International Journal of Neuropsychopharmacology (2012), **15**, 375–387. © CINP 2011
doi:10.1017/S1461145711000678

ARTICLE

CINP

Neuropeptide S alters anxiety, but not depression-like behaviour in Flinders Sensitive Line rats: a genetic animal model of depression

Gregers Wegener[1], Beate C. Finger[2,4], Betina Elfving[1], Kirsten Keller[1], Nico Liebenberg[1], Christina W. Fischer[1], Nicolas Singewald[3], David A. Slattery[4], Inga D. Neumann[4] and Aleksander A. Mathé[5]

[1] *Centre for Psychiatric Research, Aarhus University, Aarhus, Denmark*
[2] *School of Pharmacy, University College Cork, Cork, Ireland*
[3] *Department of Pharmacology and Toxicology & Centre of Molecular Biosciences Innsbruck, University of Innsbruck, Austria*
[4] *Department of Neurobiology, Institute of Zoology, University of Regensburg, Regensburg, Germany*
[5] *Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden*

Abstract

Neuropeptide S (NPS) and its receptor (NPSR) have been implicated in the mediation of anxiolytic-like behaviour in rodents. However, little knowledge is available regarding the NPS system in depression-related behaviours, and whether NPS also exerts anxiolytic effects in an animal model of psychopathology. Therefore, the aim of this work was to characterize the effects of NPS on depression- and anxiety-related parameters, using male and female rats in a well-validated animal model of depression: the Flinders Sensitive Line (FSL), their controls, the Flinders Resistant Line (FRL), and Sprague–Dawley (SD) rats. We found that FSL showed greater immobility in the forced swim test (FST) than FRL, confirming their phenotype. However, NPS did not affect depression-related behaviour in any rat line. No significant differences in baseline anxiety levels between the FSL and FRL strains were observed, but FSL and FRL rats displayed less anxiety-like behaviour compared to SD rats. NPS decreased anxiety-like behaviour on the elevated plus-maze in all strains. The expression of the NPSR in the amygdala, periventricular hypothalamic nucleus, and hippocampus was equal in all male strains, although a trend towards reduced expression within the amygdala was observed in FSL rats compared to SD rats. In conclusion, NPS had a marked anxiolytic effect in FSL, FRL and SD rats, but did not modify the depression-related behaviour in any strain, in spite of the significant differences in innate level between the strains. These findings suggest that NPS specifically modifies anxiety behaviour but cannot overcome/reverse a genetically mediated depression phenotype.

Received 30 November 2010; Reviewed 11 January 2011; Revised 24 March 2011; Accepted 30 March 2011; First published online 9 May 2011

Key words: Animal model, anxiety, gene expression, depression, NPS.

Introduction

Affective disorders belong to the diseases causing most chronic suffering and a major cause of suicide, while anxiety disorders belong to the most prevalent. In a recently published report with data from WHO Mental Health surveys, the days in which the study subjects were unable to normally perform their daily activities (days out of role) were assessed (Alonso *et al.* 2010). Psychiatric disorders, such as depression, bipolar disorder and anxiety disorders, accounted for a significant proportion of all days out of role. Together with the WHO Global Burden of Disease report (WHO, 2004), this highlights the severity of the problem, and prompts the search for novel strategies in therapeutics and prophylaxis.

The wide variety of neuropeptide effects in the regulation of central nervous system (CNS) functions have, in recent years, been exploited in CNS drug

Address for correspondence: Dr G. Wegener, Centre for Psychiatric Research, Aarhus University Hospital – Risskov, DK-8240 Risskov, Denmark.
Tel.: +45 7789 3549 *Fax*: +45 7789 3549
Email: wegener@dadlnet.dk

development (Chiou *et al.* 2007; Frank & Landgraf, 2008; Ring, 2005; Valdez, 2006; Varty *et al.* 2003). Thus, peptide receptors may constitute relevant drug targets for neuropsychiatric disorders, as exemplified by neurotensin receptor agonists in schizophrenia (Boules *et al.* 2007; Kinkead & Nemeroff, 2006) and corticotropin-releasing factor 1 or vasopressin receptor antagonists in depression and anxiety (Frank & Landgraf, 2008; Nielsen, 2006).

Recently, the receptor for a previously unknown, highly conserved (Reinscheid, 2007), 20-amino-acid peptide, neuropeptide S (NPS), was implicated in the regulation of anxiety and wakefulness (Xu *et al.* 2004). The orphan G-protein-coupled receptor was identified as the cognate NPS receptor (NPSR) (Mori *et al.* 2002). NPS acts as a NPSR agonist, increasing intracellular calcium and accumulation of cyclic adenosine monophosphate (cAMP) (Gupte *et al.* 2004; Xu *et al.* 2004).

In rodents, current evidence supports the existence of one NPSR (in contrast to the eight described human variants; Vendelin *et al.* 2005) that exhibits a high sequence similarity to the human hNPSR-A variant (Pulkkinen *et al.* 2006). In humans, a naturally occurring polymorphism, NPSR A/T ($Asn^{107}Ile$), in the NPSR has been described to result in an enhanced intracellular Ca^{2+} and cAMP signal transduction, which may be relevant in human asthma (Reinscheid *et al.* 2005). In a recently published study, the same polymorphism has been observed to have a role in panic disorder, proposed to be mediated through a heightened autonomic arousal and distorted processing of anxiety-relevant emotional stimuli (Domschke *et al.* 2010). Further, in an association analysis of genetic variants in NPS and NPSR1 in three independent study samples, NPSR1 was associated with panic disorder diagnosis and with parent-reported anxiety/depression (Donner *et al.* 2010). Similarly, carriers of the NPSR1T allele have been associated with over-interpretation of fear reactions (Raczka *et al.* 2010).

The mRNA encoding NPSR are expressed at high levels in several regions of the CNS, e.g. thalamus, hypothalamus, and amygdala (Leonard & Ring, 2011; Xu *et al.* 2004, 2007). Based on this distribution, NPS was proposed to be involved in arousal, regulation of food intake, and anxiety (Beck *et al.* 2005; Smith *et al.* 2006; Xu *et al.* 2004). These roles have been supported by behavioural studies, where centrally administered NPS decreases sleep, increases wakefulness (Xu *et al.* 2004), reduces food intake (Beck *et al.* 2005; Smith *et al.* 2006), and anxiety-related behaviours (Vitale *et al.* 2008; Xu *et al.* 2004). Consistent with these observations, NPS and NPSR have also been implicated in learning and memory (Meis *et al.* 2008). NPS also facilitates extinction of conditioned fear responses in an auditory-cued paradigm when administered into the amygdala in mice (Jungling *et al.* 2008). Furthermore, NPSR-deficient mice show significant deficits in inhibitory avoidance test compared to controls (Duangdao *et al.* 2009).

The role of NPS in depression-like behaviour has not been intensively investigated. However, central administration of NPS to mice did not show a pronounced effect in the tail suspension test (TST; Leonard *et al.* 2008) or forced swim test (FST; Guerrini *et al.* 2009). Depression is linked to cognitive deficits (Fossati *et al.* 1999, 2002; Harvey *et al.* 2004) and, interestingly, NPS improves cognitive performance in the novel object recognition task in mice (Duangdao *et al.* 2009) and facilitates spatial memory in the Morris water maze, when injected supraspinally (Li *et al.* 2009).

While the data from human polymorphism studies suggest that the NPS system may be involved in the aetiology of anxiety and depression, all the animal experimental findings are from 'normal' rodents thus making it difficult to draw translational conclusions. Consequently, we decided to test effects of NPS in a rodent model of depression.

The Flinders Sensitive Line (FSL) rat is a highly validated genetic animal model of depression (Overstreet *et al.* 2005; Wegener *et al.* in press). These animals present with exaggerated immobility in the FST, the prototypical screening procedure for antidepressant action and depression-like phenotype in rodents (Liebenberg *et al.* 2010; Porsolt *et al.* 1977, 1978; Slattery *et al.* 2005). Further, FSL animals present with several of the measurable characteristic features of 'clinical' depression, as well as increased stress responsiveness compared to their controls – the Flinders Resistant Line (FRL) and Sprague–Dawley (SD) rats, while they also respond to chronic, but not acute, treatment with antidepressants (Neumann *et al.* 2010; Overstreet *et al.* 2005; Wegener *et al.* in press). At the neurobiological level, the FSL rat displays multiple abnormalities consistent with proposed theories of depression, in particular altered serotonergic and cholinergic function as well as decreased expression of neuropeptide Y (Jimenez Vasquez *et al.* 2000, 2007; Mathé *et al.* 2007; Overstreet *et al.* 2005; Wegener *et al.* in press).

