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► **To cite this version:**

Thomas D. Prévôt, François Gastambide, Cécile Viollet, Nadia Henkous, Guillaume Martel, et al.. Roles of Hippocampal Somatostatin Receptor Subtypes in Stress Response and Emotionality. *Neuropsychopharmacology*, 2017, 42 (8), pp.1647-1656. 10.1038/npp.2016.281 . hal-03013941

**HAL Id: hal-03013941**

**<https://hal.science/hal-03013941>**

Submitted on 19 Nov 2020

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Accepted Article Preview: Published ahead of advance online publication



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**Cite this article as:** Thomas D Prévôt, François Gastambide, Cécile Viollet, Nadia Henkous<sup>1</sup>, Guillaume Martel, Jacques Epelbaum, Daniel Béracochéa, Jean-Louis Guillou, Roles of Hippocampal Somatostatin Receptor Subtypes in Stress Response and Emotionality, *Neuropsychopharmacology* accepted article preview 16 December 2016; doi: [10.1038/npp.2016.281](https://doi.org/10.1038/npp.2016.281).

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Received 22 July 2016; revised 30 October 2016; accepted 2 December 2016;  
Accepted article preview online 16 December 2016

## Roles of hippocampal somatostatin receptor subtypes in stress response and emotionality

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Short title: hippocampal sst receptors and emotionality (42 characters)

6 Key words: anxiety – depression – stress – sst<sub>2</sub> – sst<sub>4</sub> – corticosterone

Number of words:

Abstract : 218

Article body : 4040

Introduction: 518

References: 50

Number of Figures: 5

Number of Tables: 0

Supplemental information: 1

**Abstract.**

Altered brain somatostatin functions recently appeared as key elements for the pathogenesis of stress related neuropsychiatric disorders. The hippocampus exerts an inhibitory feedback on stress but the mechanisms involved remain unclear. We investigated herein the role of hippocampal somatostatin receptor subtypes in both stress response and behavioral emotionality using C57BL/6, wild type and *sst*<sub>2</sub> or *sst*<sub>4</sub> knockout mice. Inhibitory effects of hippocampal infusions of somatostatin agonists on stress-induced hypothalamo-pituitary-adrenal axis (HPA) activity were tested by monitoring peripheral blood and local hippocampus corticosterone levels, the latter by using microdialysis. Anxiolytic and antidepressant-like effects were determined in the elevated-plus maze, open field, forced swimming and stress-sensitive beam walking tests. Hippocampal injections of somatostatin analogs and *sst*<sub>2</sub> or *sst*<sub>4</sub>, but not *sst*<sub>1</sub> nor *sst*<sub>3</sub> receptor agonists produced rapid and sustained inhibition of HPA axis. *sst*<sub>2</sub> agonists selectively produced anxiolytic-like behaviors whereas both *sst*<sub>2</sub> and *sst*<sub>4</sub> agonists had antidepressant-like effects. Consistent with these findings, high corticosterone levels and anxiety were found in *sst*<sub>2</sub>KO mice and depressive-like behaviors observed in both *sst*<sub>2</sub>KO and *sst*<sub>4</sub>KO strains. Both hippocampal *sst*<sub>2</sub> and *sst*<sub>4</sub> receptors selectively inhibit stress-induced HPA axis activation but mediate anxiolytic and antidepressive effects through distinct mechanisms. Such results are to be accounted for in development of pathway-specific somatostatin receptor agents in the treatment of hypercortisolism (Cushing's disease) and stress-related neuropsychiatric disorders.

**Introduction**

Stress plays an important role in the pathogenesis of neuropsychiatric disorders such as major depression (Lin and Sibille, 2015a; McEwen *et al*, 2015; Pittenger and Duman, 2008). The main effect of stress is to activate the hypothalamo-pituitary-adrenal axis (HPA) leading to release of glucocorticoids from adrenal glands into the blood. Because of their liposolubility, glucocorticoids reach the brain by crossing the blood-brain barrier and bind to glucocorticoid and mineralocorticoid receptors (Joels, 2008). The hippocampus contains the highest concentration of glucocorticoid receptors and plays a key role for negative feedback regulations on the HPA axis (Herman *et al*, 2005; McEwen *et al*, 2015). High glucocorticoid levels contribute to hippocampal dysfunctions and may lead to hippocampal atrophy as observed in major depression or Cushing's disease (Andela *et al*, 2015; Sheline, 1996). Ablation of the dorsal hippocampus or lateral fornix disrupts the diurnal corticosteroid rhythm and elevates resting corticosteroids in rodents (Fendler *et al*, 1961). The intrinsic hippocampal mechanisms responsible for this effect remain unclear, but transynaptic glutamatergic projections from GABA neurons in the peri-paraventricular region and the bed nucleus of the stria terminalis may be involved (Herman and Mueller, 2006; Radley and Sawchenko, 2011). As mineralocorticoid and glucocorticoid receptors are predominantly expressed in principal cells, previous studies concerning corticosteroid effects on neuronal activity in the hippocampus focused mainly on these neurons (Joels, 2008). However, chronic stress also impairs GABAergic functions in the hippocampus by affecting the integrity of parvalbumin-expressing neurons (Hu *et al*, 2010) and somatostatin (SOM)-expressing cells (Czeh *et al*, 2015). In vivo, rapid SOM release occurs in the

