

Neural basis of reward anticipation and its genetic determinants

Tianye Jia^{a,b}, Christine Macare^{a,b}, Sylvane Desrivieres^{a,b}, Dante A. Gonzalez^c, Chenyang Tao^d, Xiaoxi Ji^d, Barbara Ruggeri^{a,b}, Frauke Nees^{e,f}, Tobias Banaschewski^e, Gareth J. Barker^a, Arun L. W. Bokde^g, Uli Bromberg^h, Christian Büchel^h, Patricia J. Conrod^{a,i}, Rachel Dove^c, Vincent Frouin^j, Jürgen Gallinat^k, Hugh Garavan^{l,m}, Penny A. Gowlandⁿ, Andreas Heinz^k, Bernd Ittermann^o, Mark Lathrop^p, Hervé Lemaitre^q, Jean-Luc Martinot^q, Tomáš Paus^{r,s,t}, Zdenka Pausova^u, Jean-Baptiste Poline^v, Marcella Rietschel^{e,w}, Trevor Robbins^x, Michael N. Smolka^y, Christian P. Müller^z, Jianfeng Feng^{d,aa}, Adrian Rothenfluh^{c,1}, Herta Flor^{e,f,1}, Gunter Schumann^{a,b,1,2}, and the IMAGEN Consortium³

^aInstitute of Psychiatry, Psychology and Neuroscience, King's College London, London SE5 8AF, United Kingdom; ^bMedical Research Council Social, Genetic and Developmental Psychiatry Centre, London SE5 8AF, United Kingdom; ^cDepartment of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX 75390; ^dCenter for Computational Systems Biology, Fudan University, Shanghai 200433, People's Republic of China; ^eDepartment of Child and Adolescent Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, 68159 Heidelberg, Germany; ^fDepartment of Cognitive and Clinical Neuroscience, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, 68159 Heidelberg, Germany; ^gTrinity College Institute of Neuroscience and Discipline of Psychiatry, School of Medicine, Trinity College Dublin, Dublin 2, Ireland; ^hDepartment of System Neuroscience, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; ⁱDepartment of Psychiatry, Université de Montréal, Centre Hospitalier Universitaire Ste Justine Hospital, Montréal, QC, Canada H3T 1C5; ^jNeurospin, Commissariat à l'Energie Atomique et aux Energies Alternatives, 91190 Paris, France; ^kClinic for Psychiatry and Psychotherapy, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; ^lDepartments of Psychiatry and Psychology, University of Vermont, Burlington, VT 05401; ^mInstitute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland; ⁿSchool of Physics and Astronomy, University of Nottingham, Nottingham NG7 2RD, United Kingdom; ^oPhysikalisch-Technische Bundesanstalt, Braunschweig und Berlin, D-10587 Medizinische Messtechnik, Germany; ^pMcGill University and Genome Quebec Innovation Centre, Montréal, QC, Canada H3A 0G1; ^qINSERM Commissariat à l'énergie atomique et aux énergies alternatives Unit 1000 "Imaging & Psychiatry," University Paris Sud, 91401 Orsay, France; ^rRotman Research Institute, University of Toronto, Toronto, ON, Canada M6A 2E1; ^sSchool of Psychology, University of Nottingham, Nottingham NG7 2RD, United Kingdom; ^tMontreal Neurological Institute, McGill University, Montréal, QC, Canada H3A 2B4; ^uThe Hospital for Sick Children, University of Toronto, Toronto, ON, Canada M5G 1X8; ^vHenry H. Wheeler, Jr. Brain Imaging Center, University of California, Berkeley, CA 94720; ^wDepartment of Genetic Epidemiology, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, 68159 Heidelberg, Germany; ^xBehavioural and Clinical Neurosciences Institute, Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, United Kingdom; ^yDepartment of Psychiatry and Psychotherapy, and Neuroimaging Center, Technische Universität Dresden, 01187 Dresden, Germany; ^zDepartment of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-University Erlangen-Nuremberg, 91054 Erlangen, Germany; and ^{aa}Department of Mathematics, Warwick University, Coventry CV4 7AL, United Kingdom

