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Research report

Neurokinin-1 receptor deletion modulates behavioural and neurochemical alterations in an animal model of depression

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A B S T R A C T

The substance P/NK1 receptor system plays an important role in the regulation of stress and emotional responding and as such had been implicated in the pathophysiology of anxiety and depression. The present study investigated whether alterations in the substance P/NK1 receptor system in brain areas which regulate emotional responding accompany the depressive behavioural phenotype observed in the olfactory bulbectomised (OB) mouse. The effect of NK1 receptor deletion on behavioural responding and monoamine levels in discrete brain regions of the OB model, were also examined. Substance P levels in the frontal cortex and NK1 receptor expression in the amygdala and hippocampus were enhanced following olfactory bulbectomy. Although NK1 receptor knockout (NK1−/−) mice did not exhibit altered behavioural responding in the open field test, noradrenaline levels were enhanced in the frontal cortex, amygdala and hippocampus, as were serotonin levels in the frontal cortex. Locomotor activity and exploratory behaviour were enhanced in wild type OB mice, indicative of a depressive-like phenotype, an effect attenuated in NK1−/− mice. Bulbectomy induced a decrease in noradrenaline and 5-HIAA in the frontal cortex and an increase in serotonin in the amygdala, effects attenuated in OB NK1−/− mice. The present studies indicate that alterations in substance P/NK1 receptor system underlie, at least in part, the behavioural and monoaminergic changes in this animal model of depression.

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1. Introduction

Substance P and its preferred receptor neurokinin (NK)1, are members of the tachykinin family of neuropeptides which are widely distributed in both the central and the peripheral nervous system and regulate a number of physiological responses including inflammation, pain and emesis. Over the past decade a wealth of clinical and preclinical data has implicated the substance P/NK1 receptor system in the regulation of stress and emotion, and consequently the pathogenesis of depressive disorders. Substance P neurons and NK1 receptors are highly expressed in limbic brain regions such as the amygdala, hypothalamus and hippocampus [1,2]. Recent studies have shown that pharmacological blockade of NK1 receptors modulates emotional processing in healthy volunteers, effects associated with enhanced activation of limbic brain areas [3,4]. Chronic stress has been shown to enhance circulating plasma levels of substance P in healthy subjects [5] and depressed patients display elevated levels of substance P in the plasma [6] and CSF [7]. Preclinical studies have shown that chronic mild stress increases substance P mRNA in the medial amygdala and hypothalamus [8], chronic immobilisation stress reduces NK1 receptor expression in the hippocampus [9] and maternal separation induces increased NK1 receptor internalisation, a marker of substance P release, in the basolateral amygdala of guinea pigs [10]. In a genetic animal model of depression, the Flinders Sensitive Line rat, substance P immunoreactivity is enhanced in the frontal cortex and reduced in the striatum [11]. In comparison, low plasma and hypothalamic substance P levels have been shown in the Wistar Kyoto rat, a model that exhibits both a depressive and anxiety-like phenotype [12]. Thus, region specific alterations in substance P neurotransmission may be observed in different models of stress and depression.

Pharmacological and/or genetic antagonism of the NK1 receptor has been shown to attenuate stress-induced behaviour in several paradigms [13,14] and induce an antidepressant-like phenotype in tests such as the forced swim and tail suspension test [12,15,16]. However, few studies have examined the effect of NK1 receptor antagonism in models of depression that attempt to characterise

Abbreviations: SHT, serotonin; SHAA, 5-hydroxyindole-3-acetic acid; NK1, neurokinin 1; OB, olfactory bulbectomy; WT, wild type.

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the clinically depressed state. Of those that have, antagonism of the NK1 receptor reduced immobility of Wistar Kyoto rats in the forced swim test [12] while deletion of the Tac1 gene which encodes for substance P and neurokinin A, results in an antidepressant-like phenotype in the olfactory bulbectomised (OB) mouse model of depression [16]. Several NK1 receptor antagonists been developed as potential antidepressant treatments, and although initial reports were promising [10], research is still underway to develop antagonists that exhibit enhanced efficacy compared with existing treatments.