dorsal dentate gyrus when acute stress or dexamethasone is applied to animals (Arancibia *et al*, 2001), suggesting a role for SOM in the hippocampal regulatory feedback on stress. In addition, mice lacking SOM exhibit elevated behavioral emotionality, high basal plasma corticosterone (CORT) levels and reduced gene expression of BDNF, cortistatin and GAD67, together recapitulating behavioral, neuroendocrine and molecular features of human depression (Lin *et al*, 2015a).

Five somatostatin receptor subtypes have been described, *sst*<sub>1</sub>, *sst*<sub>2</sub>, *sst*<sub>3</sub>, *sst*<sub>4</sub>, and *sst*<sub>5</sub>. The development of selective SOM analogs (Rohrer *et al*, 1998) and the availability of genetically modified animal models (Zeyda and Hochgeschwender, 2008) has contributed to a better understanding of individual *sst* receptor characteristics and their roles in brain functions and disorders including anxiety and depression (Engin *et al*, 2008; Engin and Treit, 2009; Epelbaum *et al*, 2009; Viollet *et al*, 2000). Recent studies identified a causal role for frontal and cingular SOM cells in modulating behavioral emotionality in mice (Lin *et al*, 2015a; Lin and Sibille, 2015b; Soumier and Sibille, 2014) and showed that SOM anxiolytic effects are mediated by *sst*<sub>2</sub> receptors expressed in the amygdala and the septum of the rat brain (Yeung *et al*, 2011; Yeung and Treit, 2012). While all *sst* receptor subtypes (except for *sst*<sub>5</sub>) are present in the hippocampus, SOM has been shown to modulate learning and memory formation through *sst*<sub>2</sub> and *sst*<sub>4</sub> receptors selectively (Gastambide *et al*, 2010; Gastambide *et al*, 2009; Guillou *et al*, 1993). The present study investigated the role of hippocampal SOM and its receptors in stress response and emotionality, an issue that has so far remained unexplored.

**Materials / subjects and Methods****Experimental goals**

The aims of the present study were first to determine whether any of the four sst receptor subtypes ( $sst_{1-4}$ ) expressed in the hippocampus inhibits stress-induced activation of the HPA axis and then, to investigate whether such inhibitory control could have anxiolytic or antidepressant properties. Intrahippocampal injections of selective sst receptor agonists were given prior to a stress event and plasma CORT elevation was measured. Given that plasma levels do not always parallel brain concentrations of glucocorticoids, microdialysis was conducted to assess CORT levels after sst agonist infusions and acute stress directly in the hippocampus. Then, independent groups of mice received sst agonists intrahippocampally and anxiety or depressive-like behaviors were assessed. Finally, further hippocampal microdialysis assays and emotional evaluations were conducted in  $sst_2$  or  $sst_4$  knockout mice.

**Animals**

Male mice of the C57Bl/6J strain were obtained from Charles River Laboratories (L'Arbresle, France).  $sst_2$  knockout ( $sst_2$ KO) strain was originally generated by Zheng et al. (Zheng *et al*, 1997).  $sst_4$  knockout ( $sst_4$ KO) mice were produced by integration (knockin) of LacZ gene into the *sstr4* gene (Helyes *et al*, 2009). All lines were backcrossed to the C57BL/6J background (N>11). KO and wild-type male littermates were obtained by intercrossing heterozygous +/- mice. Animals were bred in an animal facility equipped with an artificial 12h light/dark cycle (7:00AM on). Mice were 4-8-month-old at the beginning of the experiments. All experiments were conducted between 7h30 and 12h and in compliance with the

directive 2010/63/EU, Animal Care and Use Committee (Bordeaux) and approved under the number 5012098-A.

## **Surgery**

Mice were anesthetized with a ketamine (1mg/kg body weight)-xylazine (10mg/kg body weight) mix and placed on a stereotaxic frame. A microdialysis guide-cannulae (CMA/7 microdialysis probe, CMA microdialysis, Sweden) was implanted at the following coordinates from the bregma: AP: -2.2mm; L:  $\pm 1.35$ mm; V: 1mm. The laterality of the implantation was randomized between right and left side of the brain. Guide cannulae were fixed to the skull with dental cement (Palavit G, Promodentaire) and 3 screws (stainless steel,  $\varnothing$  0.5mm, L=1mm; FOM2000). For behavioral experiments, mice were implanted at the same coordinates with stainless steel guide-cannulae (Le Guellec tubular components, France). Sterile stylets were inserted in the cannulae to maintain patency. Mice were allowed to recover from surgery for 1 week before experiments.