Edited by Jean-Pierre Changeux, CNRS, Institut Pasteur, Paris, France, and approved February 11, 2016 (received for review February 19, 2015)

Dysfunctional reward processing is implicated in various mental disorders, including attention deficit hyperactivity disorder (ADHD) and addictions. Such impairments might involve different components of the reward process, including brain activity during reward anticipation. We examined brain nodes engaged by reward anticipation in 1,544 adolescents and identified a network containing a core striatal node and cortical nodes facilitating outcome prediction and response preparation. Distinct nodes and functional connections were preferentially associated with either adolescent hyperactivity or alcohol consumption, thus conveying specificity of reward processing to clinically relevant behavior. We observed associations between the striatal node, hyperactivity, and the *vacuolar protein sorting-associated protein 4A (VPS4A)* gene in humans, and the causal role of *Vps4* for hyperactivity was validated in *Drosophila*. Our data provide a neurobehavioral model explaining the heterogeneity of reward-related behaviors and generate a hypothesis accounting for their enduring nature.

fMRI | neural network | VPS4A | dopamine receptor | GWAS

Successful behavioral adaptation requires effective reward processing that determines whether a desired goal is approached and maintained. Reward processing can be separated into behavioral anticipation or reward expectancy as a consequence of learning and behavioral and subjective responses to rewarding outcomes (1). In humans, dysfunctional reward processing (in particular, dysfunctional reward anticipation) has been implicated in various externalizing disorders, including attention-deficit hyperactivity disorder (ADHD) (2) and addiction (3). Brain regions involved in reward anticipation include the ventral tegmental area, the medial forebrain bundle, and the nucleus accumbens/ventral striatum (VS; including the ventral caudate-putamen) as well as the ventromedial and insular cortices (4). More recently, observations have been reported to link reward processing in humans with cortical activation (5), including the primary somatosensory (6), primary visual (V1)

(7), and auditory (8) cortices. Dopamine is the principal neurotransmitter regulating reward processing, particularly through the mesocorticolimbic pathway (9), the neuronal projection from the ventral tegmental area to the VS and prefrontal cortex. A general feature of striatal information processing is the control by reward-related dopamine signals of direct and indirect cortical inputs from different neurotransmitter systems, including noradrenaline, glutamate, and GABA as well as acetylcholine, endogenous opioids, and cannabinoids (10). As a consequence, striatal dopaminergic activity integrates cortical and subcortical inputs with reward response. In addition to direct and indirect regulation by heteroreceptors, dopamine release is regulated by presynaptic autoreceptors of the D2 family, in particular D2 dopamine receptors (DRD2) that

Author contributions: T.J., S.D., C.P.M., J.F., A.R., H.F., and G.S. designed research; T.J., C.M., S.D., D.A.G., C.T., B.R., F.N., T.B., G.J.B., A.L.W.B., U.B., C.B., P.J.C., R.D., V.F., J.G., H.G., P.A.G., A.H., B.I., M.L., H.L., J.-L.M., T.P., Z.P., J.-B.P., M.R., T.R., M.N.S., C.P.M., J.F., and I.C. performed research; T.J., C.M., D.A.G., C.T., X.J., H.L., and Z.P. analyzed data; and T.J., C.M., C.P.M., A.R., H.F., and G.S. wrote the paper.

Conflict of interest statement: T.B. has served as an adviser or consultant to Eli Lilly, Hexal Pharma, Medice, Novartis, Otsuka, Oxford Outcomes, PCM Scientific, Shire, and Vifor Pharma; he has received conference attendance support, conference support, or speaking fees from Eli Lilly, Medice, Novartis, and Shire, and he is involved in clinical trials conducted by Eli Lilly, Shire, and Vifor Pharma. G.J.B. has received honoraria for teaching from GE Healthcare and has served as a consultant for IXICO. A.L.W.B. has received funding from Science Foundation Ireland. T.R. has served as a consultant for Cambridge Cognition, Eli Lilly, Lundbeck, Otsuka, Shire, and Teva; he has received research support from Eli Lilly, GlaxoSmithKline, and Lundbeck; editorial honoraria from Elsevier and Springer-Verlag; educational speaking honoraria from Merck, Sharp, and Dohme; and royalties from Cambridge Cognition. The other authors report no financial relationships with commercial interests.