Interaction between the substance P/NK1 receptor and the monoamine neurotransmitter systems is well recognised [for reviews see 17,18]. For example, NK1 receptors are present on GABAergic and glutamatergic interneurons within the raphe nucleus, indicating that substance P modulates serotonergic activity indirectly [19–21]. Intra-dorsal raphe administration of substance P has been shown to decrease serotonergic cell firing [22] and decrease release of serotonin in the cortex [23]. Pharmacological and genetic antagonism of the NK1 receptor results in enhanced serotonergic neuronal firing [24–25] and NK1−/− mice exhibit downregulation of 5HT1A autoreceptors in the raphe nucleus [24,26]. Despite this, extracellular serotonin does not alter between NK1−/− and wild type mice [26] or following NK1 receptor antagonist [27,28]. However, following the administration of the selective serotonin re-uptake inhibitor, paroxetine, cortical extracellular serotonin is potentiated in NK1−/− mice [26] and following pharmacological antagonism of the NK1 receptor [29]. Thus increased clearance of serotonin from the extracellular space via serotonin transporters may mask the enhanced basal serotonin release in NK1−/− mice. In addition to the regulation of serotonergic neurotransmission, the substance P/NK1 receptor system modulates noradrenergic functioning. NK1 receptors are highly expressed on noradrenergic neurons within the locus coeruleus [19,30], antagonism of which has been reported to increase neuronal burst firing [31,32] and noradrenergic release in the terminal field [33,34]. In addition, NK1−/− mice exhibit desensitisation of somatodendritic α2a-adrenoceptors which account in part for the enhanced extracellular noradrenaline in the frontal cortex [35]. Thus, modulation of stress and emotional responding by NK1 receptor ligands may be mediated via interactions with the monoamine system.

The present study examined substance P levels and NK1 receptor density in discrete brain regions involved in regulating emotional responding in a preclinical model of depression, the olfactory bulbectomised (OB) mouse. This model exhibits behavioural, neurochemical, neuroendocrine and immune alterations which parallel those observed in the clinical setting [36,37], thus providing a well validated and clinically relevant model to investigate potential neurobiological substrates underlying depressive disorders. This study expands the evaluation of region specific changes in the substance P/NK1 receptor system in a further preclinical model of depression. Interaction between the NK1 receptors and the monoaminergic systems may underlie the altered stress responsivity of OB mice. As such the effects of NK1 receptor deletion on behavioural responding and central monoamine levels following bulbectomy were also examined.

2. Experimental procedures

2.1. Animals

Experiments were conducted on male C57BL/6j (Charles River, UK), NK1 receptor knockout (NK1−/−) (129/Sv x C57BL/6 hybrids, University College London, UK) and corresponding wild type (WT) mice. NK1−/− mice were generated by transfection of embryonic stem cells with a targeting vector designed to disrupt the NK1 receptor gene, as described previously [18] and the WT mice were littermate controls generated by heterozygous matings. All mice were matched for age and weight (weight at start of experiment 25–30 g). The animals were housed in plastic

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2.6. Western blotting for NK1 receptor density

Western blotting was carried out essentially as previously described [48,49]. Following protein determination, samples were diluted in ice-cold lysis buffer to give equal protein concentrations (10 μg/15 μl) followed by the addition of sample buffer (5 μl) (50 mM Tris–HCl, 1.84% SDS, 8% glycerol, 2% bromophenol blue, and 5% 2-mercaptoethanol). Lysates, with a total volume of 20 μl each containing 10 μg protein were then heated at 95 °C for 5 min. The proteins were then separated by SDS–PAGE using 12% polyacrylamide gels and electro-blotted onto a nitrocellulose membranes (0.2 μm; VWR International, UK). Membranes were rocked in blocking solution (5% milk, 0.5% Tween 20 in TBS) for 1 h followed by incubation in blocking solution containing rabbit anti-NK1 receptor (1:1000) (AB5060; Millipore, UK) overnight at 4 °C. This antibody has been raised against the C-terminus of the rat NK1 receptor and has been previously characterised for western blotting and immunohistochemistry [50,51] and specificity confirmed in our laboratory. The following day, membranes were then incubated at room temperature in secondary antibody HRP–goat–anti-rabbit (1:10,000 in blocking solution) for 1 h. Following three 5 min washes (0.5% Tween 20 in TBS), membranes were exposed to chemiluminescent reagents (Thermo Fisher Scientific Inc., Ireland) for 5 min followed by exposure to the G-BOX image analyzer (Syngene, UK) for 10–20 min. Membranes were subsequently stripped (25 mM glycine–HCl pH 2, 1% SDS) and re-blotted for β-actin (1:10,000; Sigma Aldrich, Ireland). Densitometric analysis of integrated band density was conducted using NIH Image J software. Background integrated density values were computed and subsequently subtracted from band integrated density values to obtain corrected integrated density values. Corrected integrated density values for NK1 were then normalised with their respective corrected integrated density of β-actin. Data were expressed as NK1 receptor density/β-actin density.

