Behavioural, molecular and glutamatergic changes in a neurodevelopmental model of schizophrenia, and their reversal by a 5-HT$_6$ receptor antagonist

King MV[1], Negm OH[2], Tighe PJ[2], Knapp S[1], Wigmore PM[1], Fone KCF[1]

[1]School of Biomedical Sciences; [2]Molecular Medical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK

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Introduction & Methods

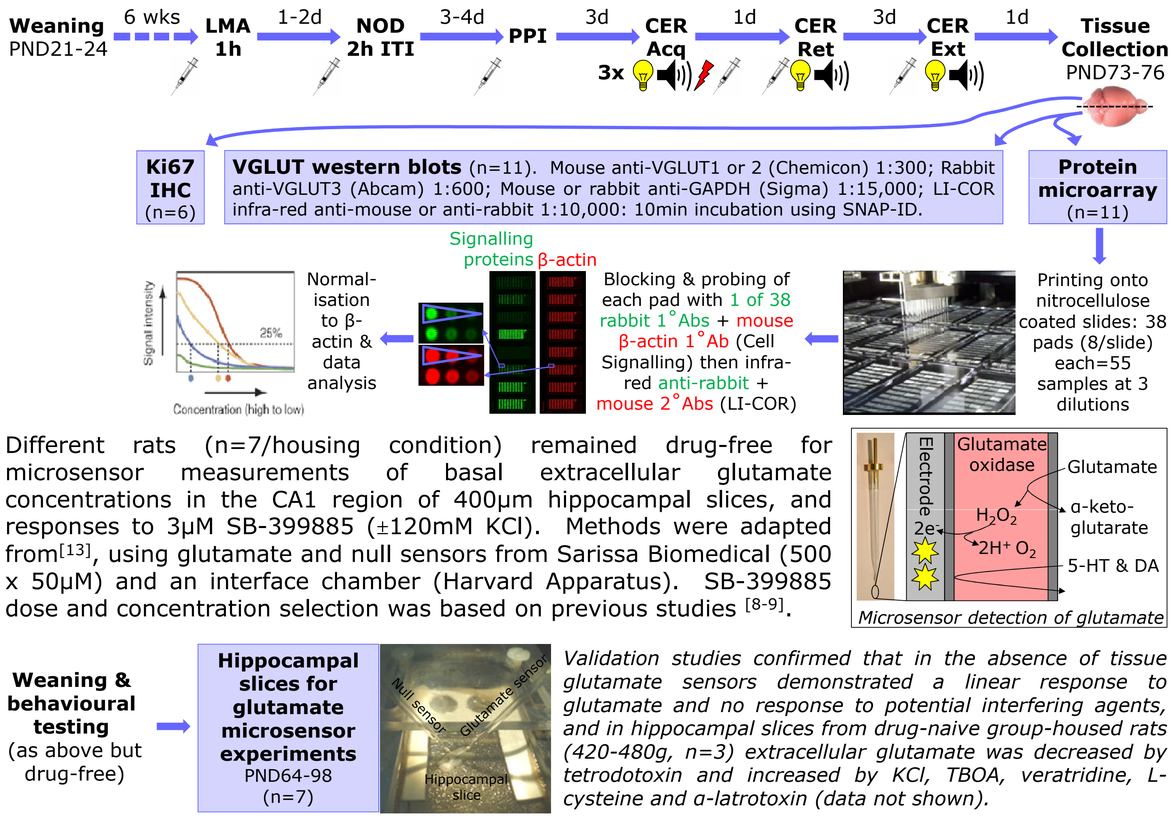
Introduction

Early-life adversity, including social isolation or alienation, is implicated in the development of psychiatric conditions like schizophrenia[1]. In the rat post-weaning social isolation impairs memory in several tasks which map to distinct cognitive domains deficient in this disorder, including hippocampal dependent novel object discrimination (NOD) and conditioned emotional responses (CER). These impairments are accompanied by structural alterations within the hippocampus, including reduced cell proliferation and survival, dendritic length and spine density[2-4]. Reduced parvalbumin & calbindin-containing GABA interneurones and decreased expression of Vesicular Glutamate Transporter 1 (VGLUT1) indicate disruption of inhibitory and excitatory neurotransmission[5] and also resemble changes seen in schizophrenia.

In contrast, 5-HT$_6$ receptor antagonists reverse time delay and pharmacologically-induced cognitive deficits, increase cell proliferation within the dentate gyrus and elevate hippocampal glutamate and ACh efflux in group housed rats[6-7], suggesting their potential to reverse isolation-induced behavioural and neurochemical deficits. **The aims of this study were to further characterise isolation-induced hippocampal changes by western blotting (for VGLUT1-3), protein microarray (for 38 selected intracellular signalling molecules), immunohistochemistry (IHC; for Ki67, a marker of proliferating cells) and the use of enzyme-coated glutamate microsensors, and examine the ability of a 5-HT$_6$ receptor antagonist, SB-399885[8], to reverse the behavioural and hippocampal alterations in this model**.