This article is a PNAS Direct Submission.

Data deposition: IMAGEN data are available from a dedicated database: <https://imagen.cea.fr>.

¹A.R., H.F., and G.S. contributed equally to this work.

²To whom correspondence should be addressed. Email: gunter.schumann@kcl.ac.uk.

³A complete list of the IMAGEN Consortium can be found in [Table S7](#).

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1503252113/-DCSupplemental.

Significance

We characterize in humans a coordinated network of brain activity describing neurobehavioral correlates of reward anticipation. The network involves nodes in striatal and cortical brain regions, which are preferentially associated with distinct externalizing behaviors—hyperactivity and alcohol consumption—suggesting that the heterogeneity of reward-related behaviors might be accounted for by different association patterns of nodes and their connecting links. In a genome-wide association study of the striatal node with subsequent functional validation in *Drosophila*, we identify molecular genetic mechanisms involving *vacuolar protein sorting-associated protein 4A (VPS4A)* in dopamine regulation, reward anticipation, and hyperactivity. Our approach might facilitate the identification of causal neural mechanisms, important for the identification of previously unidentified targets and the establishment of neurobehaviorally informed end points for clinical trials.

are coupled to inhibitory G proteins, modulate ion channel activity, and/or inhibit adenylyl cyclase. Postsynaptic dopamine receptors include DRD1, which activates the cAMP pathway and is colocalized with glutamatergic NMDA receptors in the postsynaptic density, and they are thought to contribute to the glutamate-dopamine cross-talk (11).

Despite neurobiological and molecular evidence indicating extensive corticostriatal integration in reward processing, most human neuroimaging studies have limited their investigations to regions of interest analyses of very selected brain structures, namely the VS and the orbitofrontal cortex. There is, as yet, no comprehensive analysis to investigate a coordinated network of brain activity during reward anticipation in large human datasets or study its genetic basis. Given that the behavioral heterogeneity associated with dysfunctional reward processes is too extensive to be easily explained by differences in brain activities in these regions of interest alone, such a network-based analysis might help to explain the neural underpinnings of common and distinct neuropsychological deficits associated with reward-related mental disorders.

Results

Functional Brain Network of Reward Anticipation. We investigated the pattern of brain activation during reward anticipation in the IMAGEN sample (12) by measuring the blood oxygen level-dependent (BOLD) response in functional neuroimaging [functional MRI (fMRI)] analyses of 1,544 14-y-old adolescents (Table 1) using the high win vs. no win contrast of the monetary incentive delay (MID) task (*Materials and Methods* and *SI Materials and Methods*). We applied a hypothesis-free brain-wide weighted voxel coactivation network analysis (WVCNA) (13) and obtained 1,397 modules of brain activation during reward anticipation. Of these, 21 modules fulfilling stringent methodological requirements were selected for additional analysis (*SI Materials and Methods* and Fig. S1). The modules included subcortical reward-processing areas, including the striatum and cortical areas in (but not limited to) the frontal, parietal, and occipital lobes (Tables S1 and S2). We examined relationships among these modules by generating functional connections (i.e., a partial correlation matrix) (*SI Materials and Methods* and Fig. S2A). Subsequent hierarchical clustering identified four nodes involved in reward anticipation (Fig. 1 and Fig. S2B). Node 1 consisted of the caudate nucleus, putamen, and nucleus accumbens (striatum), node 2 included occipital areas (V1/V2) involved in early visual processing, node 3 included somatosensory and motor areas, and node 4 involved occipital, parietal, and cerebellar areas. Their corresponding first principle components were used in the following analyses.

