and are not affected by nonspecific motor effects of manipulations (Markou and Koob, 1992). Thus, a major advantage of this procedure is that it provides a quantitative measure of reward (brain reward thresholds measured in μ A) that is extremely stable over periods of months under baseline conditions, allows repeated testing of subjects, and shows predictable and reliable effects of pharmacological manipulations on brain reward function (Kornetsky et al., 1979; Markou and Koob, 1992; Vlachou and Markou, 2010 in press).

In summary, in the present study, we assessed the effects of i.c.v. somatostatin-28 administration on complex locomotor and investigatory behavior, brain reward function, and sensorimotor gating in rats. Spontaneous locomotor and exploratory activity was measured in a behavioral pattern monitor (BPM; Geyer et al. 1986). Sensorimotor gating was assessed using PPI of the acoustic startle reflex. Brain reward function was measured in a discrete-trial ICSS threshold procedure (Markou and Koob, 1992). Somatostatin-28 was selected for the present studies because it is a more stable compound and has a longer plasma half-life than somatostatin-14 (Patel, 1999). Additionally, we evaluated the effects of SRA-880 ([3R, 4aR, 10aR]-1,2,3,4,4a,5,10,10a-octahydro-6-methoxy-1-methyl-benz[g] quinoline-3-carboxylic-acid-4-(4-nitro-phenyl)-piperazine-amide, hydrogen malonate), a nonpeptide sst₁ receptor antagonist (Hoyer et al., 2004), on PPI and brain reward function in the ICSS procedure. SRA-880 was selected to be used in these studies as one of the first non-peptide receptor antagonists available for basic science investigations. We have shown previously that the sst₁ receptor has an autoreceptor function in the retina, hypothalamus, and the nucleus accumbens (Thermos et al., 2006) and most recently in the hippocampus (De Bundel et al., 2010); some of these findings were made possible by the use of SRA880, both

in vitro and *in vivo*. The role of sst₁ receptors in sensorimotor gating and reward processes are not known. Because somatostatin-28 did not have a robust effect on motor behavior, the effects of SRA-880 on locomotor and investigatory activity were not evaluated.

2. Materials and Methods

2.1. Subjects

Male Wistar rats (Charles River; Raleigh, NC) weighing 300–350 g at the beginning of the experiments were housed in groups of two in a humidity- and temperature-controlled vivarium on a 12 h/12 h light/dark cycle. Rats had *ad libitum* access to food and water throughout the course of the studies except during testing. Training and testing occurred during the dark cycle. All experiments were in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Drugs

Somatostatin-28 (Novartis Pharma AG, Basel, Switzerland) was dissolved in saline and administered i.c.v.. The i.c.v. injection volume was 5 µl of drug/saline solution per side infused over 30 s using a Hamilton 10 µl microsyringe (Hamilton, Reno, NV) connected to the injection cannula via polyethylene tubing. A stainless-steel injector was 3 mm longer than the guide cannula so that its tip protruded into the ventricle. After each infusion, 7 mm stylets were placed into the cannula to prevent unnecessary blockage. SRA-880 (Novartis Pharma AG, Basel, Switzerland) was dissolved in a mixture of 20% 1-methyl-2-pyrrolidinone and 80% of a 5% D-glucose nanopure water solution. SRA-880 was injected subcutaneously (s.c.) in a volume of 1 ml/kg. Control rats were injected with appropriate vehicle solutions.

2.3. Intracranial electrode and cannula implantation surgery and placement verification

Rats were anesthetized with isoflurane (1–3%) in an oxygen vapor mixture. Subjects were prepared with bipolar stainless steel electrodes (Plastics One, Roanoke, VA) in the posterior lateral hypothalamus (anterior/posterior, –0.5 mm from bregma; lateral, ± 1.7 mm; dorsal/ventral, –8.3 mm from dura with the incisor bar elevated 5.0 mm above the interaural line; (Pellegrino et al., 1986). During the same surgery, guide cannulas (23 gauge; Plastics One, Roanoke, VA) were implanted bilaterally in the lateral ventricles (anterior/posterior, 0.6 mm from bregma; lateral, + 2.0 mm; dorsal/ventral, 3.0 mm from dura with the incisor bar elevated 5.0 mm above the interaural line; Pellegrino et al., 1986). At the end of the experiments, rats were anesthetized with pentobarbital and perfused via the ascending aorta with physiological saline (100 ml) followed by a 10% formalin solution. Coronal sections were sliced on a cryostat and stained with cresyl violet to verify the infusion sites. Animals with accurate cannula placements were included in the statistical analyses.

