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Measuring dopamine with transporter and auto-receptor blockade during cued reward behaviour: implications for attention-deficit/hyperactivity disorder mechanism and Parkinson's disease treatment

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Dopamine signalling is crucial to motivation, movement and cognitive function, and has been implicated in both attention-deficit/hyperactivity disorder (ADHD) and Parkinson's disease. Methylphenidate increases extracellular dopamine by blocking reuptake via the dopamine transporter. It is an established treatment for ADHD and has been used with poor results as a treatment for Parkinson's disease.

We investigated the dynamics of striatal dopamine release in rats after methylphenidate administration *in vivo* during a signalled reward task using fast scan cyclic voltammetry. Dulled phasic responses led us to test whether dopamine D2 receptor (D2R) mediated autoinhibition might be obscuring an effect of the drug. D2R blocker raclopride was administered as a micro dose simultaneously with methylphenidate, and compared to each drug alone and vehicle.

One-way ANOVA confirmed a significant effect of treatment ($F_{3,15}$=7.057; P=0.004) with post-hoc Tukey's tests confirming that the mean dopamine signal after combined treatment (mean±SD 11.1±3.1 nM) was significantly higher than after treatment with saline (3.1±2.0 nM; P=0.003), raclopride (5.0±4.0 nM; P=0.02), or methylphenidate alone (4.7±3.4 nM; P=0.02). There were no differences between saline, raclopride and methylphenidate treatments.

Increased response to the combined treatment implicates D2R mediated homeostatic control of phasic firing as the target for the treatment of ADHD with methylphenidate, enabling down-regulation of phasic firing via up-regulated autoinhibition. Thus, an imbalance in D2R mediated phasic firing homeostasis is likely to play a role in the mechanism of ADHD itself. The substantial rebound of dopamine signal in response to both cue and reward also suggests the potential of the two drug combination to rescue methylphenidate as a treatment for Parkinson's disease. Magnifying dopamine release in conjunction with naturally occurring stimuli has implications for improving current treatments and associated side effects.

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Understanding the subtleties of development of atherosclerosis and the effect of sex hormones

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The New Zealand Ministry of Health estimates that cardiovascular disease accounts for >32% deaths/year across all age ranges. The underlying pathological change is progressive stenosis (narrowing) of arteries as atherosclerotic lesions develop. Sex differences in atherosclerotic susceptibility are apparent, although the root cause of these differences is not fully understood. One theory is that oestrogen beneficially influences the composition and progression of lesions. However, female hormone replacement therapy has been associated with increased atherosclerotic

risk. Therefore, the aim of this study was to investigate the effect of oestrogen treatment on atherosclerotic lesions.

Twenty-five week old female ApoE$^{-/-}$ mice were treated for eight weeks with subcutaneous bi-weekly injections with either 3µg/g of 17β-estradiol (E2, n=7) or ethanol (vehicle control, n=11). Mice were euthanised by CO_2 inhalation followed by fixation with 4% paraformaldehyde. Histological sections (4µm) along the length of the brachiocephalic artery were prepared, photographed and analysed. Atherosclerotic lesions were measured using Fiji-Image J software. Images were sub-divided into four anatomical regions before the lesions were scored (0–6) using a modified clinical scoring system. In addition, different parameters of lesion composition were also scored (0–3) for initial thickening, foam cells, lipid collection and cholesterol crystals.

Comparison (Students *t*-test) of lesions of E2 treated (n=7) to vehicle control (n=11) showed (mean±SEM) no increase in lesion volume (0.14±0.03 *cf.* 0.09±0.02 mm^3, *P*=0.215), or stenosis level (31.8±4.3 *cf.* 28.1±4.4%, *P*=0.556). However, the E2 treated group had significantly increased intima thickening score (2.9±0.04 *cf.* 2.7±0.1, *P*=0.04) and a trend for increased cholesterol crystals (1.1±0.3 *cf.* 0.6±0.2, *P*=0.08). Therefore, subtle alterations to lesion composition may underpin anecdotal evidence that E2 treatment increases risk in cardiovascular disease.

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A non-coding genetic variant associated with abdominal aortic aneurysm alters *ERG* gene regulation

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Abdominal aortic aneurysm (AAA) is an irreversible weakening and enlargement of the abdominal aorta and a major cause of sudden death in the elderly. Recently, four novel single nucleotide polymorphisms (SNPs) specifically associated with AAA and not with other cardiovascular diseases or risk factors have been identified. These variants are located in non-coding DNA, and it is unclear how they contribute to AAA pathogenesis. Here, we investigated the gene regulatory function for one of the non-coding SNPs associated with AAA, rs2836411, which is located in an intron of the *ERG* gene.

We show that rs2836411 is located in an enhancer element in vascular endothelial and haematopoietic cell types, and that the risk allele significantly reduces enhancer activity in cell culture (*P*<0.0001, Sidak's multiple comparisons test, six biological replicates). Enhancers can regulate the expression of proximal or distant genes by directly contacting them via chromatin looping. To identify whether rs2836411 regulates the expression of *ERG* and/or of distant genes, we identified the chromatin interactions formed. In vascular endothelial cells, which express *ERG*, the SNP region interacts highly within the *ERG* gene, while in vascular smooth muscle cells, which do not express *ERG*, the interactions are distributed across a wider region. This indicates that rs2836411 directly contacts the *ERG* gene promoters. Furthermore, the risk allele correlates with reduced *ERG* expression in aortic and other vascular tissues.

In conclusion, our results indicate that rs2836411 likely affects *ERG* expression by altering enhancer activity. This study links a non-coding genetic association with AAA to the *ERG* gene, thereby providing evidence for a novel gene specifically involved in AAA formation. ERG is involved in vascular development, angiogenesis, and inflammation in atherosclerosis, therefore mechanistically, rs2836411 could contribute to AAA by modulating ERG levels.

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