## **Drugs**

Somatostatin analogs (RC160 and octreotide, TOCRIS, France), as well as selective agonists for sst<sub>1</sub> (L-797,591 Rahway, NJ, USA) sst<sub>2</sub> (L-054,264, TOCRIS, France), sst<sub>3</sub> (L-796,778 Rahway, NJ, USA) or sst<sub>4</sub> (L-803,087, TOCRIS, France) were all used at the dose of 5nmol. For microdialysis experiments, all drugs were diluted in artificial cerebrospinal fluid (aCSF) containing 10% of dimethylsulfoxide (Vehicle, Veh) and were delivered into the hippocampus via the microdialysis probe within 15 min. For other experiments, the drugs were prepared at the concentration of 5nmol/0.5 $\mu$ l based on previous experiments



(Gastambide *et al*, 2010) and were delivered into the hippocampus within 5 minutes in freely-moving animals as previously described (Martel *et al*, 2006). Injections were given 15 min before testing sessions.

### **Microdialysis**

Microdialysis was performed in freely moving animals to measure CORT levels in the dorsal hippocampus 1h before and 3h after acute stress as previously described (Dorey *et al*, 2012). The experiment was conducted by squad of 2 animals belonging to controls or experimental groups respectively. The day before the experiment, animals were individually placed in a 30cm-diameter chamber made of clear plexiglass walls and equipped with a grid floor. Animals had *ad libitum* access to their habitual chow and water. A microdialysis probe (CMA/7 membrane length 1mm;  $\varnothing=0.24\text{mm}$ ; CMA Microdialysis, Sweden) was inserted into the guide-cannulae. The membrane was 1mm longer than the guide-cannulae to be directly in contact with the hippocampal tissue. Probe was continuously perfused with sterile, filtered Dulbecco's solution (mock CSF, Sigma, France) at a rate of  $0.1\mu\text{l}/\text{min}$  for fluid equilibration during 1 night. After the equilibration phase, baseline dialysates were collected every 15 min with a flow rate of  $1\mu\text{l}/\text{min}$  during 1h. After baseline establishment, three inescapable foot-shocks ( $0.9\text{mA}$ ; 1sec) were applied from the grid floor within 1 min to provide stress. For pharmacological experiments, the perfusion was switched to another syringe containing the drug or the vehicle for 15 min after baseline. At the end of the injection, syringes were switched back to aCSF and the stress procedure was applied. The dialysates were collected every 15 min during 3h after stress. Samples were stored at  $-80^{\circ}\text{C}$  until free CORT determination.

**Plasma corticosterone collection**

Mice received an intrahippocampal injection of either one sst agonist or Veh solution in their home-cage. Fifteen minutes after the injection, 3 foot-shocks were delivered as described above and 15 minutes later blood was collected from the retro-orbital vessel under anaesthesia (isofluran) by a capillary tube. After centrifugation at 3500 r.p.m. for 10 min at 4°C, the supernatant was stored at –80°C until ELISA assay. An enzyme immunoassay commercial kit (Correlate-EIA, Assay Designs, Ann Arbor, MI) was used to measure plasma or hippocampal CORT concentrations. The sensitivity of the ELISA assay is 18.6 pg/ml. Baseline sample concentration was more than 10-fold above the sensitivity threshold. Data are expressed in pg/ml/15min for CSF and in ng/ml for plasma.

**Behavior****Motion recording during intrahippocampal microdialysis**

Each mouse was video recorded and its activity was quantified by videotracking (Viewpoint, Champagne au Mont d'Or, France). Three displacement thresholds were determined from pilot trials to quantify resting or very small motions, small motions and large motions respectively as function of pixel displacements/16ms. Changes of movement types were counted as a motion unit and analyzed per period of 15 minutes to assess the evolution of the activity from the baseline to the end of the post-stress evaluation.

**Elevated Plus-Maze**

The elevated plus-maze (EPM) apparatus was composed of grey polyvinylcarbonate and consisted of four arms surrounding a central platform. Each arm was 30cm long, 7cm wide, and 60cm above the ground. The four arms

joined at the center on a 7cm square platform. Two opposite arms of the plus maze ("closed arms") were bordered by 24cm-high sidewalls opened on the top, and the two other arms ("open arms") were exposed to a 70lux light intensity. At the beginning of each test, the mouse was placed in the center of the maze in a cylinder (7cm in diameter, 17cm high) for 30 sec. Then, the cylinder was removed and the animal was allowed to explore all arms of the maze freely for 8 min. An entry was counted when the mouse entered an arm with all 4 feet. The number of entries, times in open and closed arms, latencies to entry were measured.