2.4. Behavioral pattern monitor (BPM)

Behavior was measured in a BPM which consisted of a 30.5 × 61.0 cm black Plexiglas chamber equipped with 2.5 cm holes in the walls and floor as described previously (Geyer et al., 1986). Photocells in each hole detected investigatory nose pokes (hole pokes). A touchplate located 15.2 cm above the floor allowed the detection of rearings when the animal made contact between the metal floor and the metal touchplate. A 4 × 8 grid of infrared photobeams detected the animal's position in the X–Y plane. A computer continuously monitored all photobeams and the touchplate and stored the data for later analysis. The BPM system collected data on 23 behavioral measures, including the number

of holepokes, rearings, and locomotor activity. Three types of locomotor activity measures were derived. Movements were defined as the total number of X-Y beam breaks. Crossings were quantified by the number of crossings between any of eight equal square sectors within the BPM and required whole-body translocations for scoring. Region entries were defined as the number of entries into and time spent in the center and corners of the apparatus. A 60 min period of habituation to the testing room occurred before the test. The test session duration was 60 min. Data were analyzed in 10 min time bins.

2.5. Acoustic startle procedure

Four startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA) were used. The startle chambers consisted of a Plexiglas cylinder, 8.2 cm diameter, resting on a 12.5 × 25.5 cm Plexiglas frame within a sound-attenuated ventilated enclosure. Acoustic startle was produced by a loudspeaker mounted 24 cm above the Plexiglas cylinder. A piezoelectric accelerometer on the base of the cylinder frame detected and transduced vibration, which was digitized and recorded by an interface unit and computer. Sound levels (dB [A] scale) and accelerometer sensitivities within each chamber were calibrated regularly and remained constant throughout the experiment.

After the rats were placed in the startle chambers, the 70 dB background noise was presented for a 5 min acclimation period and continued throughout the test session. During a test session, all trial types were presented several times in a pseudorandom order for a total of 107 trials (18 120 dB pulse-alone trials, 53 no stimulus [nostim] trials, 18 76 dB prepulse + 120 dB pulse trials, and 18 82 dB prepulse + 120 dB pulse trials). Additionally, six pulse-alone and six nostim trials, which were not included in the calculation of PPI values (based on the observation that the most rapid habituation of the startle reflex occurs within the first few presentations of the startling stimulus; Geyer et al., 1990), were presented at the beginning of each test session, and six additional pulse-alone 120 dB and six nostim trials were presented at the end of each test session to assess startle habituation throughout the session. The time between trials averaged 15 s (range, 12–30 s), and the total duration of a test session was approximately 16.5 min. The pulse-alone trial consisted of a 40 ms 120 dB pulse of broadband noise. The prepulse + pulse trials consisted of a 20 ms noise prepulse, a 100 ms delay, then a 40 ms 120 dB startle pulse (120 ms onset-to-onset interval). Prepulse intensities were 3, 6, and 12 dB above the 70 dB background level, and each prepulse trial was designated as 73 dB prepulse + pulse, 76 dB prepulse + pulse, and 82 dB prepulse + pulse. The nostim trial consisted of background noise only and allowed the assessment of general activity in the startle chamber by the piezoelectric accelerometer when no acoustic stimuli were presented.

2.6. Intracranial self-stimulation apparatus and procedure

The discrete-trial current-threshold ICSS procedure was a modification of a procedure originally developed by Kornetsky and colleagues (Kornetsky et al., 1979) and has been described in detail by Markou and Koob (Markou and Koob, 1991, 1992). Briefly, training and testing occurred in 16 sound-attenuated Plexiglas chambers (San Diego Instruments, San Diego, CA) that contained a metal wheel manipulandum. Brain stimulation was delivered by constant-current stimulators (San Diego Instruments, San Diego, CA). At the start of each trial, rats received a noncontingent electrical stimulus (100 Hz rectangular cathodal pulses). During the following 7.5 s limited hold, if the rats responded by turning the wheel manipulandum (positive response), then they received a second contingent stimulus identical to the previous noncontingent stimulus. During a 2 s period immediately after a positive response, further responses had no consequences. If no response occurred during the 7.5 s limited hold, a negative response was recorded. The intertrial interval (ITI), which followed the limited hold period, had an average duration of 10 s (range 7.5–12.5 s).

Responses occurring during the ITI resulted in a further 12.5 s delay of the onset of the next trial. Stimulation intensities varied according to the psychophysical method of limits. Rats received four alternating series of ascending and descending current intensities, beginning with a descending series. Within each series, the stimulus intensity was altered by 5 μ A steps between each set of trials (three trials per set). The initial stimulus intensity was set at 30–40 μ A above the baseline current threshold for each rat. A test session typically lasted 30 min and provided current-intensity thresholds as the dependent variable. The threshold for each descending series was defined as the stimulus intensity between the successful completion of a set of trials (two consecutive sets), during which the rat failed to respond positively on two or more of the three trials for two consecutive steps. For the ascending series, the reverse situation defined the threshold. Current thresholds were recorded for each of the four series, and the mean of these values was taken as the threshold for that session. Baseline reward thresholds were considered stable when less than 10% variation occurred over 5 consecutive days. The latency between the onset of the noncontingent stimulus at the start of each trial and a positive response was recorded as the response latency. The response latency for each test session was defined as the mean response latency of all trials during which a positive response occurred.