Methods

Male Lister hooded rats (University of Nottingham or Charles River UK) were weaned on post-natal day (PND) 21-24 and housed individually or in same-litter groups of 3-4. They received minimal handling (single weekly weighing and cage change) until assessment (starting 5-6 weeks later) of locomotor activity (LMA), NOD, pre-pulse inhibition of acoustic startle (PPI) and contextual and cue-mediated CER. Rats received i.p. vehicle (1% Tween 80, 1ml/kg) or 10mg/kg SB-399885 (Tocris Bioscience) on six occasions (n=11/housing-treatment combination), either 30min prior to behavioural testing, or immediately after CER acquisition to preclude potential nociceptive/affective confounds. Behavioural testing[10], IHC[11] and protein microarray[12] used established protocols.



Different rats (n=7/housing condition) remained drug-free for microsensor measurements of basal extracellular glutamate concentrations in the CA1 region of 400µm hippocampal slices, and responses to 3µM SB-399885 (±120mM KCl). Methods were adapted from[13], using glutamate and null sensors from Sarissa Biomedical (500 x 50µM) and an interface chamber (Harvard Apparatus). SB-399885 dose and concentration selection was based on previous studies [8-9].

Microsensor detection of glutamate

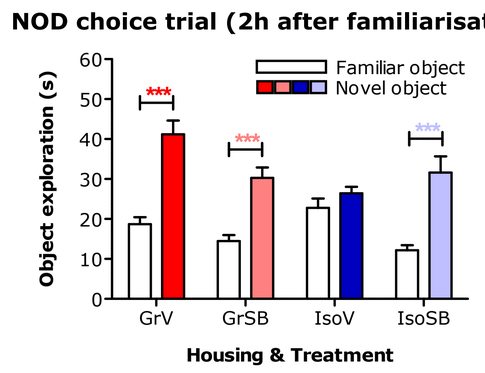
Weaning & behavioural testing (as above but drug-free) → **Hippocampal slices for glutamate microsensor experiments** PND64-98 (n=7)

Validation studies confirmed that in the absence of tissue glutamate sensors demonstrated a linear response to glutamate and no response to potential interfering agents, and in hippocampal slices from drug-naive group-housed rats (420-480g, n=3) extracellular glutamate was decreased by tetrodotoxin and increased by KCl, TBOA, veratridine, L-cysteine and α-latrotoxin (data not shown).

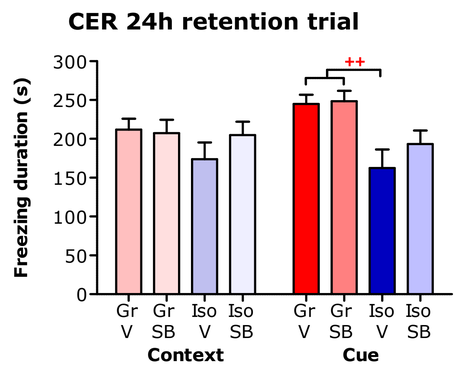
Results

1. Behaviour

Isolation-reared rats (Iso) exhibited increased ambulation 5-10min into the locomotor activity test (P<0.05) and SB-399885 decreased activity of both group housed (Gr; P<0.01) and Iso (P<0.001). There were no spatial preferences for either identical object during the NOD familiarisation trial, although SB-399885 decreased total object exploration (P<0.01). Neither housing nor treatment influenced PPI, acquisition of CER, or freezing to context alone during the 24h retention trial.



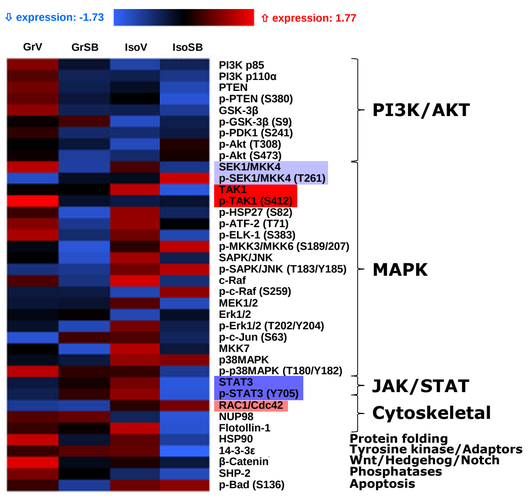
Isolation rearing prevented discrimination of the novel from the familiar object (IsoV P>0.05, whereas GrV P<0.001) and SB-399885 reversed this impairment (IsoSB P<0.001; 2-way RM ANOVA & Bonferroni post-hoc).



