damaged MBP fragment increases in compliance with increasing the duration of the disease. It is not excluded that long-term antipsychotic treatment led to increase quantity of MBP fragments in serum of schizophrenic patient.

Disclosure statement: Supported by Grant of RSF No. 14–15–00480 "The search for biomarkers of socially significant endogenous mental disorders" 2014–2016.

P.1.g.067 Altered hippocampal glutamate signalling in a rat 'dual-hit' neurodevelopmental model of schizophrenia

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Post-weaning social isolation in the rat induces behavioural and neurochemical alterations similar to those in schizophrenia [1], which are exacerbated by combined neonatal phencyclidine (PCP) to produce a more robust 'dual-hit' model [2]. This study utilised microsensors to characterise the effects of these two early-life interventions on glutamate signalling in hippocampal slices, followed by western blot analysis of glutamate transporters, GABAergic markers, and 5-HT_6 and mGlu receptors that regulate glutamate release in order to explain the observed neurochemical changes.

Male Lister hooded rats (CRUK) received saline vehicle (2 ml/kg; Veh) or PCP HCl (10 mg/kg; PCP) s.c. on post-natal day (PND) 7, 9 and 11 then from weaning (PND21) were housed in groups (3–4/cage; Gr) or individually (Iso), resulting in four housing-treatment combinations (n = 18). Locomotor activity (LMA), novel object discrimination (NOD) and pre-pulse inhibition of acoustic startle (PPI) were evaluated (PND57–63) to allow balanced allocation to microsensors (PND64–98) or western blots (PND64). 400 μm hippocampal slices [3] were transferred to an interface chamber (Harvard Apparatus) and glutamate and null sensors (Sarissa Biomedical; 0.5 mm x 50 μm diameter) inserted into CA1 to monitor basal extracellular glutamate (sensitive to 20μM tetrodotoxin) and responses to KCl (30–120mM), the glutamate reuptake inhibitor TBOA (200μM), and selective 5-HT_6 (SB-399885; 3μM), group III mGlu (CPPG; 100μM) and mGlu7 (MMPIP; 100μM) receptor antagonists (±120mM KCl), using post-slice calibration (10μM glutamate). Compounds were evaluated in separate slices and had no effect in the absence of tissue. Data are mean±s.e.m. and were analysed by two-way ANOVA with Sidak post-hoc.

Veh-Iso and PCP-Iso both exhibited impaired NOD and although PPI was unaffected only PCP-Iso showed robust locomotor hyperactivity, as expected. Isolation reduced basal glutamate ($F_{(1,28)}$=4.178, P = 0.05) by approximately 31% (Veh-Gr 2.7±0.5, PCP-Gr 2.8±0.5, Veh-Iso 1.8±03, PCP-Iso 2.0±0.3μM) but KCl and TBOA-evoked increases were unaffected. None of the antagonists influenced extracellular glutamate when administered alone, but produced significant increases (P < 0.05-P < 0.0001) when combined with 120mM KCl, which were greater than KCl alone. Responses to CPPG + KCl and MMPIP + KCl were unaffected by housing or treatment but the effect of SB-399885 + KCl was reduced by PCP ($F_{(1,28)}$=20.91, P = 0.0182), being lower in PCP-Iso (but not PCP-Gr) than both Veh-Gr (P < 0.05) and Veh-Iso (P < 0.01; Veh-Gr 20.2±3.4, PCP-Gr 21.0±4.5, Veh-Iso 13.8±1.6, and PCP-Iso 10.3±2.1μM).

The 'dual-hit' PCP-Iso combination produced more robust effects than either intervention alone; locomotor hyperactivity (thought to reflect mesolimbic dopamine hyperfunction), impaired recognition memory and deficits in both basal and drug-evoked hippocampal glutamate signalling. Although hippocampal excitotoxicity is implicated in schizophrenia [4] there is also evidence for glutamate hypofunction, albeit to a lesser extent in CA1 than other subfields [5]. Initial findings therefore advocate the use of glutamate microsensors and improved animal models to investigate the underlying neurobiology of schizophrenia and evaluate novel therapeutics. Subsequent studies will examine the ability of 5-HT_6 receptor antagonists to reverse cognitive deficits in PCP-Iso, and the longer-term aim is to expand microsensor studies to freely moving animals.

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Disclosure statement: Funded by a University of Nottingham Early Career Research and Knowledge Transfer Award.

P.1.g.068 Phosphorylation of TRPV1 located within the dorsal periaqueductal gray enhances social defeat stress-induced analgesia in mice

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One of the most studied intracellular pathways that regulates the activity of TRPV1 (transient receptor potential vanilloid type 1) is the phosphorylation of certain amino acid residues, which affect TRPV1 sensitivity to vanilloids (e.g., AEA – endocannabinoid/endovanilloid). Vanilloids have been widely investigated in the modulation of nociception. For instance, vanilloid compounds (both agonists and antagonists) modulate the latency of nociceptive responses in animals subjected to several tests of pain (e.g., tail-flick and formalin tests). In addition, stress suppresses pain probably by activating brain structures [e.g., amygdala, dorsal periaqueductal gray (dPAG)] that engage vanilloid mechanisms. Accordingly, the present study attempted to elucidate the role of phosphorylated (sensitive to AEA) TRPV1 receptors within the dPAG in the modulation of social defeat-induced antinociception in mice. Nociception was assessed using the tail-flick test. In this context five to seven days after surgical implantation of guide cannula targeted to the dPAG, male Swiss mice (n = 4–6) were individually subjected to 2 basal tail-flick latencies (TFL in seconds) 10 min and 5 min before intra-dPAG drug treatment. In Experiment 1; animals received intra-dPAG injection of cyclosporin A (0, 0.01, 0.1, 1 nmol; CsA, drug that renders TRPV1 phosphorylated and, therefore, sensitive to endovanilloid) and then were placed in the tail-flick apparatus to record TFL at 10, 15,