2.7. Experimental design

2.7.1. Experiment 1. Effects of somatostatin-28 on spontaneous locomotor behavior—Rats ($n = 32$) were prepared with i.c.v. cannulae and injected with somatostatin-28 (0, 1.0, 3.16, or 10 μ g/5 μ l per side) 10 min before the test session according to a between-subjects experimental design. Nine rats were excluded from the analyses due to lost head mounts and misplaced cannulas resulting in $n = 4$ –8 per experimental group.

2.7.2. Experiment 2. Effects of somatostatin-28 alone or combined with the ssr_1 receptor antagonist SRA-880 on the startle response and PPI—Twenty-three rats from Experiment 1 and ten drug-naïve rats were used according to a between-subjects experimental design ($n = 33$ total; four groups, $n = 8$ –9/group). Drug-naïve animals were added in Experiment 2 as controls for potential carry-over effects of somatostatin-28 administration on startle and PPI in rats used after the completion of testing in Experiment 1. Rats were assigned to treatment groups in a counterbalanced manner so that each re-used rat received a different dose of somatostatin-28 than in Experiment 1, and the same number of previously used rats were assigned to each of the four groups in Experiment 2. Somatostatin-28 (0, 1.0, 3.16, or 10.0 μ g/5 μ l per side, i.c.v.) was administered 10 min before the test session.

The same subjects ($n = 33$) were used to evaluate the effects of the somatostatin-28 and SRA-880 combination on the startle response and PPI according to a between-subjects crossover experimental design. In the first crossover design, rats were administered SRA-880 (10 mg/kg, s.c.) or vehicle and somatostatin-28 (10 μ g/5 μ l per side, i.c.v.) or vehicle. In the second crossover design, rats were administered SRA-880 (20 mg/kg, s.c.) or vehicle and somatostatin-28 (10 μ g/5 μ l per side, i.c.v.) or vehicle. Pretreatment times were 10 min for somatostatin-28 and 45 min for SRA-880.

For the crossover design experiment, five rats out of 33 were excluded from the first part of the cross-over experiment resulting in $n = 28$, and six rats were excluded from these remaining 28 resulting in $n = 22$ for the second part of this cross-over experiment. These rats were excluded due to cannulae loss or blockage.

2.7.3. Experiment 3. Effects of somatostatin-28 and SRA-880 on brain reward function—Naive rats were prepared with i.c.v. cannulae and ICSS electrodes. Rats were trained in the ICSS procedure until stable baseline responding was achieved (less than 10% variation over 5 days). Rats were divided into two groups. Somatostatin-28 (0, 1.0, 3.16, or 10.0 µg/5 µl per side, i.c.v., $n = 8$) or SRA-880 (0, 1, 5, or 10 mg/kg, s.c., $n = 9$) were administered 10 min or 45 min, respectively, before the test session according to a within-subjects Latin square design for each of the two compounds.

2.8. Statistical analyses

All analyses were performed using the Biomedical Computer Programs for Personal Computers Statistical Package (BMDP, Los Angeles, CA) using the appropriate mixed-factor analysis of variance (ANOVA). Statistically significant interactions were followed by Newman Keuls *post hoc* test. The level of significance was set at $p < 0.05$.

2.8.1. BPM data analyses—The following variables were obtained from the raw data: the number of crossings, movements, holepokes and rearings; the number of entries; and the amount of time spent in a particular region (center, walls, and corners). Data were analyzed in 10 min time bins using a two-way ANOVA, with Time the repeated measure and Somatostatin-28 dose the between-subjects factor.

2.8.2. Startle and PPI data analyses—Startle habituation was calculated as the average response to all of the pulse-alone trials presented in blocks 1–4 during the test session. Prepulse inhibition was calculated as a percentage score for each prepulse trial type: % $PPI = 100 - ([startle\ response\ for\ prepulse + pulse] / [startle\ response\ for\ pulse-alone]) \times 100$. In PPI data analyses, startle response was calculated as the average response to all of the pulse-alone trials, excluding the first and last blocks of six pulse-alone trials. Startle amplitude and PPI values were analyzed using an ANOVA, with Drug dose (Somatostatin-28 and SRA-880) the between-subjects factor and Block and Prepulse intensities the within-subjects factors.

2.8.3. ICSS data analyses—ICSS thresholds and response latencies were expressed as a percentage of baseline values assessed on the day before the drug treatments. One-way ANOVA was used for data analyses, with Drug dose (e.g., four levels) as the within-subjects factor. For the testing of *a priori* hypotheses relating to the somatostatin-28-induced threshold elevations group comparisons were made using paired *t*-tests in addition to *post-hoc* tests after statistically significant effects in the ANOVAs.