In view of the above, the aim of the current study was to investigate the possible role of NPS in anxiety and depression-like behaviours in FSL and FRL strains and an additional control, the SD rats. Therefore, we determined whether central administration of NPS differentially affected these behaviours in FSL, FRL

and SD rats, and if basal NPSR expression levels in hippocampus, amygdala, and periventricular hypothalamic nucleus (PVN) could contribute to the FSL phenotype. Further, we aimed to explore if such assessments, in a model of depression, could be useful tools in mapping the biological underpinnings discriminating between anxiety and depression. Finally, we evaluated possible gender differences in the behaviour measured by assessing the effect of NPS on female FSL, FRL, and SD animals' behaviour.

Methods

Animals

Male and female FSL and FRL rats (age 10–12 wk), from the colony maintained at the University of Aarhus (originally derived from the colony at the University of North Carolina, USA) and male and female SD rats purchased from Taconic A/S (Denmark) were used. All rats weighed 280–350 g at the start of experimental procedures, and were cage-housed individually (Cage 1291H Eurostandard Type III H, $425 \times 266 \times 185$ mm, Techniplast, Italy) at 20 ± 2 °C on a 12-h light/dark cycle (lights on 07.00 hours). Tap water and chow pellets were available *ad libitum*. The animal colony was protected from outside noise, and all experimental procedures were performed in specially equipped rooms within the animal house between 08:00 and 12:00 hours. All animal procedures were approved by the Danish National Committee for Ethics in Animal Experimentation (2007/561-1378).

Drugs

Rat NPS (H-Ser-Phe-Arg-Asn-Gly-Val-Gly-Ser-Gly-Val-Lys-Lys-Thr-Ser-Phe-Arg-Arg-Ala-Lys-Gln-OH) trifluoroacetate salt (Bachem AG, Switzerland) was diluted with Ringer's solution (145 mM Na, 3 mM K^+, 1.2 mM Ca^{2+}, 1.0 mM Mg^{2+}, pH adjusted to 7.4) to a final concentration of 0.05, 0.25, 0.5 or 1.0 nmol/5 μl (Smith *et al.* 2006). Vehicle animals received a 5 μl infusion of sterile Ringer's solution. All drugs were kept on ice during the experimental procedures. Drugs were freshly prepared daily from a frozen stock solution.

Stereotaxic surgery

All surgical procedures were performed under semi-sterile conditions. Prior to surgery rats were anaesthetized with fentanyl/fluanisone (VetaPharma Ltd, UK; 0.0945/0.3 mg/kg) and midazolam (0.25 mg/kg). The anaesthetized animals were fixed in a stereotaxic frame (David Kopf, USA) with the incisor bar set at −3.3 mm to give a flat skull surface and subsequently implanted with an intracerebroventricular (i.c.v.) guide cannula just above the right lateral ventricle (AP −1.0, L −1.6, DV −1.8, according to Paxinos & Watson, 1986), fixed with two stainless-steel screws and dental acrylic (GC Fuju Plus, GC Corp., Japan). A dummy was inserted into the guide cannula. Following surgery, the animals were injected with ampicillin (100 mg/kg s.c., 0.09 mg/kg buprenorphin s.c. (Schering-Plough A/S, Denmark) and 0.2 mg/kg Rimadyl (Pfizer A/S, Denmark), and returned to their home cage for recovery. The rats were monitored by daily weighing and handling, including removal of the dummy cannula in order to habituate the animals to the procedure of later intracerebral injection, thereby minimizing the stress reaction on the experimental day.

I.c.v. procedure

For the infusion, the upper part of a 25-mm-long 25-gauge cannula, was inserted into a 45-cm polyamine tubing (0.38 mm inner diameter, 1.09 mm outer diameter), which in turn was attached to a 25 μl Hamilton syringe (Hamilton Syringes, Swizerland). The infusion system was filled with 5 μl NPS or vehicle, which was then injected over a period of 1 min and left in place for 30 s to allow diffusion. Injection of NPS or vehicle was performed 45 min prior to the behavioural testing and the animal was returned to its home-cage for this period. Animals were randomly assigned to the subgroups and received the same treatment throughout all experiments.

Elevated plus-maze (EPM)

After a 7-d recovery from surgery, the rats were tested for anxiety-related behaviour on the EPM. A plus-shaped maze, elevated 70 cm above the ground, was constructed from black acrylic and consisted of two open arms and two closed arms (each length × width: 50×10 cm), connected by a neutral zone (10×10 cm). The closed arms were surrounded by 40 cm high walls, whereas the open arms had only a 0.5 cm high rim. Light intensity in the open arm was 80–90 lx whereas it was 20 lx in the closed arm. Behaviour of the animal on the maze during the 5-min testing period was monitored by a camera, mounted on the ceiling above and connected to a computer. The anxiety-related behaviours, including the percent of entries into open arms (%OAE) and the percent time spent on the open arms (%OA), the number of full entries (FE) into open arms (i.e. number of times the rat spent with the entire length of its body on the open arm) and

closed-arm entries (CAE) were subsequently assessed by an observer blind to the treatment. The number of entries into the closed arms was used as indicator of general locomotor activity.

Forced swim test (*FST*)

Five days after the EPM test the rats were exposed to the FST as previously described, using a modified version of the original protocol defined by Porsolt (Liebenberg *et al.* 2010; Porsolt *et al.* 1977, 1978; Slattery *et al.* 2005). Animals were subjected to a 15-min pre-swim and 24 h later a 5-min test session in a perspex cylinder (height 60 cm, diameter 24 cm) filled with water (25 °C) to a height of 40 cm. Water was changed between testing of each rat. Vehicle or NPS was injected 45 min before the test session. The depression-related behaviours including, struggling, swimming and floating during the 5 min on day 2 were blindly assessed according to previously described methods (Cryan *et al.* 2002).

Home-cage locomotor activity

In randomly selected animals, home-cage activity was measured 3–5 d after FST. The TSE Actimot system (Germany) was used. Briefly, following vehicle or NPS injection, each rat was returned to its home cage, which was subsequently placed inside the Actimot counting chamber for 2 h. The chamber consists of an aluminium frame equipped with infrared light beams, allowing continuous measurement of the animal's activity.

Tissue preparation

Separate groups of naive male FSL, FRL and SD rats were euthanized by decapitation (11:00–13:00 hours), the brains were quickly removed and frozen in a mixture of dry-ice and isopentane and, subsequently, cut (200-μm-thick slices) on a cryostat. The left and right hippocampi, amygdalae, and PVN were punched out using a brain puncher with an internal diameter of 1 mm (Stoelting, USA). The brain samples were stored in RNAse-free tubes at −80 °C until further analysis.

Measurements of mRNA transcripts with real-time quantitative polymerase chain reaction (*real-time qPCR*)

RNA extraction was performed with RNeasy Mini kit (Germany). RNA characterization, cDNA synthesis, and real-time qPCR were performed as described previously (Elfving *et al.* 2008).

Briefly, the punched tissue were homogenized in lysis buffer (Qiagen, Germany) with a mixer-mill (Retsch, Germany; twice for 40 s at 30 Hz/s). Total RNA was isolated following the manufacturer's instructions (Qiagen). Aliquots of the RNA solution were taken for both RNA quantification and quality assessment.

The integrity of RNA and the RNA concentration were determined with RNA StdSens microfluidic chips using the Experion Automated Electrophoresis System (Bio-Rad, USA). The RNA purity and the RNA concentration were determined with a NanoDrop spectrometer (Thermo Fisher Scientific, USA). To ensure the same RNA basal properties in the groups, data on quality, concentration, and purity of the extracted RNA from the FRL, FSL, and SD groups were compared with one-way ANOVA. Afterwards RNA was reversely transcribed using random primers and Superscript II Reverse Transcriptase (Invitrogen, USA) according to the manufacturer's instructions. The cDNA samples were stored undiluted at −80 °C until real-time qPCR analysis. They were diluted 1:30 with DEPC water before being used as a qPCR template.