2.7. Immunohistochemistry for NK1 receptor expression

Brains were removed from sucrose-azide solution, rapidly frozen on dry ice and coronal sections of 30 μm were cut on a cryostat and collected in 0.1 M phosphate buffer (PB). Endogenous peroxidase activity was quenched by placing sections into 0.75% hydrogen peroxide (H2O2) for 20 min and immunohistochemistry carried out essentially as previously described [46,52,53]. In brief, sections were incubated for 1 h in 0.1 M PBS containing 0.3% Triton X (Tx) and 2% normal goat serum following 24 h incubation in the same buffer containing NK1 receptor antisera raised in rabbit (1:3000; AB5060; Millipore, UK). The specificity of this antibody has been previously demonstrated [50,51] and confirmed in our laboratory. The incubated sections were subsequently washed and incubated for 90 min in biotinylated goat–anti-rabbit antisera (1:200; Jackson ImmunoResearch Europe, UK). This was followed by incubation in the avidin–biotin–peroxidase complex (ABC) (1:200; Vector Laboratories Ltd, UK) for a further 90 min sections were subsequently rinsed and incubated for 5 min in 0.05% H2O2 in PB for 5–10 min for a brown reaction product that was terminated by rinses in PB. The sections were mounted on glass-gelatinised slides and air-dried. Slides were dehydrated in graded alcohols, cleared with xylene and coverslipped with DePex mounting medium. Photomicrographs were taken with an Olympus microscope BX51 and Olympus C5060 digital camera and densitometric analysis preformed using NIH Image J software and expressed as corrected integrated density (ID).

2.8. HPLC analysis of brain tissue monoamine concentrations

Monoamine levels in discrete regions (frontal cortex, amygdala, hypothalamus and hippocampus) were assessed as previously described [54,55]. In brief, tissue was sonicated in 1 ml of mobile phase (0.1 M Citric Acid, 0.1 M NaH2PO4, 1.4 mM 1-octane sulphonic acid, 0.01 mM EDTA, 10% methanol; pH 2.8) containing 2 ng/20 μl N-methyl-5-HT as an internal standard. Homogenates were centrifuged at 4 °C for 15 min at 14,000 × g. A 20 μl sample of supernatant was injected onto Shimadzu HPLC with a reverse-phase C18 column (Lichrosorb RP-18; Phenomenex, UK) using an electrochemical detector at 1.0 μl/min and fed to determine peak heights of monoamines and their metabolites. The electrode was maintained at −0.8 V and the flow rate of the mobile phase through the system was 1 ml/min. Peak heights for standards of 2 ng/20 μl noradrenaline, serotonin and 5-hydroxyindole-3-acetic acid (5-HIAA) (Sigma–Aldrich, Dublin, Ireland) were obtained each month prior to injection of samples and after every 10 samples. The data were calculated as ng neurotransmitter/g of tissue.

2.9. Statistical analysis

SPSS (version 17.0) statistical package was used to analyse all data. Behavioural, neurochemical and western blotting data were normally distributed and analysed using either unpaired t-test (Sham vs. OB) or two-factor analysis of variance (ANOVA) with the effects of OB and NK1 receptor density. Post hoc analysis was performed by Fisher’s LSD test when appropriate. Data were considered significant when P < 0.05. Results are expressed as group means ± standard error of the mean (± SEM).