3. Results

Twenty rats total were excluded from Experiments 1 and 2 because of cannulae misplacements, loss or blockage. For the remaining subjects, the tips of the injectors were all found to be within the lateral ventricle.

3.1. Experiment 1. Effects of somatostatin-28 on spontaneous locomotor behavior

The data on the effects of somatostatin-28 on spontaneous locomotor behavior is presented in Table 1. Overall ANOVA indicated a significant Time $\times$ Somatostatin-28 treatment interaction on the number of crossings ($F_{15,95} = 2.69$, $p < 0.001$), total movements ($F_{15,95} = 2.51$, $p < 0.05$), the number of corner entries ($F_{15,95} = 2.43$, $p < 0.05$), and the number of wall area entries ($F_{15,95} = 1.82$, $p < 0.05$). However, there was no Time $\times$ Treatment interaction for the number of center entries, the number of holepokes, or the number of rears (data not shown).

Additional ANOVAs were performed on each of six 10 min intervals for each behavioral measure, and a significant Time $\times$ Treatment interaction was found (Table 1). Somatostatin-28 significantly decreased the number of corner entries and tended to decrease the number of crossings and total movements during the first 10 min of testing (Table 1). However, *post hoc* analyses did not reveal any significant differences between treatment groups.

3.2. Experiment 2. Effects of somatostatin-28 alone or combined with the sst₁ receptor antagonist SRA-880 on the startle response and PPI

Somatostatin-28 administration had no effect on startle amplitude, indicating similar habituation of the startle response between treatment groups (Table 2). ANOVA of startle amplitude values revealed a significant effect of Block ($F_{4,116} = 47.31, p < 0.0001$) but no effect of Somatostatin-28 treatment ($F_{3,29} = 0.55, p > 0.05$) and no Treatment $\times$ Block interaction ($F_{12,116} = 0.37, p > 0.05$). ANOVA of the PPI data indicated a significant effect of Somatostatin-28 treatment ($F_{3,29} = 5.4, p < 0.01$) but no effect of Prepulse intensity ($F_{1,29} = 0.06, p > 0.05$) and no Somatostatin-28 treatment $\times$ Prepulse intensity interaction ($F_{3,29} = 1.24, p > 0.05$). *Post hoc* analyses revealed that the highest somatostatin-28 dose (10 $\mu\text{g}/5 \mu\text{l}$) significantly decreased PPI at both the 76 and 82 dB prepulse intensities (Newman-Keuls *post hoc* test, $p < 0.01$; Fig. 1A).

The low SRA-880 dose (10 mg/kg) had no effect on startle response habituation (Table 2). ANOVA of the startle amplitude values revealed no SRA-880 treatment effect ($F_{1,26} = 0.16, p > 0.05$) but a significant effect of Somatostatin-28 treatment ($F_{7,182} = 40.12, p < 0.001$) and a significant Somatostatin-28 $\times$ SRA-880 interaction ($F_{7,182} = 3.30, p < 0.01$). *Post hoc* comparisons did not reveal significant differences between treatment groups (Table 2). Somatostatin-28 decreased PPI ($F_{1,26} = 8.55, p < 0.01$), but this effect was significant only at a prepulse intensity of 73 dB (Fig. 1B). The low SRA-880 dose of 10 mg/kg had no effect on PPI when administered alone ($F_{1,26} = 0.22, p > 0.05$) or when combined with Somatostatin-28 (interaction effect: $F_{1,26} = 0.03, p > 0.05$). A significant effect of Prepulse intensity was observed ($F_{2,52} = 41.24, p < 0.0001$), independent of drug treatment (no Prepulse intensity $\times$ SRA-880 $\times$ Somatostatin-28 interaction: $F_{2,52} = 1.58, p > 0.05$).

The high SRA-880 dose (20 mg/kg) had no effect on startle response habituation (Table 2). ANOVA of the startle amplitude values revealed no SRA-880 treatment effect ($F_{1,20} = 0.25, p > 0.05$) but a significant effect of Somatostatin-28 treatment ($F_{7,140} = 16.51, p < 0.001$). No significant Somatostatin-28 $\times$ SRA-880 interaction was found (Table 2). Replicating the previous results, ANOVA indicated that somatostatin-28 decreased PPI ($F_{1,20} = 8.07, p < 0.01$), and this effect was significant at prepulse intensities of 76 and 82 dB (Fig. 1C). A strong tendency was observed toward SRA-880 (20 mg/kg) reversing PPI deficits induced by somatostatin-28 (SRA-880 treatment: $F_{1,20} = 3.93, p < 0.061$; Somatostatin-28 $\times$ SRA-880 $\times$ Prepulse intensity interaction: $F_{2,40} = 2.94, p < 0.064$; Fig. 1C).