### **Open-Field**

The open-field was a white circular arena (1m diameter) surrounded by a 25 cm-high wall made of opaque plexiglass. One lamp, 2 meters above the apparatus, provided 70 lux of illumination distributed equally over the entire surface of the apparatus. Animals were placed facing to the wall and were allowed to freely explore the apparatus for 5 min. The floor was virtually divided into a periphery annulus 12cm wide and a central zone. Exploration parameters were quantified by videotracking.

### **Forced Swim Test**

Mice were placed into a glass cylinder (diameter 15cm, height 29cm) containing 20cm of water at  $25 \pm 1^\circ\text{C}$ . The protocol used is that initially described by Porsolt et al. (Porsolt *et al*, 1977) in which the animal is maintained in water during 6 min. Given that mice are very active during the first two minutes and inactive during the last two minutes of FST, the times spent in swimming, climbing or immobility were analyzed during the last four minutes for pharmacology

experiments or during the first four minutes in knockout experiments, in order to unmask respectively antidepressant and depressive-like effects.

### **Stress-sensitive Beam Walking Test**

Mice were placed at the extremity of a 1-meter long beam, 80cm high from the floor. The start platform was narrow (3x4 cm) and exposed to intense light (120 lux) to encourage the animal to walk through the beam to reach the dark compartment. The floor was lined with foam to prevent hurting if animals fall down. The time to cross the beam and the number of hesitations (stop or slowing down) were recorded and quantified by the videotrack system. Animals were first trained for 3 days (3 trials/day) with beams of increased difficulties as a function of their shape and size: 10mm square beam, 15 mm round beam, 8mm square beam. A cut-off was applied if the mouse fell down from the beam or did not cross the beam in 2-min time. The next day, mice were injected with  $sst_2$  or  $sst_4$  agonists or vehicle 15 minutes before receiving a foot-shock stress as described above. Fifteen minutes later, mice were tested on the beams experienced during training and an additional beam (10 mm round) was introduced. Results are the mean of two successive trials for each beam. Rationales and data regarding the stress sensitivity of the task are presented in Supp3.

### **Histology**

At the end of the experiments, mice were anesthetized with 27mg/0.5ml of Sodium Pentobarbital and quickly decapitated. Entire heads were immersed in a solution of 10% formaldehyde at 4°C for two weeks. The brain was removed from the skull and was then cryoprotected and coronally sectioned into slices

(60µm thickness). A Thionin acetate stain was used to check the exactness the implantations.

### Statistical Analysis

Statistical analyses were performed using the Statview 5.0 software (Statistical Analysis System Institute Inc, NC, USA). The data were analyzed using ANOVA to determine main factor effects and followed by appropriate post-hoc tests. Mean group comparisons were also performed using Student's t-tests.

### Results

Plasma CORT levels increased by 4-fold following stress exposure relative to non-stressed conditions (Figure 1). Stress-induced CORT levels were lower following intrahippocampal injections of L-054,264 ( $t(11)=3.98$ ;  $P=0.002$ ) or L-803,087 ( $t(9)=2.94$ ;  $P=0.016$ ) but not of L-797,591 or L-796-778 agonists relative to stressed controls, indicating that hippocampal  $sst_2$  and  $sst_4$ , but not  $sst_1$  or  $sst_3$  receptors, contribute to inhibit stress-induced activation of the HPA axis. L-054,264 also reduced basal CORT levels significantly ( $t(8)=1.92$ ;  $P=0.04$ ) whereas L-803,087 was inefficient.

Hippocampal CORT concentrations sampled by microdialysis rose within one hour following stress onset in control mice (Figure 2) and progressively returned to baseline levels within the next two hours. SOM analogs RC160 and octreotide, known to target  $sst_{2-3-5}$  receptor subtypes attenuated stress-induced increase in hippocampal CORT levels (Supp2; Treatment effects  $F_s \geq 4.10$ ;  $P_s \leq 0.05$  for both analogs). The non-peptidic agonists L-054,264 and L-803,087 (Figure 3) targeting  $sst_2$  and  $sst_4$  respectively, fully blocked the elevation of CORT levels in the hippocampus following acute stress (treatment effects:

$F_s \geq 7.61$ ;  $P_s \leq 0.01$  for both agonists). Inhibitory effects started immediately after infusion ( $P_s \leq 0.01$  for both agonists) and lasted for at least 3 hours post stress onset ( $P_s \leq 0.05$  for both agonists). As indicated on Figure 2B, stress induced a transient peak of hyperactivity in all groups. Then, locomotor activity in stressed mice infused with either the *sst2* or *sst4* agonist rapidly recovered the level of non-stressed controls and was significantly lower relative to stressed controls (treatment effects:  $F_s \geq 6.18$ ;  $P_s \leq 0.02$  for both agonists), indicating that both *sst2* and *sst4* receptors attenuate stress-induced reactivity. However, further behavioral investigations revealed that each of these agonists alleviates stress-induced emotional responses differently (Figure 3). L-054,264 selectively reduced anxiety-like behaviors as assessed by exploration levels of open places either in the EPM ( $P \leq 0.009$  for both entries and time in open arms) or in the open-field ( $P = 0.01$ ). In the EPM, L-054,264 also shortened the latency to enter an open arm ( $P = 0.001$ ). Both L-054,264 and L-803,087 modified behaviors in the FST but the latter increased the attempts to escape by climbing on the wall of the glass cylinder ( $P = 0.04$ ) whereas the former increased the time spent in swimming ( $P = 0.02$ ). Finally, only L-803,087 improved performance in the beam walking test. The *sst4* agonist prevented both stress-induced hesitations ( $P = 0.007$ , data not shown) and increases in time ( $P < 0.01$ ) to cross the beams as a function of difficulty and novelty, further highlighting that hippocampal *sst2* and *sst4* receptors control different emotional behaviors.

Consistent with such findings, behaviors of *sst2*KO and *sst4*KO mice in the EPM and in the FST were altered in an opposite manner (Figure 4). Relative to wild type littermates, *sst2*KO mice displayed increased latency to enter an open arm ( $P = 0.04$ ) and lower exploration time in open arms ( $P = 0.001$ ) whereas no significant effect was detected between *sst4*KO and wild type. In the FST, both

$sst_2$ KO and  $sst_4$ KO mice displayed increased immobility relative to their wild type littermates ( $P=0.001$  and  $P=0.004$ , respectively). However, swimming decreased selectively in  $sst_2$ KO mice ( $P=0.002$ ) whereas climbing decreased selectively in  $sst_4$ KO mice ( $P=0.02$ ) indicating that active behaviors are qualitatively altered depending on genotypes.

Basal levels of plasma CORT were significantly higher in  $sst_2$ KO mice ( $P=0.04$ ) than in WT littermates and consistently, basal CORT levels in hippocampal dialysates were also higher in  $sst_2$ KO mice (Figure 5). A significant genotype effect was observed both before ( $F(1,16)=9.91$ ;  $P=0.006$ ) and after stress exposure ( $F(1,16)=6.86$  ;  $P=0.019$ ). The time lag to produce a significant increase (45 min,  $P=0.009$ ) was shorter and the maximal ( $P=0.02$ ) and recovery ( $P=0.01$ ) levels of CORT concentrations were significantly higher in  $sst_2$ KO than in WT, indicating that the HPA axis is constitutively up-regulated and is more reactive to stress in the absence of  $sst_2$  receptors. No change in CORT levels was observed in  $sst_4$ KO mice ( $P=0.78$ ). Finally, and to further investigate the inhibitory control induced by  $sst_4$  receptor activation, intrahippocampal injections of L-803,087 were tested in  $sst_2$ KO mice (Figure 5C). L-803,087 lowered stress-induced elevation of hippocampal CORT levels in  $sst_2$ KO mice ( $F(1,12)=5.76$ ;  $P=0.03$ ), thus confirming that hippocampal  $sst_4$  receptors contribute to the inhibition of the HPA axis. However, the inhibitory effect of L-803,087 occurred only later (+1h30) whereas it occurred immediately after stress onset in experiments with C57BL/6 mice (see Figure 2), indicating that the presence of hippocampal  $sst_2$  receptors is required for the fast-acting inhibition of the HPA axis by somatostatinergic neurotransmission.

## Discussion

The hippocampus is important for episodic and spatial memory but is now also recognized for its role in mood regulation (McEwen *et al*, 2015). There is evidence for a connection between hippocampal-HPA axis dysregulations and stress-related illnesses such as depression, generalized anxiety, post-traumatic stress disorders and memory deficits (Lucassen *et al*, 2014). Chronic stress exposure (Sapolsky, 2000), acute exposure to psychogenic stressors (Popoli *et al*, 2012) or acute therapies with high-dose of corticoids (Brown, 2009) negatively impact hippocampal neuroplasticity, neurogenesis and neuronal or glial survival in relationship with depression (Banasr and Duman, 2007; Banasr *et al*, 2011; Czeh and Lucassen, 2007; Pittenger *et al*, 2008). Studies have also linked depression to an increase in the excitatory-inhibitory ratio which may be attributed to a loss of GABAergic neurons. Parvalbumin and SOM subpopulations in both the dorsal and ventral hippocampus are particularly vulnerable to chronic stress exposure (Czeh *et al*, 2015). We observed a causal inhibitory effect of RC160, octreotide and selective  $sst_2$  or  $sst_4$  receptor agonists on the acute stress response, as measured by alterations in both plasma and hippocampal CORT levels, demonstrating the critical role played by SOM neurotransmission in inhibitory hippocampal feedback on the HPA axis. The levels and the time course of stress-induced increase in hippocampal CORT were comparable to previous experiments using the same protocol (Chauveau *et al*, 2010; Dorey *et al*, 2012; Tronche *et al*, 2010). Consistent with the finding that acute stress or dexamethasone produces rapid SOM release in the hippocampus (Arancibia *et al*, 2001), SOM inhibitory effects were detectable within 15 minutes after infusion, indicating that activation of the hippocampal somatostatinergic network by glucocorticoids controls the stress response rapidly.  $sst_2$  and  $sst_4$  receptor