3. Results

3.1. OB-induced behavioural hyperactivity is associated with alterations in substance P levels and NK1 receptor expression in discrete brain regions

OB mice displayed a characteristic increase in distance moved on exposure to the open field test, when compared to Sham-operated controls (t26 = 4.65 P < 0.001; Fig. 1a). Examination of whether this OB-related hyperactivity was associated with alterations in substance P neurotransmission revealed that OB mice exhibited increased substance P levels in the frontal cortex [t15 = 3.15 P = 0.007], but not other brain regions examined, when compared to Sham-operated controls (Fig. 1b). Western blotting analysis revealed an increase in NK1 receptor density in the amygdala [t10 = 2.75 P = 0.021] and hippocampus [t10 = 3.40 P = 0.008] but not
in the hypothalamus or frontal cortex, of OB mice when compared to Sham-operated controls (Fig. 1c). Further immunohistochemical analysis revealed that the increase in NK1 receptor density in the amygdala was confined to the medial amygdaloid nuclei [Sham: 21.48 ± 1.16 ID vs. OB: 30.80 ± 1.45 ID; t4 = 5.02 P = 0.007] (Fig. 2).

3.2. NK1 receptor knockout mice exhibit reduced OB-related increase in nocturnal home cage activity and distance moved in the open field

On exposure to a novel open field arena, OB WT mice exhibited a significant increase in distance moved, confirming development of the OB syndrome [OB: F1,37] = 55.39 P < 0.001; Fig. 3a and b]. Deletion of the NK1 receptor reduced distance moved of Sham-operated mice when compared to their wild type counterparts, however this effect failed to reach statistical significance. In comparison, OB NK1−/− mice demonstrated a significant reduction in distance moved in the open field when compared to wild type counterparts [F1,37] = 7.81 P = 0.008; OB vs. OB NK1−/− P < 0.01]. Temporal analysis revealed that although NK1−/− mice did not display enhanced locomotor activity in the open field test for the first minute of exposure, distance moved was comparable between OB NK1−/− and OB WT by the 3rd minute of the test (Fig. 3b), demonstrating that NK1−/− mice exhibit a delayed OB-induced increase in locomotor activity [time: F2,74] = 21.63 P < 0.001; time × OB: F2,74] = 9.25 P < 0.001; OB: F1,37] = 55.41 P < 0.001; genotype: F1,37] = 7.81 P = 0.008]. Examination of exploratory behaviour in the open field revealed that OB WT mice exhibited increased time in the centre of the arena [OB: F1,37] = 6.76 P = 0.013; Sham WT vs. OB WT P < 0.01], an effect not observed in OB NK1−/− mice (Fig. 3c).

3.3. Alterations in noradrenaline concentration in discrete brain regions following olfactory bulbectomy and/or NK1 receptor deletion

Olfactory bulbectomy significantly reduced noradrenaline concentration in the frontal cortex of wild type and NK1 receptor knockout mice [OB: F1,32] = 28.03 P < 0.001] when compared to their Sham-operated counterparts (Sham WT vs. OB WT P < 0.05) (Table 1). Deletion of the NK1 receptor resulted in increased noradrenaline levels in the amygdala [genotype: F1,33] = 9.69 P = 0.004], hippocampus [genotype: F1,32] = 12.73 P = 0.001] of Sham-operated mice (Sham WT vs. Sham NK1−/− P < 0.05), an effect not observed following bulbectomy (OB WT vs. OB NK1−/−). In comparison, OB NK1−/− mice exhibited a significant increase in noradrenaline levels in the frontal cortex when compared to wild type counterparts [genotype: F1,32] = 7.89 P = 0.008; OB WT vs. OB NK1−/− P < 0.05], and a slight but nonsignificant increase Sham-operated controls (Sham WT vs. Sham NK1−/−). There was no effect of bulbectomy or NK1 receptor deletion on noradrenaline levels in the hypothalamus.