Real-time qPCR

The real-time qPCR reactions were performed in 96-well PCR plates using an Mx3000P (Stratagene, USA) and SYBR Green. The gene expressions of NPSR1 and eight different reference genes [18 s subunit ribosomal RNA (*18 s rRNA*), β-actin (*ActB*), cyclophilin A (*CycA*), glyceraldehyde-3-phosphate dehydrogenase (*Gapd*), hydroxy-methylbilane synthase (*Hmbs*), hypoxanthine guanine phosphoribosyl transferase 1 (*Hprt1*), ribosomal protein L13A (*Rpl13A*), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (*Ywhaz*) were investigated. The reference genes were selected as previously described (Bonefeld *et al.* 2008). The primers were designed and tested according our previous work (Elfving *et al.* 2008), see Table 1.

The primers were obtained from DNA Technology A/S (Denmark). Each SYBR Green reaction (20 μl total volume) contained 1x SYBR Green master mix (Bio-Rad), 0.5 μM primer pairs, and 6 μl diluted cDNA. The mixture was heated initially to 95 °C for 3 min in order to activate hot-start iTaq DNA polymerase and then 40 cycles with denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 60 s were applied. To verify that only one PCR product was detected the samples were subjected to a heat dissociation protocol; after the final cycle of PCR, the reactions were heat-denatured by increasing the temperature from 60 °C to 95 °C. All samples and the

Table 1. The forward and reverse primers used in real-time PCR

Gene		Sequence	Base pair length
NPSR1	Forward	CTGTTCTCCATCCCCACACT	228
	Reverse	GCAGTTGGAAATCACCGTCT	
18 s rRNA	Forward	ACGGACCAGAGCGAAAGCAT	310
	Reverse	TGTCAATCCTGTCCGTGTCC	
ActB	Forward	TGTCACCAACTGGGACGATA	165
	Reverse	GGGGTGTTGAAGGTCTCAAA	
CycA	Forward	AGCACTGGGGAGAAAGATT	248
	Reverse	AGCCACTCAGTCTTGGCAGT	
Gapd	Forward	TCACCACCATGGAGAAGGC	168
	Reverse	GCTAAGCAGTTGGTGGTGCA	
Hmbs	Forward	TCCTGGCTTTACCATTGGAG	176
	Reverse	TGAATTCCAGGTGAGGGAAC	
Hprt 1	Forward	GCAGACTTTGCTTTCCTTGG	81
	Reverse	CGAGAGGTCC TTTTCACCAG	
Rpl13A	Forward	ACAAGAAAAAGCGGATGGTG	167
	Reverse	TTCCGGTAATGGATCTTTGC	
Ywhaz	Forward	TTGAGCAGAAGACGGAAGGT	136
	Reverse	GAAGCATTGGGGATCAAGAA	

standard curve were run in duplicate. A standard curve was generated on each plate.

Data analysis and statistics

Gene expression normalization was performed according to our recent work (Bonefeld *et al.* 2008; Elfving *et al.* 2010; Wegener *et al.* 2010). Briefly, we first measured mRNA levels for the eight reference genes. Stability comparison of the expression of the reference genes was then conducted with Normfinder software (Andersen *et al.* 2004). Values for each individual test gene were subsequently normalized with the optimal reference genes, based on the Normfinder mathematical algorithm (Andersen *et al.* 2004). The differential response to NPS within the FSL, FRL, or SD strains was analysed using one-way ANOVA or Student's *t* test (GraphPad Prism 5.0; USA). When appropriate, the ANOVA was followed by Bonferroni's *post-hoc* test. Intra-strain gender differences were assessed using Student's *t* tests. Differences were considered significant at $p<0.05$. Data in the figures are shown as $\pm$standard error of the mean (S.E.M.). The numbers of animals in each group are provided in the figures.

Results

Anxiety-related behaviour of FSL, FRL, and SD male and female rats in the EPM (Table 2)

The basal behaviour of male and female FSL/FRL and SD rats in the EPM is shown in Table 2 (the same basal values are also shown in Figs 1 and 2). We found no differences in anxiety-related behaviour between the FSL and FRL strains (both sexes), although in females, a trend to higher anxiety-like behaviour, as estimated by the percentage of open-arm exploration time (%OA), was found in FSL compared to FRL rats ($p=0.08$, $t=1.86$, d.f.$=16$, $p=0.081$). However, FSL and FRL males showed significantly less anxiety-like behaviour than SD males, as reflected by differences in the percentage of open-arm entries (%OAE) ($F_{2,24}=3.31$, $p=0.05$), %OA ($F_{2,24}=9.27$, $p<0.001$) and full entries into open arms (FE) ($F_{2,24}=3.63$, $p=0.04$). Similarly, female FRL animals showed less anxiety-like behaviour compared to female SD rats, as indicated by the significantly lower %OA ($F_{2,23}=6.75$, $p=0.005$). Gender differences could only be identified in FSL, but not FRL and SD, rats: FSL females showed significantly less anxiety-like behaviour compared to FSL males as reflected by an increased %OAE ($t=2.30$, d.f.$=17$, $p=0.034$).

Depression-related behaviour of FSL, FRL and SD male and female rats in the FST (Table 2)

The basal behaviour of FSL, FRL and SD animals in the FST (immobility) is shown in Table 2 (the same basal values are also shown in Figs 3 and 4). A comparison of the basal immobility confirmed the previously reported and expected differences between FSL and FRL males ($F_{2,29}=4.04$, $p=0.02$) with FSL males displaying more depression-like behaviour compared to FRL rats

Table 2. Basal behavioural values as measured in the elevated plus-maze (EPM) and forced swim test (FST)

	Males			Females		
Parameter	FSL	FRL	SD	FSL	FRL	SD
%OAE	41.91 ± 1.64	44.81 ± 6.88	36.38 ± 1.72[a]	50.63 ± 3.86[d]	49.63 ± 4.35	39.64 ± 3.15
%OA	41.60 ± 2.56	37.81 ± 6.89	19.80 ± 2.81[ab]	38.51 ± 6.78	56.58 ± 6.77	23.76 ± 5.29[c]
CAE	11.27 ± 1.05	11.43 ± 1.57	11.89 ± 1.36	7.75 ± 0.88[e]	9.10 ± 1.09	10.13 ± 0.83
FE	3.46 ± 0.42	2.14 ± 0.55	1.89 ± 0.46[a]	3.38 ± 0.94	4.10 ± 1.07	2.13 ± 0.67
Immobility	125.3 ± 10.76	77.14 ± 16.07	88.64 ± 13.50	58.57 ± 11.99[e]	22.14 ± 13.53[d]	56.20 ± 7.98[d]

%OAE, Percentage open-arm entries; %OA, percentage time spent on open arm; CAE, closed-arm entries; FE, full entries. Values shown are means ± S.E.M.
[a] $p < 0.05$ SD *vs.* FSL males; [b] $p < 0.05$ SD *vs.* FRL males; [c] $p < 0.05$ SD *vs.* FRL females (one-way ANOVA, Boferroni's *post-hoc*); [d] $p < 0.05$ gender difference; [e] $p < 0.01$ gender difference (Student's *t* test).