Isolation rearing reduced cued (5s) freezing when assessed 24h after acquisition (++P<0.01 versus GrV; 2-way ANOVA & Bonferroni post-hoc), and SB-399885 partially reversed this deficit (P>0.05 versus both GrV and IsoV).

2a. Hippocampal protein expression 24h after the final dose of SB-399885

Signalling molecules (protein microarray)



Highlighted proteins in the heat map to the left showed significant changes in expression (2-way ANOVA & Bonferroni post-hoc), as summarised below and shown in graphs, top right.

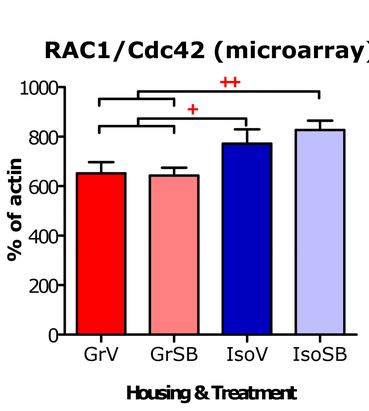
Isolation rearing increased expression of Rac1/Cdc42, irrespective of treatment.

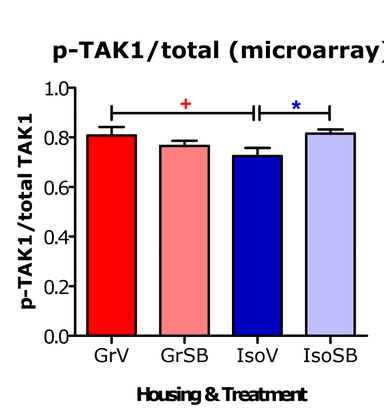
SB-399885 decreased expression of SEK1/MKK4 and increased p-SEK1/MKK4 as a proportion of total in isolation reared rats.

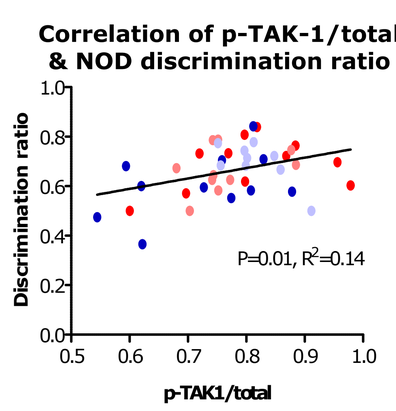
STAT3 and p-STAT3 were both decreased in IsoSB, so that p-STAT3 as a proportion of total STAT3 was unaffected.

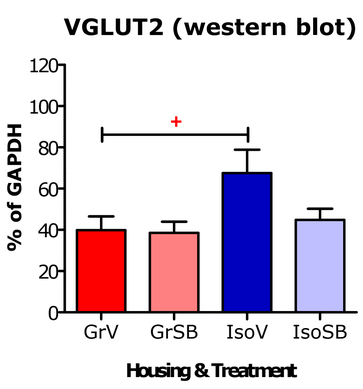
p-TAK1 as a proportion of total TAK1 was decreased by 10% in IsoV, normalised by a 13% increase in IsoSB, and correlated positively with the NOD choice trial discrimination ratio (P=0.01)

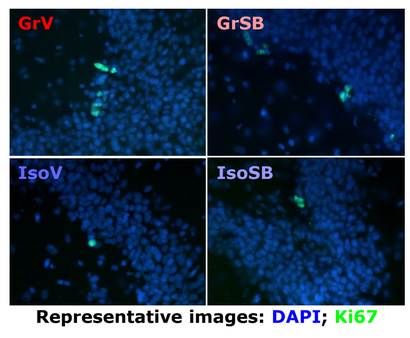
2b. Hippocampal protein expression continued

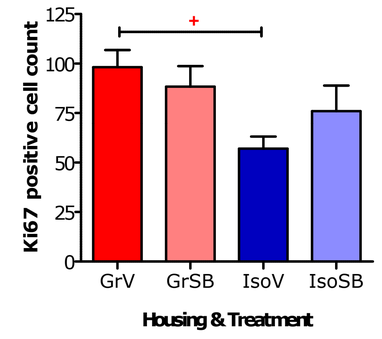






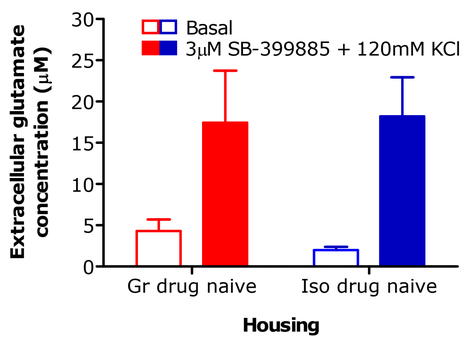






Isolation rearing elevated hippocampal VGLUT2 expression and decreased the number of Ki67-positive cells within the dentate gyrus. SB-399885 partially reversed both of these effects.