agonists produced stronger effects than RC160 and octreotide, in agreement with better stability and longer half-life than peptidergic analogs (Rohrer *et al*, 1998). Mice reacted to footshocks by expressing a transient peak of locomotor activity which was comparable in all groups, indicating that sst receptor functions do not alter sensory thresholds in this context. Both sst<sub>2</sub> and sst<sub>4</sub> receptor agonists reduced locomotor activity, an inhibitory effect which occurred rapidly and persisted for the entire experiment. The time course of decreased activity in mice infused with sst<sub>2</sub> or sst<sub>4</sub> agonists suggests that the effect represents a more complete adaptation (habituation) to the aversive context than to be a motor deficiency. Accordingly, L-054,264 had no effect and L-803,087 improved performance in the beam walking test. Both agonists also had similar inhibitory effects on immobility in the FST. Interestingly, in this latter test, they produced different active behaviors, implying that distinct mechanisms underlie their behavioral effects. The sst<sub>2</sub> receptor agonist increased swimming behavior without affecting climbing behavior, a type of behavioral pattern which is characteristic of serotonergic activity. In sharp contrast, the sst<sub>4</sub> receptor agonist increased climbing behavior without affecting swimming as produced by antidepressants with noradrenergic activity (Detke *et al*, 1995; Reneric *et al*, 2001). Though this dissociation is less clear in mice than in rats, the behavioral profile produced by L-054,264 (decreased immobility/increased swimming) was similar to that previously observed in rats which received intracerebroventricular injections of SOM (Engin *et al*, 2008) or L-779,976, another sst<sub>2</sub> selective agonist (Engin *et al*, 2009). In addition, the hippocampal injection of sst<sub>2</sub>, but not the sst<sub>4</sub> agonist, also produced anxiolytic-like behavior in EPM and open field testing, corroborating the idea that hippocampal sst<sub>2</sub> and sst<sub>4</sub> regulate different emotional responses. These results indicate that the affinities for the sst receptor

subtypes of L-054,264 (1250-fold superior for  $sst_2$  than for  $sst_4$ ) and L-803,087 (6700-fold superior for  $sst_4$  than for  $sst_2$ ) are sufficient to produce selective behavioral changes (Rohrer *et al*, 1998; Yang *et al*, 1998).

Consistent with the pharmacological results and previous studies (Viollet *et al*, 2000),  $sst_2$ KO, but not  $sst_4$ KO mice displayed increased anxiety-like behaviors. CORT levels were also constitutively higher in  $sst_2$ KO mice in agreement with the increased ACTH release previously described in these mice (Viollet *et al*, 2000) and the increased basal level of CORT reported in SOMKO mice (Lin *et al*, 2015a), an effect undetected in  $sst_4$ KO mice. In addition, increased immobility in the FST associated with decreased swimming in  $sst_2$ KO mice whereas climbing was decreased in  $sst_4$ KO mice corroborate the idea that  $sst_2$  and  $sst_4$  regulate emotionality by different mechanisms. Recent data showing increased immobility in the FST in  $sst_4$ KO mice whereas intraperitoneal treatments with J-2156, another highly selective  $sst_4$  agonist, decreased immobility in the tail suspension test in C57BL/6 mice, support the idea that the  $sst_4$  receptor subtype is involved in depression-like behaviors (Scheich *et al*, 2016). We previously showed that injections of  $sst_4$  agonists in the dorsal hippocampus produced a shift from flexible cognitive to habit memory systems to rescue task performance (Gastambide *et al*, 2009) as similarly produced by stress (Schwabe and Wolf, 2013). Together with the present results, this suggests that dorsal hippocampal  $sst_4$  receptors control both emotional and cognitive responses to cope with stressful situations.