3.3. Experiment 3. Effects of somatostatin-28 and SRA-880 on brain reward function

Mean group absolute values for ICSS thresholds and response latencies obtained on the days prior to somatostatin-28 administration ranged from $110.31 \pm 18.31 \mu\text{A}$ to $115.63 \pm 18.5 \mu\text{A}$ and from $3.15 \pm 0.18 \text{ s}$ to $3.40 \pm 0.26 \text{ s}$, respectively. Somatostatin-28 significantly elevated brain reward thresholds ($F_{3,21} = 3.34, p < 0.05$) at the highest doses of 3.16 and 10 $\mu\text{g}/\mu\text{l}$ compared with vehicle (Table 3). Somatostatin-28 treatment had no effect on response latencies ($F_{3,21} = 1.97, \text{n.s.}$).

Mean group absolute values for ICSS thresholds and response latencies obtained on the days prior to SRA-880 administration ranged from $150.37 \pm 18.95 \mu\text{A}$ to $159.58 \pm 25.12 \mu\text{A}$ and

from 3.05 ± 0.16 s to 3.21 ± 0.13 s, respectively. SRA-880 treatment had no effect on brain reward thresholds ($F_{3,24} = 2.77$, n.s., Table 3) or response latencies ($F_{3,24} = 1.10$, n.s., Table 3).

4. Discussion

In the BPM, i.c.v. injections of somatostatin-28 decreased spontaneous locomotor activity during the first 10 min after injection with no apparent changes in exploratory activity (i.e., the number of hole-pokes and rears) in rats. The highest somatostatin-28 dose (10 μ g/5 μ l per side) significantly decreased PPI with no effect on the amplitude of the acoustic startle response or startle response habituation. Administration of SRA-880 tended to reverse the somatostatin-induced deficits in PPI. SRA-880 had no effect on either the startle response or PPI when administered alone. Administration of somatostatin-28, but not SRA-880, induced elevations in brain reward thresholds, reflecting a decrease in brain reward function.

In the present study, i.c.v. administration of somatostatin-28 decreased spontaneous locomotor, but not exploratory activity, mainly due to decreases in crossings, corner entries and movements during the first 10 min of testing. Previously published findings on the effects of somatostatin on locomotor activity provided mixed results. Specifically, somatostatin, at doses of 1 μ g or 4 μ g i.c.v., increased locomotor activity, 10 μ g decreased rearings, and 6 nM decreased locomotor activity and rears during a 3 min open field test (Vecsei et al., 1984; Vecsei and Widerlov, 1988b, 1990). Additionally, administration of cysteamine, which is known to deplete somatostatin levels in the brain (Sagar et al., 1982), markedly decreased open field activity (Vecsei et al., 1989) and attenuated the motor response to dopaminergic receptor agonists after either systemic or central administration (Martin-Iverson et al., 1986; Lee et al., 1988). However, the somatostatin-induced increases in locomotion in these studies may be attributed to anxiety-related behaviors induced by the open field test (Holmes et al., 2002). Recent studies implicated somatostatins in anxiety and depression (Kluge et al., 2008; Pallis et al., 2009). Studies using *sst₂* receptor knockout mice reported decreased locomotor and exploratory responses in stress-inducing situations measured in an open field (Viollet et al., 2000) and unchanged levels of locomotor activity in undemanding tasks (Zeyda et al., 2001; Allen et al., 2003). Furthermore, recent findings showed anxiolytic- and antidepressant-like effects of i.c.v.-administered somatostatin in the elevated plus maze and forced swim test, respectively (Engin et al., 2008). In the fear-potentiated startle procedure, which assesses anxiety-related behaviors and is predictive of anxiolytic properties of drugs (Davis et al., 1993), octreotide blocked fear-potentiated startle, further implicating somatostatin in anxiety-like behaviors (Fendt et al., 1996) see also (Kluge et al., 2008). Thus, increases in locomotor activity after somatostatin administration can be observed in anxiogenic situations. In the present study, locomotor activity was assessed under no or low stress conditions, and somatostatin decreased spontaneous locomotor activity with no significant effects on exploratory behavior.

Additionally, differential effects of somatostatin on locomotor behavior have been shown to be brain site-specific. Intra-nucleus accumbens injections of somatostatin (3.2–100 ng/side) significantly increased locomotor activity, and this effect was mediated by *sst₂* receptors (Raynor et al., 1993). Somatostatin also dose-dependently increased locomotor activity when administered into the globus pallidus (Marazioti et al., 2008), decreased locomotor activity when administered into the ventral pallidum/substantia innominata (Marazioti et al., 2005), and had biphasic effects when administered into the caudate putamen (Tashev et al., 2001). Altogether, these findings demonstrate that somatostatin and its receptors located in nuclei of the basal ganglia are implicated in motor control and locomotion when somatostatin is administered into specific brain sites, but not after i.c.v. administration as in the present study.

