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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Flight behaviour in humans is intensified by a candidate genetic risk factor for panic disorder: evidence from a translational model of fear and anxiety

Molecular Psychiatry (2011) **16,** 242–244; doi:10.1038/mp.2010.2; published online 23 February 2010

Panic disorder (PD) is a serious and common psychiatric condition[1] characterized mainly by recurrent episodes of intense, uncontrollable fear known as panic attacks.[2] The underlying causal mechanism for PD is unknown;[3] however, the discovery that drugs with clinical effectiveness against PD preferentially alter rodent flight behaviour suggests that PD reflects alterations in the brain systems that govern flight.[4] An association between PD and flight in humans is supported anecdotally by the tendency for PD sufferers to feel a strong urge to flee from the location where a panic attack occurs.[2] Here we provide the first human empirical evidence for a PD–flight link, showing that flight behaviour is significantly more intense in carriers of a candidate genetic risk factor for PD than in non-carriers.

Human flight behaviour was measured using a computerized translation of a rodent runway task (Figure 1a) designed to index fear proneness behaviourally, as the intensity of flight effort in response to a pursuing threat stimulus.[5] The genetic risk factor for PD used in this study was the C allele of the 102T/C single-nucleotide polymorphism (rs6313) within the serotonin 2a receptor gene (*HTR2A*) on chromosome 13q14.2; the C allele in this SNP is known to be associated with increased susceptibility to pure but not co-morbid PD,[6] as well as to increased intensity of panic symptoms.[7] All 200 participants (107 of whom were male) gave informed consent and self-identified as healthy Caucasians. Buccal cells were collected and DNA extracted using established methods (see Supplementary Information).

The genotype distribution of rs6313 SNP in *HTR2A* was in Hardy–Weinberg equilibrium ($\chi^2 = 0.632$, d.f. = 2, $P = 0.73$). There were no significant genotype effects on flight intensity ($F(1, 192) = 2.69$, $P = 0.070$); however, carriers of the C risk allele showed significantly greater flight intensity than TT individuals ($F(1, 194) = 4.90$, $P = 0.033$; Figure 1b). The construct validity of flight intensity as a specific measure of fear proneness was supported by its significant positive association with scores on tissue damage fear (measured by the Fear Survey Schedule) ($F(1, 194) = 5.92$, $P = 0.022$). The construct validity of flight intensity was also supported by the absence of a significant association with scores on Spielberger trait anxiety ($F(1, 194) = 0.01$, $P = 0.998$), which is a widely accepted questionnaire measure of anxiety proneness.[8] Sex did not affect flight intensity in this model ($F(1, 194) = 1.50$, $P = 0.222$), nor was there a significant allele $\times$ sex interaction ($F(1, 194) = 1.56$, $P = 0.213$). There was no significant interaction between tissue damage fear and rs6313 carrier status (C carrier or TT homozygote) ($F(1, 191) = 0.44$, $P = 0.511$).

In overview, therefore, our study is the first molecular genetic investigation of human defensive behaviour and the first study empirically to support in humans the hypothesis that PD stems from alterations in the brain systems governing flight behaviour. However, although the *HTR2A* gene on chromosome 13q4-21 has previously been associated with PD, as a caveat it should be noted that the rs6313 SNP in exon 1 of the coding sequence of the *HTR2A* gene is a synonymous (or silent) polymorphism. Therefore, its previously observed effects at the phenotypic level may be mediated not by changes in protein structure but by other mechanisms such as gene expression. As rs6313 is part of a four-SNP haplotype (rs6311, rs1328674, rs6313, rs6314), future attempts at understanding