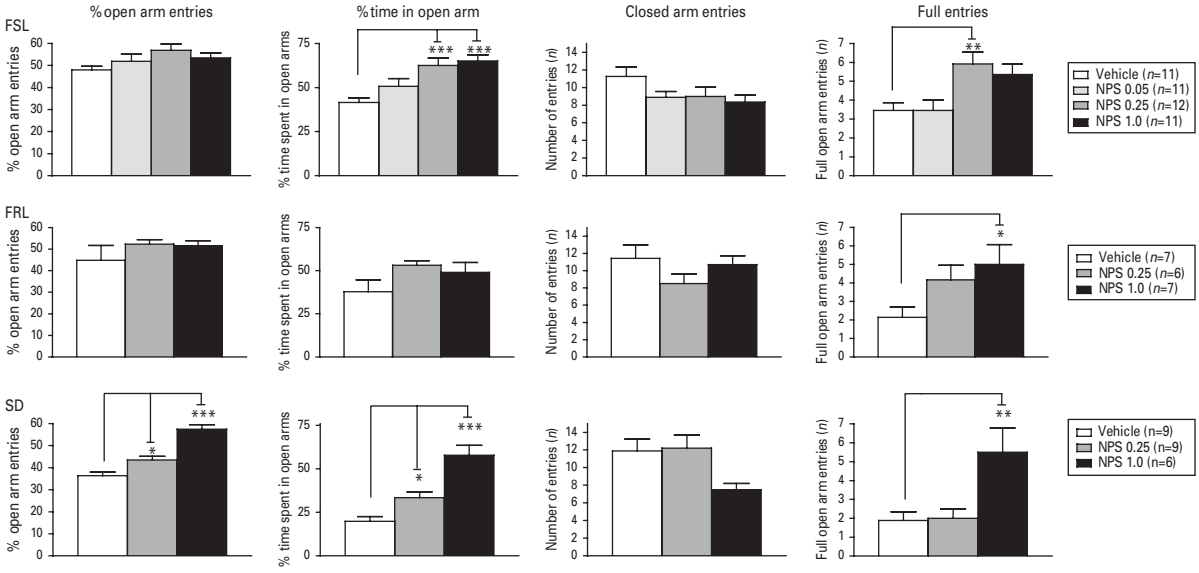

Fig. 1. The effect of different doses (in nmol/5 μl) of NPS on male elevated plus-maze behaviour in FSL, FRL, and SD rats. Data represent mean ± S.E.M. Two-way ANOVA, *post-hoc* Bonferroni's correction were performed (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

($p < 0.05$). Moreover, there was a tendency for FRL females to be less immobile than their FSL and SD counterparts ($F_{2,21} = 3.30$, $p = 0.057$). Regarding gender differences, female rats of all three strains showed lower depression-like behaviour compared to male rats as indicated by the lower immobility in female FSL ($t = 3.67$, d.f. = 21, $p = 0.0014$), FRL ($t = 2.62$, d.f. = 12, $p = 0.023$) and SD ($t = 2.12$, d.f. = 17, $p = 0.049$) rats.

Effects of NPS on anxiety-like behaviour on the EPM in FSL, FRL and SD rats

Males (*Fig. 1*)

As expected, NPS had a dose-dependent anxiolytic-like effect in all three rat strains when tested on the EPM. The effect was most pronounced in SD males, where differences were found in %OAE ($F_{2,21} = 32.32$, $p < 0.0001$), %OA ($F_{2,21} = 23.46$, $p < 0.0001$) and FE ($F_{2,21} = 7.26$, $p = 0.004$), with no effect on CAE ($F_{2,21} = 3.22$, $p = 0.06$), although it may be considered a trend. Bonferroni's *post-hoc* analysis revealed a dose-dependent increase in %OAE, %OA and FE after NPS, when compared to vehicle-treated controls.

NPS also affected anxiety-related behaviour in FSL male rats, specifically %OA ($F_{3,41} = 8.21$, $p = 0.0002$) and FE ($F_{3,41} = 5.52$, $p = 0.028$), but no differences in %OAE ($F_{3,41} = 2.07$, $p = 0.11$) and CAE ($F_{3,41} = 1.67$, $p = 0.13$). Bonferroni's *post-hoc* analysis revealed a dose-related increase in %OAE and FE after NPS

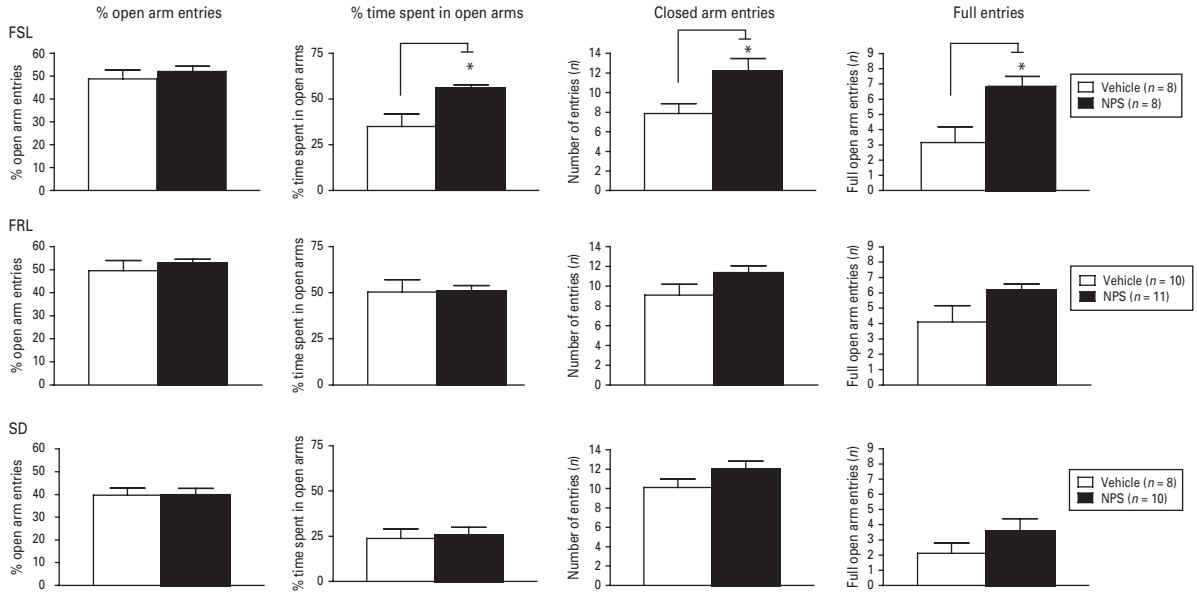

Fig. 2. The effect of NPS (1 nmol/5 μl) on female elevated plus-maze behaviour in FSL, FRL and SD rats. Data represent mean $\pm$ S.E.M. Student's t test was performed (* $p < 0.05$).

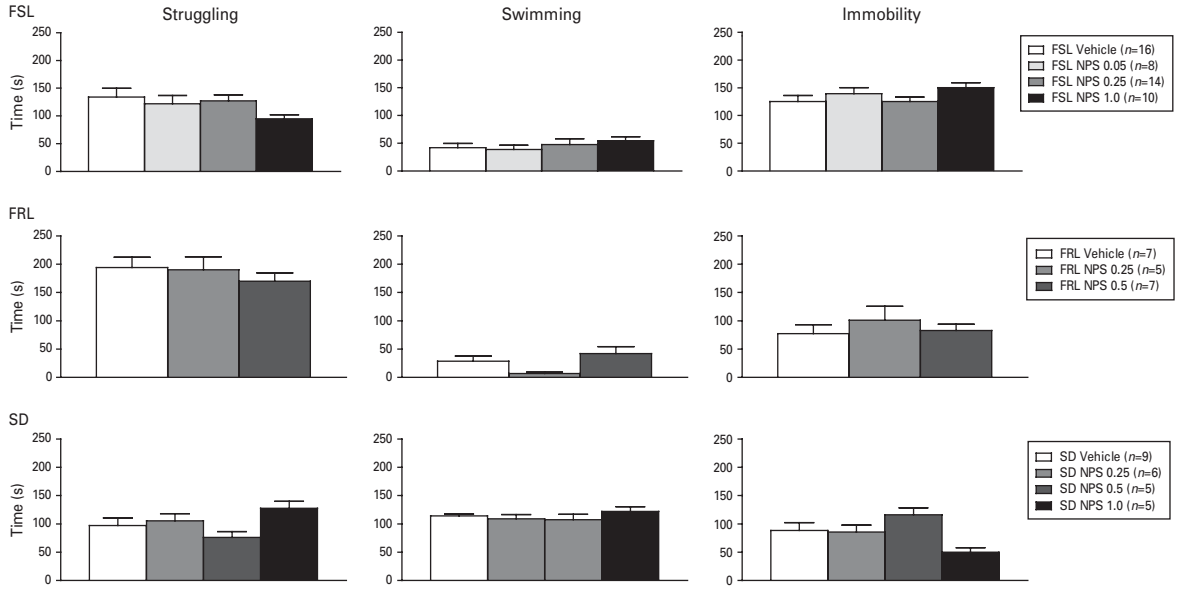

Fig. 3. The effect of different doses (in nmol/5 μl) of NPS on male forced swim test behaviour in FSL, FRL, and SD rats. Data represent mean $\pm$ S.E.M. Two-way ANOVA and *post-hoc* Bonferroni's correction were performed. No differences were observed.