The role of somatostatin in sensorimotor gating has not been studied extensively. Early work implicated somatostatins in the maturation of sensorimotor information processing by showing that chronic application of cysteamine, a sulfhydryl agent that decreases somatostatin levels, in developing rats significantly reduced startle amplitude in rats on postnatal day 18 (Kungel et al., 1996). In the present study, i.c.v. administration of somatostatin-28 had no effect on acoustic startle amplitude and habituation of the startle reflex. Our results are consistent with previous studies showing that octreotide had no effect on baseline startle amplitude when administered into the caudal pontine reticular nucleus (Fendt et al., 1996). Furthermore, somatostatin-28 induced PPI deficits when administered at the highest dose (10 µg/5 µl per side, i.c.v.). Somatostatin-induced increases in dopaminergic neurotransmission (Thermos et al., 1996; Hathway et al., 1998; Pallis et al., 2001; Rakovska et al., 2002; Rakovska et al., 2003; Marazioti et al., 2008) may underlie the observed PPI deficits, similar to the PPI-disruptive effects of psychostimulants, a putative model of the positive symptoms of schizophrenia (Geyer and Ellenbroek, 2003). Thus, somatostatin antagonists or depletors may exert dopamine-inhibitory effects, suggesting neuroleptic-like properties. Consistent with this hypothesis, cysteamine administration reversed amphetamine-induced PPI deficits (Feifel and Minor, 1997), similar to the reversal of psychostimulant-induced PPI deficits by typical and atypical antipsychotics (Geyer et al., 1990; Geyer et al., 2001; Ralph and Caine, 2005; Geyer, 2006). In the present study, the somatostatin-induced PPI deficit was partially reversed by SRA-880 administration. Somatostatin-induced increases in dopaminergic transmission have been shown to be mediated by sst₂ receptors (Hathway et al., 1999). SRA-880 is a selective sst₁ receptor antagonist with no effect on other types of somatostatin receptors; therefore, only partial reversal of somatostatin-induced PPI deficits by SRA-880 was observed in the present study. Alternatively, in the presence of inhibitory tone, the sst₁ autoreceptor antagonist SRA-880 may produce an increase in local somatostatin release (De Bundel et al., 2010), resulting in the partial reversal of somatostatin-induced PPI deficits. Administration of the sst₁ receptor antagonist SRA-880 alone had no effect on either startle amplitude or PPI. Similarly, systemic administration of cysteamine had no effect on baseline startle response or PPI (Feifel and Minor, 1997). Additionally, in genetically modified mice, both the acoustic startle response and the level of PPI did not differ between wildtype and sst₂^{lacZ/lacZ} knockout mice (Allen et al., 2003) or somatostatin peptide knockout mice (Zeyda et al., 2001).

In the present study, somatostatin-28 (3.16 and 10 µg/5 µl per side) induced threshold elevations, indicating deficits in brain reward function. SRA-880 had no effect on brain reward function at any dose tested. These results are consistent with previous studies showing that somatostatin induced robust decreases in self-stimulation behavior in the rate-frequency self-stimulation procedure (Vecsei et al., 1982, 1983; Balazs et al., 1988). Somatostatin-induced response depression in the rate frequency ICSS procedure was potentiated by co-administration with the dopamine D₂ receptor antagonist haloperidol, the GABA_A receptor antagonist bicuculline, the α adrenergic receptor antagonist phenoxybenzamine, and the rather nonselective serotonin 5-HT₁/5-HT₂ receptor ligand methysergide (Vecsei et al., 1982, 1983; Balazs et al., 1988). By contrast, atropine, a muscarinic receptor antagonist, partially reversed the effects of somatostatin (Vecsei et al., 1983). These findings clearly indicate that multiple neurotransmitter systems may be involved in the effects of somatostatin on brain reward function.

Dopaminergic neurotransmission is one the systems that mediate motivation and reward, suggesting its role in somatostatin-induced anhedonia. Drugs that block dopamine neurotransmission (e.g., dopamine receptor antagonists) are well known to induce reward deficits, reflected by elevations in brain reward thresholds (e.g., Baldo et al., 1999; Bruijnzeel and Markou, 2005). Conversely, drugs that indirectly facilitate dopamine