3. Glutamate microsensors



Isolation rearing tended to lower basal extracellular glutamate in hippocampal slices, from 4.32 ± 1.40µM to 1.99 ± 0.41µM (P=0.0708).

Although 5-HT$_6$ receptor blockade elevates hippocampal glutamate efflux in conscious rats[6], 10min perfusion with 3µM SB-399885 alone had no effect in hippocampal slices, possibly due to the absence of tonic 5-HT input.

The combination of 3µM SB-399885 + 120mM KCl (15min) stimulated glutamate release to the same extent irrespective of housing (P=0.0087; 2-way RM ANOVA).

Conclusions

Cognitive deficits in the isolation-reared rat neurodevelopmental model of schizophrenia are accompanied by elevated hippocampal Rac1/Cdc42 & VGLUT2 and reduced p-STAT3 & p-TAK1 expression, decreased cell proliferation in the dentate gyrus and a trend for reduced basal extracellular CA1 glutamate, supporting the value of this model to investigate the underlying neurobiology of schizophrenia. The pro-cognitive effects of SB-399885 are maintained in this model, and six injections over two weeks fully reversed the isolation-induced decrease in p-TAK1 and partially reversed alterations in VGLUT2 and cell proliferation (24h after the final injection), further supporting the use of 5-HT$_6$ receptor antagonists to treat cognitive dysfunction.

In normal rats chronic 5-HT$_6$ receptor antagonist administration increased expression of polysialylated neural cell adhesion molecule (PSA-NCAM; linked to newly generated granule cells) in the dentate gyrus 24h after the final dose, without enhancing neurogenesis[7]. Future research will examine the long-term survival of newly proliferated dentate gyrus cells in isolation-reared rats. Previously reported isolation-induced decreases in VGLUT1 expression[5] were not observed in the current study. Although the current increase in VGLUT2 expression appears to contrast with the possible reduction in basal extracellular glutamate levels this VGLUT2 upregulation may be a compensatory adaption to other alterations in inhibitory or excitatory neurotransmission.

The signalling proteins altered in this study all contribute to the SAPK/JNK branch of the MAP kinase cascade, which regulates cell proliferation, differentiation, migration, apoptosis and cytoskeletal integrity[14], and may modulate learning and memory[15]. Rac1, Cdc42 and TAK1 activate MKK4/7 (directly or via MEKK1/3) leading in turn to JNK activation and STAT3 inhibition. However, JNK itself and other targets of this cascade (c-Jun, ATF-2 and Elk-1) were unaffected in the current study. It remains to be seen whether the observed signalling protein changes are consistent either with isolation-induced cognitive impairment (particularly since deficits in frontal cortical Cdc42-mediated signalling are implicated in schizophrenia[16]) or reversal of isolation-induced cognitive deficits by the 5-HT$_6$ receptor antagonist.

[1] Stilo & Murray 2010 *Dialogues Clin Neurosci* 12:305 **[2]** Lu *et al* 2003 *Exp Neurol* 183:600 **[3]** Roberts & Greene 2003 *Brain Res* 991:271 **[4]** Silva-Gomez *et al* 2003 *Brain Res* 983:128 **[5]** Reynolds & Harte 2007 *Biochem Soc Trans* 35:433 **[6]** Fone 2008 *Neuropharmacology* 55:1015 **[7]** Foley *et al* 2008 *Neuropharmacology* 54:1166 **[8]** Hirst *et al* 2006 *Eur J Pharmacol* 553:109 **[9]** Wesołowska & Nikiforuk 2007 *Neuropharmacology* 52:1274 **[10]** Shortall *et al* 2012 *Eur Neuropsychopharmacol* Epub ahead of print **[11]** ELBeltagy *et al* 2012 *Brain Res Bull* 88:514 **[12]** Aleskandarany *et al* 2012 *Breast Cancer Res Treat* 136:419 **[13]** Oldenziel *et al* 2007 *J Neurosci Methods* 160:37 **[14]** Nishina *et al* 2004 *J Biochem* 136:123 **[15]** Sherrin *et al* 2010 *J Neurosci* 30:13348 **[16]** Chen *et al* 2012 *Int J Biochem Cell Biol* 44:447.

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