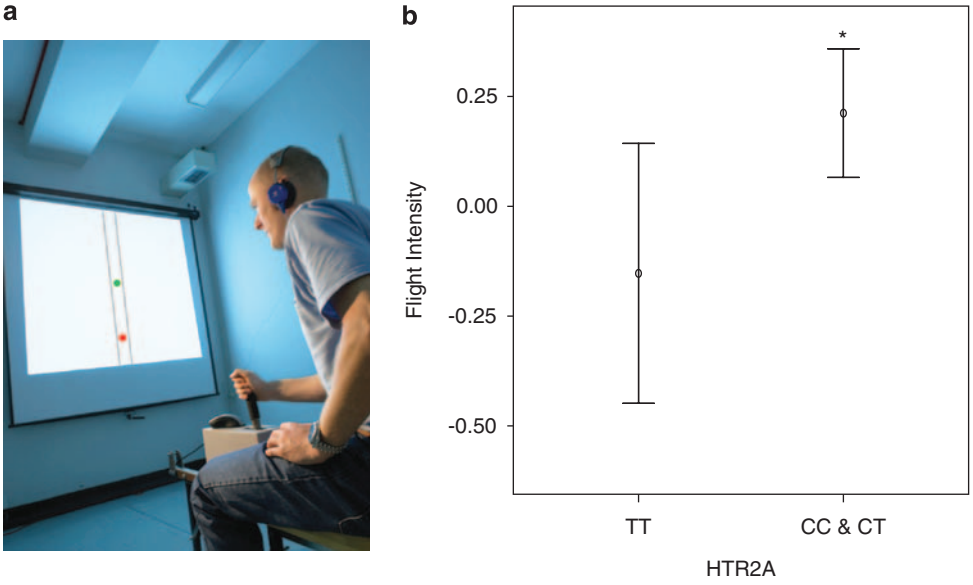

Figure 1 (**a**) The Joystick Operated Runway Task, a computerized human translation of the Mouse Defense Test Battery. The participants used a force-sensing joystick apparatus (PH-JS1, Psyal, London, UK) to control the speed of a cursor (green dot) pursued along an on-screen runway by a threat stimulus (red dot). In order to provide aversive motivation for flight, if the red dot caught the green dot participants received an unpleasant but harmless 115-dB white noise burst lasting 250 ms. In order to mimic the calorie cost of flight in real threat situations, the velocity of the green dot was increased in proportion to the force applied to the joystick. In order to control for individual differences in strength and motivation, the minimum force required for the green dot cursor to reach the escape velocity was set at 50% of the maximum force that the participant exerted during an earlier calibration session. (**b**) Flight intensity was significantly increased by carrying the C allele of the 102T/C polymorphism (rs6313) within the serotonin 2a receptor gene (*HTR2A*; error bars represent 1 s.e.m.; $*P < 0.033$).

the causative mechanisms underlying the association between the C allele in rs6313 and flight intensity should ultimately consider these other linked polymorphisms.[9,10]

Although our findings suggest that PD is mediated by the brain systems that govern flight behaviour, our molecular genetic design could not reveal which brain systems may be implicated. In rats electrical stimulation of the dorsal periaqueductal grey prompts flight behaviour, suggesting that this structure may be particularly relevant to determining susceptibility to PD. Therefore, a desirable next step would be functional neuroimaging studies using our runway task that explore brain activity during flight. In addition, it would be desirable from an individual differences perspective to explore whether participants that flee intensely and report being especially prone to fear show particularly intense activity in the target brain systems.

Finally, a strength of the use of healthy participants in this study is that the heightened flight reactions of C allele carriers cannot be easily explained as an outcome of the acute symptomatic effects of PD or as a side effect of medication for PD, but instead may be part of an inherited trait of fearfulness/flight proneness[3] that could ultimately constitute an endophenotype[11] for PD. However, this view should be tempered with the consideration that individuals without the C allele (that is, who had the TT genotype) are relatively rare (only 18 male and 12 female participants in the present sample). The minority status of the TT individuals, therefore, implies that molecular genetic studies of the present type should invert the interpretation of their results, portraying TT carriers as unusually resistant to fear or PD rather than carriers of the C allele as particularly prone to fear or PD.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Biological embedding of stress through inflammation processes in childhood

Molecular Psychiatry (2011) **16,** 244–246; doi:10.1038/mp.2010.5; published online 16 February 2010

Children exposed to adverse psychosocial experiences show elevated disease risk in adulthood.[1] It is therefore important to characterize the biological mechanisms through which children may acquire such lasting vulnerability to disease, namely, the mechanisms of biological embedding.[2]

Recent studies suggest that inflammation could be an important developmental mediator translating childhood psychosocial into biological risk. We previously showed that adult individuals exposed to childhood maltreatment had elevated levels of inflammation biomarkers.[3] The elevation in inflammation levels was most evident in adults exposed to childhood maltreatment who also experienced depression at the time of inflammation assessment.[4] These epidemiological findings from a population-representative birth cohort are supported by experimental evidence in animal models.[5] In turn, elevated inflammation levels in adulthood have been linked to elevated risk of mental and physical illness.[6]

A key unanswered question is when the effect of childhood stress on inflammation emerges. The significance of this question lies in its potential to uncover the origins of enduring disease vulnerability in children exposed to adverse psychosocial experiences, and to suggest the best timing for effective interventions.

To test the possible emergence of the effect of stress on inflammation in childhood, we studied a sample of 12-year-old children participating in the Environmental Risk Longitudinal Twin Study.[7] We studied children from 41 homes where we found evidence of physical maltreatment. We compared them with children from homes where we found no evidence of maltreatment. These two groups of children were matched with regard to family socio-economic status, gender and zygosity. Children's exposure to physical maltreatment was prospectively assessed during their first decade of life using a standardized interview with mothers having documented validity and reliability. Childhood depressive symptoms at age 12 were assessed using the Children's Depression Inventory, and children scoring higher than its validated clinical cut-off point (CDI $\geqslant$ 20) were classified as depressed. Inflammation at age 12 was assessed based on levels of high-sensitivity C-reactive protein (hsCRP) collected through blood spots.[8] Measures of body temperature and waist–hip ratio, two important potential intervening variables, were also collected at the time of blood spot collection (see Supplementary Methods).

Based on our previous findings that elevated inflammation levels were concentrated among adults exposed to childhood maltreatment who also experienced depression at the time of inflammation assessment,[4] we hypothesized that maltreated children who also experienced depression at the time of inflammation assessment would show elevated hsCRP levels. We therefore divided our sample into four groups: children from maltreatment-free homes without depression (controls), children from maltreatment-free homes with current depression (depressed-only), children from homes with maltreatment but no depression (maltreated-only), and children from homes with maltreatment with current depression (depressed + maltreated). We found significant mean differences in hsCRP across these four groups (Table 1, panel 1). Depressed + maltreated children showed a significant mean elevation in hsCRP levels compared with control children. In contrast,