(effective from 0.25 nmol/5 μl), when compared to vehicle-treated controls.

NPS also affected anxiety in FRL male rats, specifically FE ($F_{2,17} = 3.29$, $p = 0.05$), whereas %OA ($F_{2,17} = 2.02$, $p = 0.16$), %OAE ($F_{2,17} = 0.84$, $p = 0.44$) and CAE ($F_{2,17} = 1.36$, $p = 0.28$) were not altered.

Females (*Fig. 2*)

An anxiolytic effect of NPS was also revealed in female rats, but this effect was strain-dependent. Specifically, in FSL females (Fig. 2, top panels), NPS (1 nmol/5 l) caused an increase in %OA ($t = 2.95$, d.f. $= 7$, $p = 0.021$),

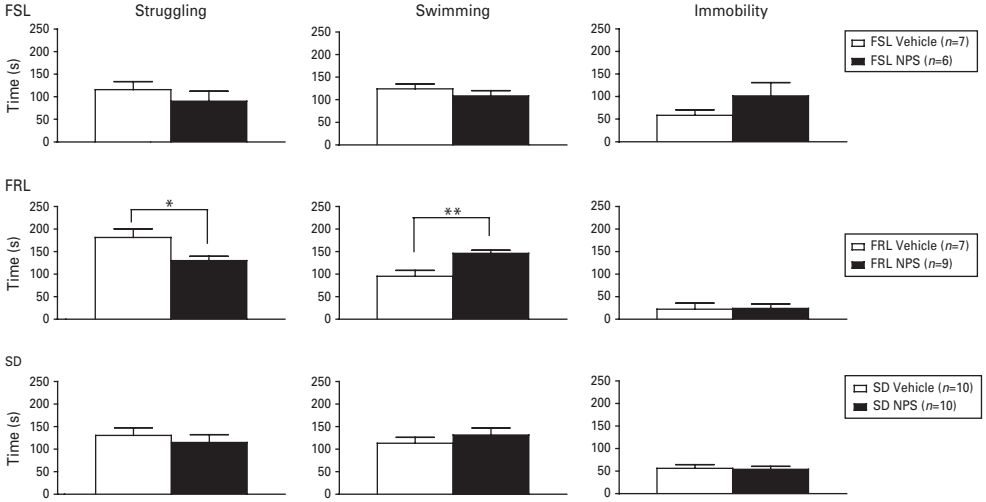

Fig. 4. The effect of NPS (1 nmol/5 μl) on female forced swim test behaviour in FSL, FRL and SD rats. Data represent mean $\pm$ S.E.M. Student's t test was performed (* $p<0.05$, ** $p<0.01$).

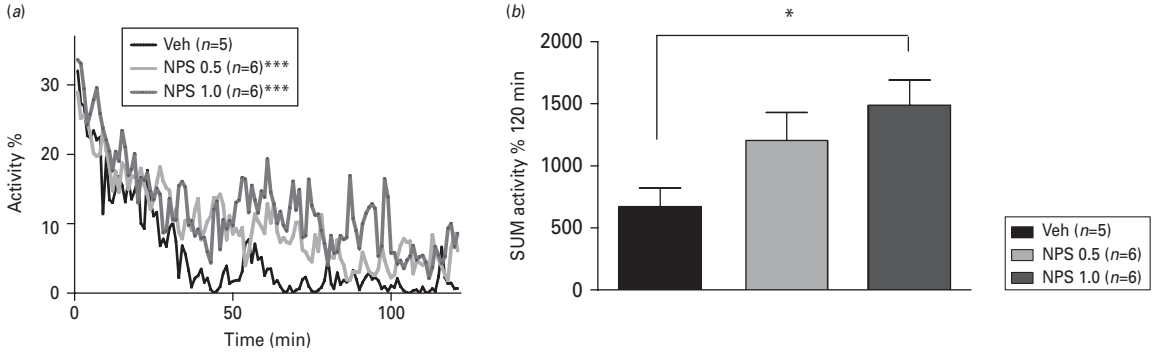

Fig. 5. The effect of two doses (in nmol/5 μl) of NPS on home-cage locomotion behaviour in (*a*) male FSL rats collected continuously over 120 min. One-way ANOVA with repeated measures and *post-hoc* Bonferroni's correction were performed (*** $p<0.001$, compared to controls). (*b*) Sum of activity during 120 min; data represent mean $\pm$ S.E.M. One-way ANOVA and *post-hoc* Bonferroni's correction were performed (* $p<0.05$).

but also influenced FE ($t=2.92$, d.f. $=9$, $p=0.016$) compared to control. In contrast, in FRL females (Fig. 2, middle panels), NPS (1 nmol/5 μl) only tended to increase the number of FE ($t=1.82$, d.f. $=11$, $p=0.095$) reflecting reduced anxiety, but failed to alter %OA ($t=0.09$, d.f. $=9$, $p=0.93$) and %OAE ($t=0.75$, d.f. $=11$, $p=0.46$).

Similarly, in SD females, NPS (1 nmol/5 μl) only tended to increase the number of FE ($t=1.425$, d.f. $=15$, $p=0.17$), but did not affect %OAE ($t=0.073$, d.f. $=15$, $p=0.94$) or %OA ($t=0.315$, d.f. $=13$, $p=0.76$) compared to vehicle-treated controls.

NPS also altered locomotor activity, particularly in female FSL rats where it increased CAE ($t=2.16$, d.f. $=9$, $p=0.028$). In contrast, in FRL and SD female rats, NPS only tended to increase locomotion (FRL: $t=1.745$, d.f. $=15$, $p=0.102$; SD: $t=1.582$, d.f. $=15$, $p=0.14$).

Effects of NPS on depression-like behaviour in the FST in FSL, FRL and SD rats

Males (*Fig. 3*)

Neither the high nor the low NPS dose modified any of the three components of depressive-like behaviour (struggle, swimming, immobility) in FSL, FRL, or SD male rats (Fig. 3). However, in SD rats, a difference in the dataset for immobility was observed ($F_{3,21}=3.53$, $p=0.03$). The highest NPS dose tended to reduce immobility; however, *post-hoc* testing did not reveal significant differences between the groups.

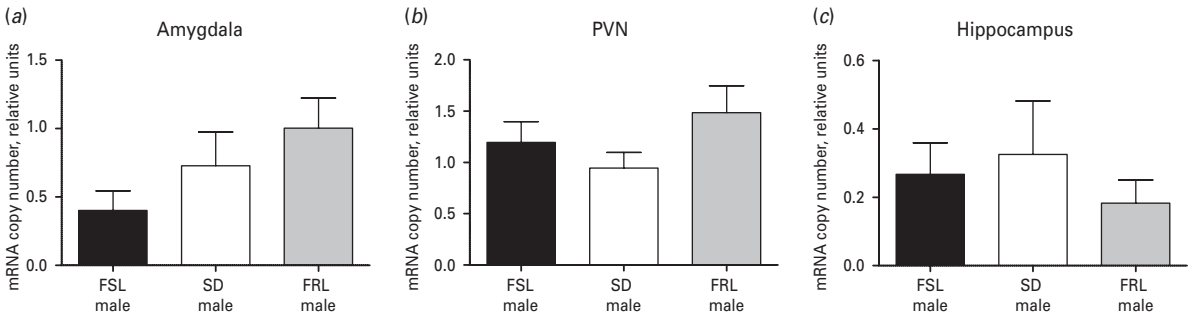

Fig. 6. Expression of NPSR1 in (*a*) hippocampus, (*b*) amygdala and (*c*) PVN of male FSL, FRL SD rats displayed as mRNA copy number. Data represent mean ± S.E.M. ($n = 6$/group). One-way ANOVA and *post-hoc* Bonferroni's correction were performed.