3.4. Alterations in serotonin and 5HIAA concentration in discrete brain regions following olfactory bulbectomy and/or NK1 receptor deletion

NK1 receptor deletion resulted increased levels of serotonin in the frontal cortex [genotype: F1,33] = 7.78 P = 0.009], a slight but nonsignificant increase in the amygdala and hippocampus, and reduced 5HIAA levels in the hypothalamus [genotype: F1,29)] = 2.26 P = 0.048] of Sham-operated mice [Sham WT vs. Sham NK1−/−: P = 0.05] (Table 1). Olfactory bulbectomy significantly reduced 5HIAA levels in the frontal cortex of WT mice [OB: F1,31] = 4.03 P = 0.050; Sham WT vs. OB WT P < 0.05], an effect attenuated in NK1−/− mice [F1,31] = 3.92 P = 0.050; OB WT vs. OB NK1−/−

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<th>The effect of bulbectomy and/or NK1 receptor deletion on monoamine levels in discrete brain regions.</th>
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<td>Sham WT</td>
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<td>Noradrenaline</td>
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<td>Frontal cortex</td>
<td>460 ± 56</td>
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<td>Amygdala</td>
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<td>Serotonin</td>
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<td>Hypothalamus</td>
<td>1237 ± 335</td>
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<tr>
<td>5HIAA</td>
<td></td>
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<tr>
<td>Frontal cortex</td>
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<td>Amygdala</td>
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<tr>
<td>Hippocampus</td>
<td>859 ± 80</td>
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<tr>
<td>Hypothalamus</td>
<td>1284 ± 65</td>
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Data expressed as mean (ng/g neurotransmitter)± SEM. N = 6–11. Sham, Sham-operated; OB, olfactory bulbectomised; WT, wild type; NK1−/−, NK1 receptor knockout.

* P < 0.05 vs. Sham WT.

* P < 0.05 vs. Sham NK1−/−.

* P < 0.05 vs. OB WT.
system in critical brain regions involved in the regulation of emotional responding, namely increases in the content of substance P in the cortex and increases in NK1 receptor expression in the amygdala and hippocampus. Modulation of this system by deletion of the NK1 receptor reduced the OB-induced increase in locomotor activity and exploratory behaviour on exposure to a novel open field. Furthermore, NK1 receptor deletion attenuated OB-induced changes in serotonin and 5HIAA concentration in the amygdala and frontal cortex, respectively. Thus interaction between the substance P and serotonergic system may underlie the behavioural changes observed in the model.

Enhanced activity of the substance P/NK1 receptor system has been reported clinically [6,7] and in several animal models of depression [8,10–12]. The present study expands on these findings demonstrating that in the OB mouse, substance P levels in the frontal cortex are elevated, as is the expression of NK1 receptors in the amygdala and hippocampus. Therefore alterations in the substance P/NK1 receptor system in critical brain regions involved in regulation of emotional responding occur in this model of depression. Correlating with our findings, substance P levels are also increased in the frontal cortex of Flinders Sensitive Line rats, a genetic animal model of depression [11]. The amygdala has been shown to be a critical site for substance P modulation of emotional responses to stress [for reviews see [17,56,57]]. Exposure to various stressors can induce the release of substance P in the amygdala, an effect which modulates anxiety-related and emotional behaviour [13,58,59]. In addition, the medial amygdala plays an important role in mediating defensive behaviours due to its monosynaptic substance P projections to the medial hypothalamus [60,61]. Disinhibition of the amygdala has been proposed to underlie the behavioural changes observed following olfactory bullectomy including emotional blunting and deficit in defensive behaviours [62–66]. The present study demonstrated enhanced NK1 receptor expression in the medial amygdaloid nuclei following bullectomy, and thus it is tempting to speculate that the increased receptor density observed in the amygdala may underlie the altered behavioural response to stress observed in the model.

The expression of NK1 receptors was also demonstrated to be enhanced in the hippocampus of OB mice. OB mice have been repeatedly demonstrated to exhibit deficits in learning and memory [43,44,67–69] and substance P has been shown to induce memory promoting and reinforcing effects [70,71]. However, NK1 receptors are located primarily on GABAergic interneurons in the hippocampus [72], activation of which may enhance or inhibit hippocampal output depending on whether interneurons or pyramidal neurons are inhibited as a consequence [73,74]. Further studies are required in order to determine the role of hippocampal NK1 receptors in mediating the OB-induced changes in learning and memory.

