Non-linear relationship between SERT expression and frequency sensitivity of 5-HT signals
Jennings KA 1*, Lesch KP 2, Sharp T 3 & Cragg SJ 1,4
1University Department of Physiology, Anatomy and Genetics, Oxford, UK; 2Molecular Psychiatry, Department of Psychiatry, Psychosomatics and Psychotherapy, Wurzburg, Germany; 3University Department of Pharmacology, Oxford, UK; 4Oxford Parkinson’s Disease Centre, Oxford, UK
katie.jennings@dpag.ox.ac.uk

Introduction
Variation in expression and function of the human 5-hydroxytryptamine (5-HT, serotonin) transporter (SERT) is associated with psychiatric disturbances [1,2]. Such associations remain controversial however, and the underlying neurobiology is not understood. One point of discordance is that whilst SERT levels seem to inversely correlate with brain 5-HT levels, the relationship between SERT levels (or expression-modifying polymorphisms) and psychiatric phenotype appears to be more complex; i.e. both high and low SERT expressing alleles associate with detrimental psychiatric phenotypes. How SERT levels impose changes in brain 5-HT levels to modulate psychiatric vulnerability is an important question for understanding the behavioural consequences of serotonergic dysfunction. However, better understanding of how SERT regulates 5-HT is needed since previous studies assayed brain 5-HT on a timescale too low to resolve the dynamic fluctuations in extracellular levels that are predicted by reported patterns of activity in vivo [3,4].

Methods
We used fast-scan cyclic voltammetry at a carbon-fibre microelectrode (CFM) to detect electrically evoked extracellular 5-HT ([5-HT]o) in the substantia nigra pars reticulata (SNr) in acute brain slices (300 µm) from SERT-overexpressing (OE) and wildtype littermate mice (male, CBAxC57BL6J; 20-35 g [5]) and from homozygote (-/-) and heterozygote (-/+ ) SERT knockout (KO) mice and their wildtype littermates (male, C57BL6J; 20-35 g [6]). Stimulus trains of 20 pulses at a range of frequencies (10, 20, 50 and 100 Hz; 200 µsec pulse width) were delivered by a local bipolar concentric electrode (~100 µm from the CFM). Data were analysed using two-way repeated measures ANOVA for genotype (G), frequency (F) and treatment (T) and post hoc protected Fisher’s LSD test, α=0.05.

Results and Discussion
In the SNr of both strains of wildtype mice evoked [5-HT]o was frequency-dependent, with the lowest stimulus frequencies evoking the least [5-HT]o (Fig 1). SERT expression showed a clear linear relation to the magnitude of evoked [5-HT]o (Fig 1, inset). Accordingly, SERT OE mice showed less evoked [5-HT]o compared to wildtype littermates at equivalent stimulation frequencies (Fig 1A),
whilst in SERT -/- KO mice the magnitude of evoked 5-HT was greater than in -/+ KO mice, which in turn was greater than in wildtype mice (Fig 1B).

**Figure 1.** SERT expression shows a linear relationship to magnitude of evoked [5-HT]o. Averaged evoked [5-HT]o profiles ± SEM versus time in (A) wild-type (WT) and SERT overexpressing (OE) mice and (B) wild-type (WT), -/+ (HET) and -/- SERT knockout (KO) mice (n = 5–6). Stimuli (arrows) indicate start of 20-pulse trains. Linear relationship between magnitude of evoked [5-HT]o (by 20 pulse, 50 Hz stimulation) and SERT expression is illustrated by inset. G*F*T interaction NS; G*T and G*F interactions; *post hoc* comparisons of all genotypes versus WT in drug-free conditions were significant.

By contrast to the linear relationship between SERT levels and [5-HT]o magnitude, the relationship to frequency sensitivity was non-linear. Thus, both SERT -/- KO and OE mice showed a marked loss of frequency sensitivity of evoked [5-HT]o, whilst SERT -/+ KO mice showed similar frequency sensitivity to their wildtype littermates (Fig 2).

**Figure 2.** SERT expression shows a non-linear relationship to frequency-sensitivity of evoked [5-HT]o. Mean peak [5-HT]o ± SEM from profiles in Figure 1, expressed as a percentage of the smallest WT response ([5-HT]o evoked at 10 Hz). For abbreviations see Figure 1. * and • denote significant G*F*T interaction followed by significant *post hoc* test versus WT and HET (*) or HET only (●).

We next explored whether this impaired frequency sensitivity reflected a simple change in 5-HT reuptake rate, as opposed to the occurrence of neuroadaptive mechanisms. Blockade of SERT by citalopram (100 nM) did not further affect frequency sensitivity in SERT -/- KO or OE mice nor did it restore frequency sensitivity (Figure 3). However, in WT and SERT -/+ KO mice citalopram reduced frequency sensitivity, partially mimicking the SERT -/- KO phenotype and suggesting that changes in reuptake rates at least in part underpin our observations in SERT -/- KO and OE mice.
In summary, our findings show that SERT expression levels impact differently on distinct aspects of 5-HT signaling. In turn, these data offer insight into how variation in SERT expression might relate to both dialysate 5-HT levels and behavioural abnormalities. Thus, although the magnitude of evoked [5-HT]o is modulated by SERT expression in a linear way, with high SERT expression yielding reduced [5-HT]o and vice versa as reported by previous microdialysis studies [5,6], both large increases and decreases in SERT expression lead to a profound loss of frequency sensitivity of [5-HT]o. Encoding of information within firing patterns is a critical component of neuronal computation. Impaired ability to generate appropriate 5-HT signals in response to dynamically changing firing patterns will perturb information processing and downstream behaviors. That 5-HT neurons exhibit dynamic switching of firing frequencies [3] that are linked to behavioural motivation [4] suggests that the loss of frequency sensitivity described here may drive the maladaptive behaviours in disorders linked to both high (e.g. OCD, ADHD) and low (e.g. depression, anxiety) expressing alleles of the human SERT.

References