Females (*Fig. 4*)

In agreement with the findings in male FSL and SD animals, no overall effect of NPS (1 nmol/5 μl) on FST behaviours was observed in FSL or SD females. However, in FRL females, NPS resulted in a significant increase in swimming ($t = 3.61$, d.f. = 14, $p = 0.002$) and a decrease in struggling behaviour ($t = 2.58$, d.f. = 14, $p = 0.028$).

Effects of NPS on home-cage locomotion in FSL rats (*Fig. 5*)

Home-cage activity of FSL was recorded for 120 min directly following NPS infusion (Fig. 5). The percentage of activity/time was found to be altered in male FSL rats treated with NPS at 0.5 and 1 nmol/5 μl ($F_{2,120} = 59.77$, $p < 0.0001$). Bonferroni's *post-hoc* analysis revealed a significant difference between all groups ($p < 0.001$), and NPS increased locomotion in a dose-dependent fashion.

Expression of NPSR1 in hippocampus, amygdala, and PVN (*Fig. 6*)

Figure 6 shows the NPSR1 receptor mRNA copy number as measured in the hippocampus, amygdala, and PVN in naive male FSL, FRL, and SD animals. No difference in the expression pattern was evident in any of the areas, although a trend towards lower NPSR1 expression in FSL rats in the amygdala was found ($F_{2,22} = 1.94$, $p = 0.16$), and a simple comparison only between FSL and FRL rats using unpaired t test revealed a significant increase in NPSR1 receptor mRNA copy number in FRL male rats compared to FSL males ($t = 2.22$, d.f. = 13, $p = 0.045$).

Discussion

The main finding in the present work is the marked dose-dependent anxiolytic effect of NPS in male FSL, FRL, and SD rats without any effect on the depression-related parameters in any strain assessed. In contrast, while NPS was anxiolytic in female FSL and SD rats, it did not alter anxiety-related behaviour in female FRL rats. These results are in line with the previously reported anxiolytic action of NPS in male mice and rats, i.e. centrally administered NPS increased the time animals spent exploring the less protected or brighter areas of their respective environment (open field, four-plate test, EPM, elevated zero-maze, light-dark box) (Leonard *et al.* 2008; Rizzi *et al.* 2008; Xu *et al.* 2004) and extend them to female rodents for the first time. In addition, an overall anxiolytic-like effect of NPS has also been demonstrated, as NPS reduced the time mice spent burying unfamiliar objects (defensive marble-burying test) (Vitale *et al.* 2008; Xu *et al.* 2004). The reduction in anxiety-related behaviour after i.c.v. NPS was particularly pronounced in FSL and SD male rats, but not in FRL rats, demonstrating that the anxiolytic properties of NPS may indeed be dependent on the genetic background of the animals (Slattery *et al.* 2008; Wegener *et al.* 2008).

Of note, the basal behaviour in the EPM was similar in FSL and FRL strains whereas SD male and female rats displayed more anxiety-related behaviour than both Flinders strains. This result is in agreement with previous work (Schiller *et al.* 1991). and is of interest since it clearly separates the depressive-like and anxiety-like phenotypes Consistent with such reasoning and in line with the evidence that FSL is a good model of depression but not anxiety, administration of diazepam equally increased the time FSL and FRL rats spent in the open arms of the EPM (Overstreet *et al.* 1995). Young FSL rats (PND 40) display less anxiety in the open field and EPM compared to SD controls (Braw *et al.* 2006), as was also observed in the present study using adult rats. We are not aware of other studies directly comparing the anxiety status of FSL with SD controls, but the finding clearly highlights the

FSL/FRL model exclusively as a model of depression. This feature of the FSL indicates that the model also has a heuristic value in exploring the neurobiology of depression *vs.* anxiety, e.g. as shown in differences in neuropeptide Y expression in FSL and HAB strains, which display high anxiety and comorbid depression (Mathé *et al.* 2006). Further, HAB mice display anxiety without comorbid depression (Bunck *et al.* 2009).

As far as we are aware, this study is the first to investigate gender differences in anxiety-like behaviour between FSL and FRL animals, and gender-dependent effects of NPS. Therefore, the observation that FSL, but not FRL or SD females, present with increased anxiety-like behaviour in the EPM compared to their male counterparts is a novel and intriguing finding (see Table 2). However, the underlying mechanism(s) remains unresolved and this finding needs to be repeated in order to strengthen its reliability as a genuine characteristic of the FSL phenotype.

Interestingly, NPS exerted differential anxiolytic-like effects in male and female FSL, FRL and SD rats, whereas anxiolytic effects were found in male rats of all three strains, significant effects on anxiety levels could only be found in female FSL rats (Fig. 2). These gender-dependent effects of NPS are important for the development of further therapeutic strategies. However, the NPS effect was comparatively weak in FRL rats, and only detectable in the number of full entries into the open arms, whereas the %OA time and %OAE, the main established parameter of anxiety-related behaviour on the EPM, were unaffected by NPS. The neurobiological substrate underlying this finding remains obscure, but recent studies suggest that the amygdala is the core brain area responsible for NPS-mediated anxiolytic-like actions (Jungling *et al.* 2008; Meis *et al.* 2008; Xu *et al.* 2004), and strain differences regarding the amygdala response to NPS may underlie this result. Interestingly, our real-time qPCR results showed a trend for increased amygdala NPSR1 expression in male FRL compared to FSL rats. In addition, NPS produced a robust anxiolytic-like effect in male SD rats whereas this strain was shown to have intermediate levels of NPSR1 compared to FSL and FRL strains. Furthermore, the expression levels of NPSR1 in amygdala did not show a correlation with the behaviours in the EPM (data not shown).

NPS produced a significant hyperlocomotion in both male animals, when the home-cage locomotion activity was assessed during 2 h following i.c.v. injection of NPS. This is a confirmation of previous data, demonstrating that NPS increases arousal as indicated by hyperlocomotion, righting reflex and wakefulness (Leonard *et al.* 2008; Rizzi *et al.* 2008; Xu *et al.* 2004). In support, increased locomotor activity, as reflected by the increased number of entries into the closed arms of the EPM, was found in female rats, which reached statistical significance in female FSL rats. Therefore, it may be hypothesized that the anxiolytic-like effects of NPS are related to increased arousal. However, this finding does not agree with the finding that the CAE in the EPM, which is considered an indicative parameter of locomotion (Walf & Frye, 2007), were not affected by NPS in the three male strains tested. Thus, it is possible that NPS exerts its arousing effects only under non-stressful circumstances, i.e. in the home cage. This further suggests a specificity of the anxiolytic effect over locomotor effects. These results are of note, since they clearly separate effects of NPS from those of the classical antidepressants, both the tricyclics and the SSRIs, which are also anxiolytics. Moreover, our finding strengthens the suggestion that NPS agonists could be usable as a focused treatment for anxiety.

Epidemiological and clinical studies have shown a high degree of comorbidity between anxiety disorders and depression (Judd *et al.* 1998; Wittchen *et al.* 1994), and antidepressants are used routinely to treat symptoms of anxiety. It was, therefore, relevant to determine whether the behavioural effects of NPS also include antidepressant-like activity. Consistent with previous results, FSL rats compared to FRL rats showed greater immobility in the FST, confirming the well documented phenotype differences (El Khoury *et al.* 2006; Overstreet *et al.* 2005; Yadid *et al.* 2000). An interesting finding in the current study is that female FSL, FRL, and SD rats consistently showed reduced depression-like behaviour in the FST compared to their male counterparts (see Table 2). Similarly, a previous study reported decreased immobility in the FST for female FSL rats compared to FSL males (Kokras *et al.* 2009), whereas the same study found the opposite to be the case for SD rats. Furthermore, and similar to our results, other groups have found lower immobility in female Long-Evans (Mourlon *et al.* 2010) and Wistar (Barros & Ferigolo, 1998) rats compared to males. Together, these sex differences emphasize the importance of including both female and male subjects when evaluating depression-like behaviour using the FST.