transmission (e.g., monoamine reuptake inhibitors) induced brain reward facilitation, reflected by decreases in brain reward thresholds (Lin et al., 2000; Kenny et al., 2003; Mague et al., 2005). *In vivo* microdialysis studies in rats showed that somatostatin increased dopamine release in the striatum and nucleus accumbens (Thermos et al., 1996; Hathway et al., 1998; Pallis et al., 2001; Rakovska et al., 2002; Rakovska et al., 2003; Marazioti et al., 2008). Several lines of evidence indicate that the nucleus accumbens shell is functionally associated with the mesolimbic dopamine system involved in reward processes, and the nucleus accumbens core is functionally related to the nigrostriatal dopamine system associated with motor function (Deutch and Cameron, 1992; Deutch, 1993; Pontieri et al., 1995; David et al., 2005; Bassareo et al., 2007). Thus, somatostatin-induced dopamine release in the striatum and nucleus accumbens core may mediate the effects of somatostatin on motor behavior. Although the effects of somatostatin on dopamine release in the nucleus accumbens shell are not known, based on the demonstrated somatostatin-induced reward deficits, somatostatin may decrease dopaminergic neurotransmission in the nucleus accumbens shell.

In conclusion, the results of the present study provide further information on the behavioral effects of somatostatin-28. Based on the demonstrated involvement of somatostatin-28 in brain reward function and sensorimotor gating, our findings suggest that increased somatostatin transmission may be one of the neurochemical mechanisms underlying anhedonia, one of the negative symptoms of schizophrenia, and sensorimotor gating deficits associated with cognitive impairments in schizophrenia patients.

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Figure 1.

Effects of somatostatin-28 alone (A) and combined with SRA-880 on prepulse inhibition (PPI). Data are expressed as mean % PPI $\pm$ SEM. PPI was tested at three prepulse intensities (73, 76, and 82 dB). SRA-880 at the low dose of 10 mg/kg (B) and high dose of 20 mg/kg (C) was administered either alone or combined with somatostatin-28 (10 μ g/5 μ l per side). * p < 0.05, ** p < 0.01, significant difference from control group (Newman-Keuls *post hoc* test).

Dose (1–10 µg/5 µl per side, i.c.v.)-response relationship of somatostatin-28 treatment on locomotor activity across a 60 min testing session.

Table 1

Dose	N	1–10 min	11–20 min	21–30 min	31–40 min	41–50 min	51–60 min
Crossings							
veh	4	244.0 ± 22.1	125.7 ± 20.4	61.5 ± 23.3	34.8 ± 20.6	2.8 ± 1.4	50.0 ± 29.3
1	7	160.9 ± 15.2	82.9 ± 15.0	59.3 ± 11.9	38.0 ± 10.7	28.1 ± 7.1	47.1 ± 10.3
3.16	4	193.5 ± 35.9	100.5 ± 29.0	63.2 ± 24.8	41.8 ± 19.3	55.5 ± 25.3	34.0 ± 10.5
10	8	143.2 ± 29.4	91.9 ± 15.7	66.9 ± 8.3	44.3 ± 11.4	25.6 ± 8.54	34.5 ± 9.8
		$F_{3,19} = 2.84$	$F_{3,19} = 1.0$	$F_{3,19} = 0.08$	$F_{3,19} = 0.11$	$F_{3,19} = 3.2$	$F_{3,19} = 0.41$
		$p < 0.065$, ns	ns	ns	ns	$p < 0.05$	ns
Wall region entries							
veh	4	209.8 ± 18.7	134.3 ± 16.8	73.5 ± 29.8	40.5 ± 20.1	7.5 ± 6.8	59.0 ± 31.8
1	7	170.1 ± 11.1	95.9 ± 14.7	71.0 ± 11.1	47.7 ± 12.9	36.6 ± 9.7	59.1 ± 11.0
3.16	4	168.8 ± 20.1	109.0 ± 21.3	70.0 ± 20.9	52.2 ± 30.2	57.0 ± 22.8	37.3 ± 8.1
10	8	148.8 ± 24.6	106.1 ± 11.3	83.5 ± 11.9	61.8 ± 17.1	38.5 ± 11.1	47.5 ± 13.3
		$F_{3,19} = 1.54$	$F_{3,19} = 1.0$	$F_{3,19} = 0.22$	$F_{3,19} = 0.36$	$F_{3,19} = 2.21$	$F_{3,19} = 0.44$
		ns	ns	ns	ns	ns	ns
Corner entries							
veh	4	145.0 ± 17.7	99.0 ± 5.0	54.3 ± 18.0	31.5 ± 13.2	7.5 ± 5.6	7.3 ± 3.7
1	7	128.3 ± 11.5	70.1 ± 10.3	53.9 ± 9.5	33.7 ± 8.3	27.0 ± 7.3	7.7 ± 1.4
3.16	4	109.75 ± 13.8	76.8 ± 17.1	52.7 ± 17.0	41.0 ± 7.5	43.8 ± 16.8	5.0 ± 1.2
10	8	90.9 ± 13.2	72.9 ± 8.0	58.4 ± 8.6	44.5 ± 11.4	31.9 ± 9.2	4.25 ± 1.4
		$F_{3,19} = 3.41$	$F_{3,19} = 1.55$	$F_{3,19} = 0.06$	$F_{3,19} = 0.36$	$F_{3,19} = 2.0$	$F_{3,19} = 1.16$
		$p < 0.05$	ns	ns	ns	ns	ns
Movements							
veh	4	656.5 ± 60.8	393.5 ± 54.4	207.8 ± 66.0	124.8 ± 62.1	20.5 ± 15.5	177.2 ± 97.4
1	7	482.0 ± 31.9	279.4 ± 41.8	185.0 ± 30.7	134.3 ± 29.9	91.1 ± 21.9	144.9 ± 29.9
3.16	4	499.8 ± 51.1	298.8 ± 57.8	203.8 ± 69.6	143.0 ± 50.0	154.5 ± 62.8	109.0 ± 33.3
10	8	412.8 ± 72.2	297.9 ± 34.6	222.1 ± 27.0	152.6 ± 36.8	96.4 ± 29.4	121.3 ± 31.9
		$F_{3,19} = 2.90$	$F_{3,19} = 1.28$	$F_{3,19} = 0.22$	$F_{3,19} = 0.10$	$F_{3,19} = 2.36$	$F_{3,19} = 0.43$
		$p < 0.062$, ns	ns	ns	ns	ns	ns