Previous results showed that the functionality of hippocampal  $sst_4$  receptors is lost following pharmacological blockade or knockout of  $sst_2$  receptors. L-803,087-mediated increases in glutamatergic excitability and bursting frequency in hippocampal CA1 region, as well as L-803,087-mediated

shifts toward the use of striatal memory systems are both blocked by  $sst_2$  antagonists or  $sst_2$  gene deletion (Cammalleri *et al*, 2006; Gastambide *et al*, 2010; Moneta *et al*, 2002). In line with these results we show here that in the absence of  $sst_2$  receptors, the  $sst_4$ -mediated inhibition on the HPA axis is lost during induction of the stress response while it remains effective during the stress recovery phase. Based on immunohistochemistry detections (Gastambide *et al*, 2010), binding experiments (Viollet *et al*, 2000) and mRNA mapping (Vanetti *et al*, 1994) in the mouse brain, the non-overlapping distribution of  $sst_2$  and  $sst_4$  respectively found in the dentate gyrus and in the CA1 subfield suggests that interactions between these sst receptor functions occur indirectly. The non-overlapping distribution may also account for the differential effects of  $sst_2$  and  $sst_4$  on anxiety-like and depressive-like behaviors. Insofar as the role of the CA1 subfield in emotions remains unclear, the present results suggest that dentate gyrus  $sst_2$  and CA1  $sst_4$  receptors conjointly control behavior in response to glucocorticoids by providing anxiolytic and antidepressant effects. Given the general consensus for involvement of the ventral hippocampus in regulation of stress responses and emotionality, it is interesting that the effects were produced by injections into the dorsal hippocampus. Whether the effects are relative to spread of injections in the ventral hippocampus and/or to intrahippocampal communication remains to be determined. Notwithstanding, a recent study demonstrated that optogenetic control of granule cells activity in the dorsal dentate gyrus selectively results in a dramatic increase in exploratory behavior in novel environments while affecting the ventral dentate gyrus causes an equally robust anxiolytic-like behavior (Kheirbek *et al*, 2013).

In conclusion, SOM is a key regulator of the HPA axis activity not only at the pituitary level but also at the brain level and besides cortical, hypothalamic,

amygdalar and septal involvements, hippocampal SOM neurotransmission critically controls both HPA function and emotionality through  $sst_2$  and  $sst_4$  receptors. This must be taken into account for the development and management of multiselective sst receptor ligands such as pasireotide which is currently used to control hypercortisolism in the Cushing's disease, a syndrome most often associated with anxiety, irritability, depression and memory troubles (Colao *et al*, 2014).

### **Funding and Disclosures**

This study was supported by the Région Aquitaine and by the Programme Interdisciplinaire du CNRS "Longévité et Vieillesse". The authors declare no competing financial interests or conflict of interest in relation to the work described.

### **Acknowledgements**

We thank the CPN and the INCIA small animal facility staffs for technical assistance and animal care. We thank Christophe Tronche, Una Avdic (Erasmus fellow), Marie Mennesson and Bertrand Beauchoux for their assistance during experiments. We also thank Dr. Gary Gilmour for helpful comments on the manuscript.

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## Legends to the Figures

### **Figure 1: Hippocampal infusions of $sst_2$ and $sst_4$ receptor agonists prior to an acute stress decrease the reactivity of the HPA axis.**

**A.** Animals received hippocampal infusion of Veh. (NS: Non-Stressed, N=5; and Stressed, N=6),  $sst_1$  (N=4),  $sst_2$  (N=7),  $sst_3$  (N=4) or  $sst_4$  (N=5) receptor agonists, 15 minutes before acute foot-shock stress. Plasma was collected 15 minutes after stress for ELISA analysis. Both  $sst_2$  and  $sst_4$  receptor agonists attenuated CORT response to acute stress exposure ( $P=0.0022$  and  $P=0.0164$ , respectively) whereas other agonists had no effect. The  $sst_2$  agonist (N=5;  $P=0.04$ ) but not the  $sst_4$  agonist (N=5) also reduced basal CORT levels in NS mice (**B**). Data are expressed in ng/ml, means + s.e.m. \* $P<0.05$ , \*\* $P<0.01$  relative to stressed Veh. or to NS Veh respectively in A and B (Student's *t*-test).

### **Figure 2: Hippocampal concentrations of CORT and general motion activity are decreased by infusions of both $sst_2$ and $sst_4$ receptor agonists.**

**A**-Time-course evolution of CORT level in the dorsal hippocampus sampled every 15 minutes for 1 hour before and 3 hours following acute stress. The bar on the time scales indicates the infusion of the sst agonist. The



grey arrow indicates when the acute foot-shock stress (S) was applied. Both  $sst_2$  (red square; N=10) and  $sst_4$  (blue triangle; N=7) receptor agonists rapidly decreased hippocampal CORT levels (within 15 minutes) and inhibited CORT elevation in response to acute stress as compared to stressed Veh. (white circle; N=16). The dots and gray lines represent the measures performed on controls which did not received foot-shocks (No stress, N=7). Results are expressed as mean  $\pm$  s.e.m. hippocampal CORT concentration in pg/ml/15min. **B**- Time-course evolution of stress-induced activity. Both agonists decreased stress reactivity in a long-lasting manner. Results are expressed as mean  $\pm$  s.e.m. of motion units/15min. \*P<0.05, \*\*P<0.01, compared to stressed Veh with Student's *t*-tests.