Of note, NPS failed to influence depression-like behaviour in any of the doses given. This is in agreement with a previous study where NPS had anxiolytic-like effects, but did not influence the depression-like behaviour of mice in the tail suspension test (Leonard *et al.* 2008). In a recent review, a similar, but unpublished observation by Rizzi *et al.* using the FST is cited (Guerrini *et al.* 2009). The current study extends these

findings and demonstrates that NPS does not reduce depression-like behaviour in FSL rats, a genetic animal model of depression. In analogy to the anxiolytic-like effects, it was expected that hyperlocomotion following NPS administration would also enhance mobility in the FST. However, this was not observed, again suggesting that the arousing effects of NPS administration are more observable in non-stressful situations.

The brain structures and physiological mechanisms underlying the effects of NPS observed here remain to be established. Previously, it was reported that NPSR mRNA is expressed in several distinct brain areas related to stress responses, including amygdala, bed nucleus of the stria terminalis, hypothalamus, raphe nucleus, and ventral tegmental area (Leonard & Ring, 2011; Xu *et al.* 2004). However, in relation to a possible effect on depression-related parameters, it is relevant to emphasize that the density of NPSR is very low in hippocampus (Leonard & Ring, 2011; Xu *et al.* 2004), a structure of importance in depression (Lee *et al.* 2002; Videbech & Ravnkilde, 2004) and the FST (Airan *et al.* 2007). It is tempting to speculate that the absence of antidepressant properties of NPS is due to this distinct pattern in the regional NPSR expression.

In conclusion, our results confirm the FSL line solely as an animal model of depression and we demonstrate specific gender differences in this genetic model regarding behaviour in both the EPM and FST. In addition, we confirm that central NPS administration is anxiolytic and we extend this finding to a psychopathological animal model. Moreover, our data suggests that NPS may mediate distinct neurobiological aspects of anxiety in a gender-dependent way and can discriminate between anxiety and depression phenotypes.

Acknowledgements

G.W., B.E., N.L., K.K. and C.F.W. were supported by grants from The Danish Medical Research Council (grants 271-08-0768, 22-04-0566, and 271-08-1007), the Augustinus Foundation (grant 06-3280), Aase og Ejnar Danielsens fond and the Research Foundation for County Midtjylland. A.A.M. received support from the Swedish Medical Research Council, grant 10414 and the Karolinska Institutet. I.D.N., D.S., and B.F. received support from the Elite network of Bavaria, and Deutsche Forschungsgemeinschaft. N.S. was supported by the Austrian Science Fund (FWF): P22931-B18.

Statement of Interest

None.

References

Airan RD, Meltzer LA, Roy M, Gong Y, *et al.* (2007). High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. *Science* **317**, 819–823.

Alonso J, Petukhova M, Vilagut G, Chatterji S, *et al.* (2010). Days out of role due to common physical and mental conditions: results from the WHO World Mental Health surveys. *Molecular Psychiatry*. Published online: 12 October 2010. doi:10.1038/mp.2010.101.

Andersen CL, Jensen JL, Orntoft TF (2004). Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Research* **64**, 5245–5250.

Barros HM, Ferigolo M (1998). Ethopharmacology of imipramine in the forced-swimming test: gender differences. *Neuroscience and Biobehavioral Reviews* **23**, 279–286.

Beck B, Fernette B, Stricker-Krongrad A (2005). Peptide S is a novel potent inhibitor of voluntary and fast-induced food intake in rats. *Biochemical and Biophysical Research Communications* **332**, 859–865.

Bonefeld BE, Elfving B, Wegener G (2008). Reference genes for normalization: a study of rat brain tissue. *Synapse* **62**, 302–309.

Boules M, Shaw A, Fredrickson P, Richelson E (2007). Neurotensin agonists: potential in the treatment of schizophrenia. *CNS Drugs* **21**, 13–23.

Braw Y, Malkesman O, Dagan M, Bercovich A, *et al.* (2006). Anxiety-like behaviors in pre-pubertal rats of the Flinders Sensitive Line (FSL) and Wistar-Kyoto (WKY) animal models of depression. *Behavioural Brain Research* **167**, 261–269.

Bunck M, Czibere L, Horvath C, Graf C, *et al.* (2009). A hypomorphic vasopressin allele prevents anxiety-related behavior. *PLoS One* **4**, e5129.

Chiou LC, Liao YY, Fan PC, Kuo PH, *et al.* (2007). Nociceptin/orphanin FQ peptide receptors: Pharmacology and clinical implications. *Current Drug Targets* **8**, 117–135.

Cryan JF, Markou A, Lucki I (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in Pharmacological Sciences* **23**, 238–245.

Domschke K, Reif A, Weber H, Richter J, *et al.* (2010). Neuropeptide S receptor gene – converging evidence for a role in panic disorder. *Molecular Psychiatry*. Published online: 6 July 2010. doi:10.1038/mp.2010.81.

Donner J, Haapakoski R, Ezer S, Melen E, *et al.* (2010). Assessment of the neuropeptide S system in anxiety disorders. *Biological Psychiatry* **68**, 474–483.

Duangdao DM, Clark SD, Okamura N, Reinscheid RK (2009). Behavioral phenotyping of neuropeptide S receptor knockout mice. *Behavioural Brain Research* **205**, 1–9.

El Khoury A, Gruber SH, Mork A, Mathé AA (2006). Adult life behavioral consequences of early maternal

separation are alleviated by escitalopram treatment in a rat model of depression. *Progress in Neurobiology and Biological Psychiatry* **30**, 535–540.

Elfving B, Bonefeld BE, Rosenberg R, Wegener G (2008). Differential expression of synaptic vesicle proteins after repeated electroconvulsive seizures in rat frontal cortex and hippocampus. *Synapse* **62**, 662–670.

Elfving B, Plougmann PH, Muller HK, Mathé AA, *et al.* (2010). Inverse correlation of brain and blood BDNF levels in a genetic rat model of depression. *International Journal of Neuropsychopharmacology* **13**, 563–572.

Fossati P, Amar G, Raoux N, Ergis AM, *et al.* (1999). Executive functioning and verbal memory in young patients with unipolar depression and schizophrenia. *Psychiatry Research* **89**, 171–187.

Fossati P, Coyette F, Ergis AM, Allilaire JF (2002). Influence of age and executive functioning on verbal memory of inpatients with depression. *Journal of Affective Disorders* **68**, 261–271.

Frank E, Landgraf R (2008). The vasopressin system – from antidiuresis to psychopathology. *European Journal of Pharmacology* **583**, 226–242.

Guerrini R, Salvadori S, Rizzi A, Regoli D, *et al.* (2009). Neurobiology, pharmacology, and medicinal chemistry of neuropeptide S and its receptor. *Medicinal Research Reviews* **30**, 751–777.

Gupte J, Cutler G, Chen JL, Tian H (2004). Elucidation of signaling properties of vasopressin receptor-related receptor 1 by using the chimeric receptor approach. *Proceedings of the National Academy of Sciences USA* **101**, 1508–1513.

Harvey PO, Le Bastard G, Pochon JB, Levy R, *et al.* (2004). Executive functions and updating of the contents of working memory in unipolar depression. *Journal of Psychiatric Research* **38**, 567–576.

Jimenez-Vasquez PA, Diaz-Cabiale Z, Caberlotto L, Bellido I, *et al.* (2007). Electroconvulsive stimuli selectively affect behavior and neuropeptide Y (NPY) and NPY Y(1) receptor gene expressions in hippocampus and hypothalamus of Flinders Sensitive Line rat model of depression. *European Neuropsychopharmacology* **17**, 298–308.

Jimenez Vasquez PA, Salmi P, Ahlenius S, Mathé AA (2000). Neuropeptide Y in brains of the Flinders Sensitive Line rat, a model of depression. Effects of electroconvulsive stimuli and d-amphetamine on peptide concentrations and locomotion. *Behavioural Brain Research* **111**, 115–123.

Judd LL, Kessler RC, Paulus MP, Zeller PV, *et al.* (1998). Comorbidity as a fundamental feature of generalized anxiety disorders: results from the National Comorbidity Study (NCS). *Acta Psychiatrica Scandinavica* (Suppl.) **393**, 6–11.

Jungling K, Seidenbecher T, Sosulina L, Lesting J, *et al.* (2008). Neuropeptide S-mediated control of fear expression and extinction: role of intercalated GABAergic neurons in the amygdala. *Neuron* **59**, 298–310.