Values (including arbitrary units) are expressed as mean $\pm$ SEM. *N*, number of subjects per group; ns, not significant.

Table 2

Effects of somatostatin-28 (1–10 µg/5 µl per side, i.e.v.) alone or combined with SRA-880 (10 or 20 mg/kg, s.c.) on startle response amplitude.

Treatment	N	Block 1	Block 2	Block 3	Block 4
vehicle	8	1116.8 ± 220.3	403.5 ± 89.1	541.9 ± 142.5	372.5 ± 102.9
SST 1.0	8	954.6 ± 96.6	491.3 ± 115.5	485.1 ± 118.9	402.3 ± 71.5
SST 3.16	9	1083.5 ± 144.3	461.4 ± 112.5	461.2 ± 114.8	391.7 ± 75.0
SST 10.0	8	1239.4 ± 256.7	707.5 ± 159.7	631.0 ± 194.8	580.5 ± 179.2
vehicle+vehicle	8	856.3 ± 92.3	471.4 ± 73.4	412.6 ± 76.7	268.2 ± 58.9
vehicle+SRA-880 10.0	7	1107.5 ± 134.5	552.2 ± 120.3	362.5 ± 42.4	213.1 ± 33.5
SST 10.0+vehicle	7	1059.7 ± 148.2	500.0 ± 95.1	436.5 ± 90.1	270.8 ± 56.4
SST 10.0+SRA-880 10.0	6	766.9 ± 88.6	388.0 ± 62.1	317.9 ± 36.1	297.9 ± 56.1
vehicle+vehicle	6	455.3 ± 137.3	566.0 ± 170.6	469.6 ± 141.6	554.8 ± 167.3
vehicle+SRA-880 20.0	6	522.3 ± 174.1	494.5 ± 164.9	282.8 ± 94.3	643.0 ± 214.3
SST 10.0+vehicle	5	327.0 ± 98.6	334.3 ± 100.8	520.1 ± 156.8	455.8 ± 137.4
SST 10.0+SRA-880 20.0	5	548.3 ± 182.5	328.1 ± 109.4	198.2 ± 66.1	105.3 ± 35.1

Values (arbitrary units) are expressed as mean startle amplitude ± SEM. Startle response is presented in blocks 1–4. SST, somatostatin-28; N, number of subjects per group.

Table 3

Brain reward thresholds and response latencies after acute administration of somatostatin-28 and SRA-880.

Dose	Threshold (% baseline)	Response latency (% baseline)
<i>Somatostatin-28 (μg/5 μl, i.c.v., n = 8)</i>		
vehicle	100.07 ± 1.66	103.86 ± 3.28
1	104.52 ± 4.11	95.34 ± 3.15
3.16	121.72 ± 10.98*#	101.89 ± 4.27
10	117.95 ± 6.57#	105.25 ± 2.76
<i>SRA-880 (mg/kg, s.c., n = 9)</i>		
vehicle	105.92 ± 3.34	103.03 ± 3.68
1	105.18 ± 2.94	109.47 ± 3.83
5	113.75 ± 3.62	103.98 ± 3.48
10	103.93 ± 1.74	100.27 ± 3.03

Data are expressed as a percentage of baseline thresholds and response latencies measured the day before the drug treatment day (mean ± SEM).

* $p < 0.05$, significant difference from the corresponding vehicle condition (Dunnett's multiple comparison *post hoc* test);

$p < 0.05$, significant difference from vehicle condition (**paired *t*-test**).