The open field test is used for a variety of purposes in the literature. Often these exposures are relatively long in duration (10–60 min) in order to evaluate habituation responses. In examining OB-related behavioural deficits, a short duration (3–5 min) is most commonly employed in rodents [16,40,43,45]. The present study revealed that NK1−/− mice exhibited a nonsignificant reduction in locomotor activity on exposure to the open field for this short duration, correlating with previous studies [15,16,38]. However, several studies have demonstrated a hyperactive response following deletion of the NK1 receptor [34,75,76], most probably due to the increased exposure times employed (10–60 min) that may reflect a delay in habituation rather than initial reactivity to the test arena. Time spent in the inner arena of the open field has been used as a measure of anxiety-related behaviour, a parameter which did not differ between NK1−/− and wild type mice in the present study. Similarly, genetic and pharmacological blockade of NK1 receptors has been demonstrated not to affect anxiety-related behaviour in the elevated plus maze [13,15,38] but

4. Discussion

The present study demonstrated that olfactory bullectomy results in alterations in elements of the substance P/NK1 receptor

\[ P < 0.05 \]. In comparison, OB induced an increase in serotonin levels in the amygdala of wild type but not NK1−/− mice [interaction: \( F_{1,31} = 5.78 \ P = 0.022 \)]. There was no effect of bullectomy or NK1 receptor deletion on serotonin or 5HIAA levels in the hippocampus or serotonin turnover (5HT/5HIAA) in any of the regions examined.

**Fig. 3.** The effect of bullectomy and/or NK1 receptor deletion on (a and b) distance moved and (c) time spent in the centre of the open field. Data expressed as mean ± SEM. N = 7–14. **P < 0.01 Sham WT vs. OB WT. “P < 0.05; “P < 0.01 OB WT vs. OB NK1−/−. Sham, Sham-operated; OB, olfactory bullectomised; WT, wild type; NK1−/−, NK1 receptor knockout.**
attenuate stress- and substance P-induced increases in anxiogenic behaviour in this test paradigm [13]. In addition, NK1 receptor blockade results in anxiolytic-like behaviour in tests associated with high levels of stress such as the social interaction test and fear conditioning [77,78]. Therefore, the tonic activity of the substance P/NK1 system may influence how NK1 receptor blockade ultimately affects behavioural responding to stress. Olfactory bulbectomy resulted in the characteristic hyperactivity in the open field, seen as an increase in general distance moved, as well as an increase in the time spent in the inner arena of the open field. These changes are the most commonly observed behavioural alterations in the model and properties that are attenuated by chronic antidepressant treatment [40,43,45]. This OB-induced hyperactivity was attenuated in NK1−/− mice, suggesting that NK1 receptor blockade reveals an antidepressant phenotype. Deletion of the Tac1 gene, which encodes for substance P and neurokinin A, prevents the development of the OB-induced increase in rearing [16] and decrease in sucrose preference [79]. However, in comparison with the present study, OB-induced hyperactivity in the open field was reduced in Tac1−/− mice [16]. Decreased neurokinin A in conjunction with substance P, and as such activation of other tachykinin receptors, following deletion of the Tac1 gene may account for the lack of effect in this latter study. It should be noted that towards the end of the trial, locomotor activity of OB NK1−/− mice returns to that of wild type counterparts, indicating that, although the substance P/NK1 receptor system may be involved in the initial reactivity of OB mice to a novel stressor, other transmitter systems are likely to also be involved in the altered behavioural responding in the model.

Alterations in monoaminergic neurotransmission have been the most widely studied neurochemical change examined in the OB model, with reduced serotonin and noradrenaline function- ing (typically measured as steady state neurotransmitter levels) observed in discrete brain regions such as the frontal cortex, amygdala, hippocampus and midbrain [42,62,80–85]. In accordance with these findings, the present study demonstrated that the sero- tonin metabolite 5HIAA and noradrenaline levels were significantly reduced in the frontal cortex of OB mice. In comparison, sero- tonin levels in the amygdala were increased in OB mice when compared to Sham-operated wild type controls. Methodological differences such as strain/species of animal used, time post surgery, time post behavioural testing and technique used may account for the discrepancy between our finding and those previously report- ing reduced serotonin functioning in the amygdala of OB animals [82,83]. Chronic antidepressant treatment has been demon- strated to normalise not only many of the behavioural but also the neurochemical changes observed following bulbectomy [for review see [36,37]]. The present study demonstrated that the OB-induced changes in monoamine levels in the frontal cortex and amygdala were attenuated in NK1−/− mice. However it should be noted that the attenuation of the OB-induced reduction in noradrenaline concen- trations observed in NK1−/− OB mice cannot be ascribed to an antidepressant effect per se, due to the increase (albeit non- significant) in noradrenaline observed in Sham NK1−/− mice.