**Figure 3: Hippocampal  $sst_2$  and  $sst_4$  receptors regulate emotional behaviors differently.**

Elevated-plus maze, open field, FST and beam walking tests were performed on the same animals with intra-hippocampal Veh (N=10),  $sst_2$  (L-054,264; N=8) or  $sst_4$  (L-803,087, N=8) agonist infusions.

**A.** Anxiety-like behavior evaluated in the elevated-plus maze showed that infusion of L-054,264 increased the time spent and the open arms entries whereas infusion of L-803,087 had no effect. Data are expressed as mean percentage of time and entries (+s.e.m.) related to total time spent or entries in all arms during the test.

**B.** Anxiety-like behavior evaluated in the open-field showed that both L-054,264 and L-803,087 decreased latency to reach the center of the

arena but only L-054,264 increased the time spent in the center area. Data are expressed as mean latency to reach the center in seconds (+s.e.m.) and mean percentage of time spent in the center (+s.e.m.) relatively to the entire arena. **C.** Recording of the last four minutes in the FST, showed that L-054,264 increased the time spent in swimming whereas no effect was observed on climbing nor, albeit almost significant, on immobility ( $P=0.07$ ). L-803,087 decreased immobility to the benefit of increased climbing but not of swimming responses. Results are expressed in percentage of time spent (+s.e.m.) for each behavior. **D.** Beam Walking testing showed that the time to cross the beams was decreased by L-803,087 and reached a significant level in the hardest difficulty tested. Square or round and the associated number indicate the shape and the section size of the beam. Data are expressed as mean time (second +s.e.m.) to cross the beam.

\* $P<0.05$  and \*\* $P<0.01$  relative to the Veh group using Student's t-tests.

**Figure 4:  $sst_2$  and  $sst_4$  receptor gene deletion differently modulate anxiety- and depressive-like behaviors.**

Elevated-plus maze testing aimed at evaluating anxiety-like behaviors showed that the latency to enter an open arm (seconds) was increased while percentage of time spent in open arms was decreased in  $sst_2$ KO mice (**A**) but not in  $sst_4$ KO mice (**B**) relative to their WT littermates.  $sst_2$ KO, N=12; WT, N=12;  $sst_4$ KO, N=10 WT N=8.

Recording of the first four minutes of the FST [aimed at evaluating depressive-like behavior] revealed increased immobility in both *sst*<sub>2</sub>KO (**C**) and *sst*<sub>4</sub>KO (**D**) mice. Active behaviors differed between genotypes in that swimming decreased in *sst*<sub>2</sub>KO whereas climbing was affected in *sst*<sub>4</sub>KO mice. *Sst*<sub>2</sub>KO, N=12; WT, N=12; *sst*<sub>4</sub>KO, N=10; WT, N=10.

\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 relative to wild type littermates with Student's *t*-tests

**Figure 5: *sst*<sub>2</sub> gene deletion leads to elevated plasma and hippocampal CORT concentrations and L-803,087 decreases CORT levels only during recovery of acute stress.**

**A.** Basal plasma CORT concentration measured in *sst*<sub>2</sub>KO mice (-/-, N=8) was higher than in wild-type mice (+/+, N=5) but not in *sst*<sub>4</sub>KO mice (-/- N=8; +/+ N=12) **B.** Levels of CORT concentration measured in hippocampal dialysates were also higher in *sst*<sub>2</sub>KO (N=9) than wild-type (N=9) mice, both before and after the application of electric foot-shocks. **C.** Hippocampal infusion of the *sst*<sub>4</sub> agonist L-803,087 in *sst*<sub>2</sub>KO mice (N=5) did not alter the early increase in CORT levels (from 0 to 90 minutes) following acute stress. However, L-803,087 infusion accelerated recovery (from 90 to 180 minutes) and even decreased baseline CORT levels, as compared to Veh-infused *sst*<sub>2</sub>KO mice(N=9). The bar on the time scale indicates when L-803,087 was infused. The grey arrow indicates the time when acute stress was applied. Data are expressed as

mean CORT concentration (ng/ml for plasma levels or pg/ml/15min for intrahippocampal levels)  $\pm$  s.e.m. \*P<0.05, \*\*P<0.01 vs wild-type groups or relative to the Veh group using Student's t-tests.

Accepted manuscript