Kinkead B, Nemeroff CB (2006). Novel treatments schizophrenia: targeting the neurotensin system. *CNS and Neurological Disorders – Drug Targets* **5**, 205–218.

Kokras N, Antoniou K, Dalla C, Bekris S, *et al.* (2009). Sex-related differential response to clomipramine treatment in a rat model of depression. *Journal of Psychopharmacology* **23**, 945–956.

Lee AL, Ogle WO, Sapolsky RM (2002). Stress and depression: possible links to neuron death in the hippocampus. *Bipolar Disorders* **4**, 117–128.

Leonard SK, Dwyer JM, Sukoff Rizzo SJ, Platt B, *et al.* (2008). Pharmacology of neuropeptide S in mice: therapeutic relevance to anxiety disorders. *Psychopharmacology* (*Berlin*) **197**, 601–611.

Leonard SK, Ring RH (2011). Immunohistochemical localization of the neuropeptide S receptor in the rat central nervous system. *Neuroscience* **172**, 153–163.

Li W, Chang M, Peng YL, Gao YH, *et al.* (2009). Neuropeptide S produces antinociceptive effects at the supraspinal level in mice. *Regulatory Peptides* **156**, 90–95.

Liebenberg N, Wegener G, Harvey BH, Brink CB (2010). Investigating the role of protein kinase-G in the antidepressant-like response of sildenafil in combination with muscarinic acetylcholine receptor antagonism. *Behavioural Brain Research* **209**, 137–141.

Mathé AA, Biederbeck DI, Gruber S, El Khoury A, *et al.* (2006) Neuropetide Y, calcitonin gene-related peptide and CRH in brain of rats bred for high anxiety-related behaviors. *American College of Neuropsychopharmacology* (*ACNP*), *45th Annual Meeting* (pp. 51–52). Hollywood, Florida: American College of Neuropsychopharmacology (ACNP).

Mathé AA, Husum H, El Khoury A, Jimenez-Vasquez P, *et al.* (2007). Search for biological correlates of depression and mechanisms of action of antidepressant treatment modalities. Do neuropeptides play a role? *Physiology & Behavior* **92**, 226–231.

Meis S, Bergado-Acosta JR, Yanagawa Y, Obata K, *et al.* (2008). Identification of a neuropeptide S responsive circuitry shaping amygdala activity via the endopiriform nucleus. *PLoS One* **3**, e2695.

Mori M, Hayashi K, Miya H, Sato S, *et al.* (2002). Novel polypeptide; DNA thereof and use of the same. Patent application: PCT/JP2002/009446.

Mourlon V, Baudin A, Blanc O, Lauber A, *et al.* (2010). Maternal deprivation induces depressive-like behaviours only in female rats. *Behavioural Brain Research* **213**, 278–287.

Neumann ID, Wegener G, Homberg JR, Cohen H, *et al.* (2010). Animal models of depression and anxiety: what do they tell us about human condition? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. Published online: 1 December 2010. doi:10.1016/j.pnpbp.2010.11.028.

Nielsen DM (2006). Corticotropin-releasing factor type-1 receptor antagonists: the next class of antidepressants? *Life Sciences* **78**, 909–919.

Overstreet DH, Friedman E, Mathé AA, Yadid G (2005). The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. *Neuroscience & Biobehavioral Reviews* **29**, 739–759.

Overstreet DH, Pucilowski O, Rezvani AH, Janowsky DS (1995). Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression. *Psychopharmacology* (*Berlin*) **121**, 27–37.

Paxinos G, Watson C (1986). *The Rat Brain in Stereotaxic Coordinates*. Sydney: Academic.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. *European Journal of Pharmacology* **47**, 379–391.

Porsolt RD, Bertin A, Jalfre M (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie* **229**, 327–336.

Pulkkinen V, Majuri ML, Wang G, Holopainen P, *et al.* (2006). Neuropeptide S and G protein-coupled receptor 154 modulate macrophage immune responses. *Human Molecular Genetics* **15**, 1667–1679.

Raczka KA, Gartmann N, Mechias ML, Reif A, *et al.* (2010). A neuropeptide S receptor variant associated with overinterpretation of fear reactions: a potential neurogenetic basis for catastrophizing. *Molecular Psychiatry* **15**, 1067–1074.

Reinscheid RK (2007). Phylogenetic appearance of neuropeptide S precursor proteins in tetrapods. *Peptides* **28**, 830–837.

Reinscheid RK, Xu YL, Okamura N, Zeng J, *et al.* (2005). Pharmacological characterization of human and murine neuropeptide s receptor variants. *Journal of Pharmacology and Experimental Therapeutics* **315**, 1338–1345.

Ring RH (2005). The central vasopressinergic system: examining the opportunities for psychiatric drug development. *Current Pharmaceutical Design* **11**, 205–225.

Rizzi A, Vergura R, Marzola G, Ruzza C, *et al.* (2008). Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. *British Journal of Pharmacology* **154**, 471–479.

Schiller GD, Daws LC, Overstreet DH, Orbach J (1991). Lack of anxiety in an animal model of depression with cholinergic supersensitivity. *Brain Research Bulletin* **26**, 433–435.

Slattery DA, Desrayaud S, Cryan JF (2005). GABAB receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. *Journal of Pharmacology and Experimental Therapeutics* **312**, 290–296.

Slattery DA, Finger BC, Malumby R, Wegener G, *et al.* (2008). Neuropeptide S alters affective behaviour in high (HAB) but not low anxiety-related (LAB) rats. Washington, D. C.: Society for Neuroscience.

Smith KL, Patterson M, Dhillo WS, Patel SR, *et al.* (2006). Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. *Endocrinology* **147**, 3510–3518.

Valdez GR (2006). Development of CRF1 receptor antagonists as antidepressants and anxiolytics: progress to date. *CNS Drugs* **20**, 887–896.

Varty GB, Cohen-Williams ME, Hunter JC (2003). The antidepressant-like effects of neurokinin NK1 receptor antagonists in a gerbil tail suspension test. *Behavioural Pharmacology* **14**, 87–95.

Vendelin J, Pulkkinen V, Rehn M, Pirskanen A, *et al.* (2005). Characterization of GPRA, a novel G protein-coupled receptor related to asthma. *American Journal of Respiratory Cell and Molecular Biology* **33**, 262–270.

Videbech P, Ravnkilde B (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. *American Journal of Psychiatry* **161**, 1957–1966.

Vitale G, Filaferro M, Ruggieri V, Pennella S, *et al.* (2008). Anxiolytic-like effect of neuropeptide S in the rat defensive burying. *Peptides* **29**, 2286–2291.

Walf AA, Frye CA (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols* **2**, 322–328.

Wegener G, Finger BC, Slattery DA, Neumann ID, *et al.* (2008). Neuropeptide S alters depression- and anxiety-like behaviors in the Flinders Sensitive Line rats, a genetic animal model of depression. Washington, DC: Society for Neuroscience.

Wegener G, Harvey BH, Bonefeld B, Muller HK, *et al.* (2010). Increased stress-evoked nitric oxide signalling in the Flinders sensitive line (FSL) rat: a genetic animal model of depression. *International Journal of Neuropsychopharmacology* **13**, 461–473.

Wegener G, Mathé AA, Neumann ID (in press). Selectively bred rodents as models of depression and anxiety. In: Cryan, J, Reif, A (Eds), *Behavioral Neurogenetics*. Springer Verlag.

WHO (2004). The global burden of disease: 2004 update. *WHO Reports*.

Wittchen HU, Zhao S, Kessler RC, Eaton WW (1994). DSM-III-R generalized anxiety disorder in the National Comorbidity Survey. *Archives of General Psychiatry* **51**, 355–364.

Xu YL, Gall CM, Jackson VR, Civelli O, *et al.* (2007). Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. *Journal of Comparative Neurology* **500**, 84–102.

Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, *et al.* (2004). Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. *Neuron* **43**, 487–497.

Yadid G, Nakash R, Deri I, Tamar G, *et al.* (2000). Elucidation of the neurobiology of depression: insights from a novel genetic animal model. *Progress in Neurobiology* **62**, 353–378.