NK1 receptor deletion itself induced a number of alterations in monoamine levels, most specifically increases in noradrenaline concentration in the amygdala and hippocampus, an increase in serotonin in the frontal cortex and a reduction in 5HIAA in the hypothalamus. As highlighted earlier, a close associated exists between the substance P/NK1 receptor and the monoamine sys- tems [for review see [17,18]]. Although NK1−/− mice exhibit increased serotonin neuronal firing in the dorsal raphe nucleus [24], cortical serotonin efflux does not differ between NK1−/− and wild type mice [26]. In comparison, the present studies demon- strate an increase in serotonin tissue levels in the frontal cortex of NK1−/− mice. The lack of increase in cortical serotonin release in NK1−/− mice has been attributed to an increase in clearance of this transmitter [26]. However, the present study assessed tissue- level of serotonin encompassing both intra- and extra-cellular serotonin levels, which may account for the discrepancy between studies. NK1−/− mice also exhibited an increase in noradrenaline in the amygdala and hippocampus and a slight but non-significant increase in the frontal cortex of Sham mice. These findings corre- late with previous reports of enhanced noradrenaline release in the cortex of NK1−/− mice [34,35] and in the cortex and hippocam- pus following pharmacological antagonism of the NK1 receptor [27]. This increase in noradrenaline has been proposed to be due to desensitisation of α2A-adrenoceptors autoreceptors [35] and/or increased burst activity of noradrenergic neurons [31] in the locus coeruleus of NK1−/− mice. However, it has been suggested that the effect of the substance P/NK1 receptor system on noradrenergic neurotransmission may differ under stress conditions. For example, NK1 receptor antagonism in the locus coeruleus increases norada-renaline release in the prefrontal cortex, however in the presence of a stressful stimulus, this effect is abolished [33]. Accordingly, the present study demonstrated that the increase in noradrenaline in the amygdala of NK1−/− mice was not observed in the stress-reactive OB model.

In conclusion, the present findings demonstrate region spe- cific alterations in the substance P/NK1 receptor in the OB mouse, a neurochemical abnormality that can be added to those previ- ously observed in central monoaminergic functioning in this model. Furthermore, deletion of the NK1 receptor provides evidence of an antidepressant phenotype following bulbectomy. Further work to support the role of the substance P/NK1 receptor system would include assessing whether these neurochemical changes can be reversed by conventional antidepressant treatment in the OB model, as well as investigating whether NK1 receptor antago- nism would reverse the deficits in behaviour and monoaminergic neurotransmission in the OB model, analogous to currently mar- keted antidepressants. Such studies would be important in adding to our understanding of the pathophysiology and development of novel treatment strategies for stress-related disorders such as depression.

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References

to the medulla oblongata in mice [51].

With respect to the role of neurokinin-1 in pain processing, a recent study demonstrated that administration of a neurokinin-1 receptor antagonist significantly reduced the pain responses in a rat model of chronic pain [52]. This finding is consistent with previous studies that showed a role for neurokinin-1 in pain transmission and nociception [53].

Furthermore, the neurokinin-1 receptor antagonist has been shown to reduce the intensity of inflammatory and neuropathic pain in animal models [54,55]. It has also been demonstrated that neurokinin-1 receptor antagonists are effective in reducing the pain responses in human volunteers [56].

In summary, the neurokinin-1 receptor antagonist has been shown to reduce pain responses in both animal and human models. This finding suggests that targeting the neurokinin-1 receptor may be a promising approach for the treatment of pain disorders.