20, 30 and 40 minutes after intra-dPAG drug treatment. Two-way ANOVA (factor 1: treatment; factor 2: time) followed by Duncan post hoc test revealed that intra-dPAG injection of CsA 1 nmol increased TFL in animals [treatment factor ($F_{3,15}=28.39$; $p < 0.05$); interaction ($F_{18,90}=3.23$; $p < 0.05$)]. In Experiment 2; animals were intra-dPAG injected with AM251 (10 pmol; a cannabinoid receptor antagonist, in order to prevent AEA binding to the cannabinoid system) or vehicle followed ten minutes later by local injection of CsA 0.1 nmol (an intrinsically inactive dose on nociception). Five minutes later, each animal was placed into the homecage of an aggressive conspecific mouse for a social defeat stress. The aggressive interaction was interrupted when the intruder mouse exhibited a submissive posture (i.e., defensive upright posture for a 3-sec period). Then, the intruder mouse was placed inside a transparent and perforated plastic bottle (7 cm x 7 cm x 15 cm) and kept inside the aggressor's cage for further five minutes (psychological stress). After that, further five TFL measures were recorded at 0, 5, 10, 20 and 30 minutes post-stress. Two-way ANOVA followed by Duncan post hoc test revealed that animals pre-treated with AM251 followed by CsA had potentiated the social defeat stress-induced analgesia [treatment factor ($F_{1,8}=11.08$; $p < 0.05$); time factor ($F_{6,48}=57.68$; $p < 0.05$); interaction ($F_{6,48}=3.92$; $p < 0.05$)]. Intra-dPAG injection of CsA, at an intrinsically-inactive dose (0.1 nmol), potentiated and prolonged the social defeat stress-induced analgesia. Present results are suggestive that the role of vanilloid receptors located within the dPAG in the modulation of social defeat stress-induced analgesia in mice appears to be dependent on its phosphorylated state.

Disclosure statement: This research was supported by the grant no. 0034/IP1/2013/72 from the MNSW and the statutory funds of the Institute of Pharmacology.

P.1.g.069 Relationship between dose, plasma concentration and GABA-A receptor occupancy by partial GABA-A receptor modulators

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Background: Insufficient GABA neurotransmission in key neural circuits may lead to anxiety disorders. Enhancement of GABAA channel function by positive modulators such as diazepam has been proven a successful anxiolytic therapy. Although diazepam and other benzodiazepines (BZs), agonists of GABAA receptors are highly efficacious, significant adverse effects, such as sedation, amnesia, ataxia, and abuse liability limit their clinical usefulness in anxiety [1].

GABAA receptors are ligand (GABA)-gated, heteropentameric chloride ion channels. About 16 different receptor subunits are arranged into pentameric assemblies. Spectrum of the clinical effects of BZs is mediated by the a1-, a2-, a3- and a5-containing GABAA subtypes.

AZD7325 and AZD6280 are potent, selective GABAAα2,3 receptor modulators. Both experimental drugs are functionally selective for the GABAAα2 and GABAAα3 receptor subtypes, with high affinity to α2/3 subunit (Ki <30nM) and low affinity to α5 subunit. Preclinical pharmacological studies have indicated that AZD7325 and AZD6280 were fast-acting anxiolytics with reduced effects on cognition and without sedative side effects. Clinical neurophysiological studies using EEG suggest that AZD6280 and AZD7325 induced dose-dependent effects on saccadic peak velocity, marker of anxiolytic effect. Meanwhile both compounds in the Phase I clinical development program have demonstrated significantly smaller effect on body sway and cognitive performance compared to lorazepam [2,3].

Purpose: The aim of the present two PET studies was to determine receptor occupancy of AZD7325 and AZD6280 in humans using the GABAA receptor radioligand [^{11}C]flumazenil.

Methods: Each of the PET studies consisted of two panels, with repeated weekly PET measurements, at baseline and after single dose administration. In total (both studies), twelve men, healthy volunteers, 21 to 34 years of age were examined. PET images were analysed using simplified reference tissue model, with pons as a reference region. Regional binding potentials (BPND) were obtained. The relationship between dose, plasma concentration of AZD7325, AZD6280 and GABAA receptor occupancy was examined and apparent dissociation constant Kiplasma was estimated. Assessments of safety and tolerability of both tested drugs included recording of adverse events, vital signs, electrocardiogram, and laboratory tests.

Results: Dose-dependent, saturable binding of AZD6280 and AZD7325 was demonstrated. The pattern of regional drug binding to GABAA receptors was dependent on the combination of the difference between non-selective binding of [^{11}C]flumazenil to the GABAA receptors, subtype selectivity of tested drugs and regional GABAA receptor subtype distribution. Maximum apparent receptor occupancy could be determined for both drugs using tolerated doses. The estimated Kiplasma for AZD7325 was 4 nmol/L, ID50 1.3 mg and for AZD6280 at approximatelty 90 nmol/L and 9 mg, respectively. There were no serious adverse events related to either of the tested drugs. In volunteers receiving AZD6280 doses of 20 mg and above CNS-related adverse events were observed.

Comments: The present PET studies of two novel a2/3 receptor subtype selective partial GABAA receptor modulators confirmed their pharmacological activity as of partial agonists. High GABAA receptor occupancy(>50%) by AZD7325 and AZD6280 could be reached without clear sedative effects.

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Disclosure statement: I am AstraZeneca employee.

P.1.g.070 Amygdala inactivation produces antinociception without changing threatening-induced pain inhibition in mice

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Exposure of animals to environmentally aversive situations [e.g., an open elevated plus maze (oEPM: 4 open arms) or to a predator]