Characteristics of the Functional Brain Network. We assessed associations of these nodes with neuropsychological tests (Table 1) related to reward processing through Cambridge Neuropsychological Test Automated Battery (Cantab) (www.cambridgecognition.com), including (i) the affective go/no go task, which measures selective attentional bias to affective stimuli, and (ii) the spatial working memory task, which is akin to an optimal foraging task for reward (14) and has been associated with ADHD (15), and the delay discounting task (Monetary Choice Questionnaire), a measure of delayed gratification and impulsiveness (16), taking into account that damage to the rat nucleus accumbens impairs delayed reward discounting (17). Because activity in different nodes may not be independent, we identified the predominant node driving the association with performance in these tests by carrying out partial correlation analyses controlling for the effects of all remaining nodes (Table 2 and Table S3). Predominant association was defined as a $P < 0.1$ after partial correlation analysis. The predominant association in striatal node 1 was with fewer errors in the spatial working memory task ($R = -0.12$; $P_{\text{corrected}} = 0.0001$; $df = 1,495$) and reduced delay discounting of small ($R = -0.08$; $P_{\text{corrected}} = 0.0461$; $df = 1,507$) and medium gains ($R = -0.08$; $P_{\text{corrected}} = 0.0209$; $df = 1,507$). Activation of node 2 (V1/V2) revealed predominant association with fewer omissions of responses under negative ($R = -0.09$; $P_{\text{corrected}} = 0.0090$; $df = 1,323$) stimuli in the affective go/no go task. Node 3 (somatosensory/motor) was predominantly associated with less delay discounting of large rewards ($R = -0.08$; $P_{\text{corrected}} = 0.0190$; $df = 1,507$). In node 4, we detected no predominant association with any of the neuropsychological measures. Other associations that were significant after permutation (Table 2) but were not predominant after partial correlation analysis are not described here. Although all four identified nodes are part of a reward anticipation network, their different anatomical localization as well as their distinct neuropsychological characteristics may suggest that these nodes represent functional correlates of a coordinated process underlying reward anticipation (6, 7).

Functional Network and Externalizing Behaviors. We next explored the relation of the fMRI nodes with indicators of psychopathology by searching for associations with behavioral outcomes relevant for ADHD and addictive behavior (Table 1). These outcomes included measures of hyperactivity from the Strengths and Difficulties Questionnaire (18) and lifetime alcohol consumption from the European School Survey Project on Alcohol and Drugs (19) (Table 3 and Table S4). We found that lower activation in striatal node 1 was associated with higher parent-rated hyperactivity ($R = -0.07$; $P_{\text{corrected}} = 0.0310$; $df = 1,523$). This association was only observed in boys ($R = -0.10$; $P = 6.53 \times 10^{-3}$; $df = 712$). A similar but weaker association was also observed in the small win vs. no win contrast of anticipation phase (*SI Materials and Methods*) ($R = -0.05$; $P = 0.0439$; $df = 1,427$ in the full sample and $R = -0.06$; $P = 0.104$; $df = 670$ in boys), suggesting that the association strength is proportional to the strength of reward stimuli. The occipital cortical node 2 showed the most significant association with reduced lifetime alcohol consumption ($R = -0.09$; $P_{\text{corrected}} = 0.0038$; $df = 1,484$). However, alcohol consumption was not only dependent on one node alone but also related to a link between nodes 1 and 2. It was associated with both caudate nucleus ($R = 0.09$; $P = 6.16 \times 10^{-4}$; $df = 1,483$) in node 1 and V1/V2 activation ($R = 0.08$; $P = 1.57 \times 10^{-3}$; $df = 1,483$) in node 2, which were significantly correlated ($R = 0.11$; $P_{\text{corrected}} = 9.43 \times 10^{-3}$; $df = 1,514$) (Fig. S2A).

To further assess the relation of activation in node 1 with hyperactivity, we selected extreme cases with clinically relevant hyperactivity scores ≥ 7 ($n = 113$; 76 boys), cases with mild hyperactivity scores = 4 ($n = 179$; 91 boys), and cases with no indication of hyperactivity (scores = 0) as controls ($n = 256$; 80 boys; www.sdqinfo.com). In extreme cases vs. controls, we found a two-fold increase in the effect size of the correlation between hyperactivity and node 1 activation ($R = -0.12$; $P = 0.0197$; $df = 358$) compared with a quantitative analysis in the full IMAGEN sample ($R = -0.069$). Similar to the full sample, the association in extreme

Table 1. Summary of descriptive statistics for the IMAGEN sample

Sample information	Descriptive statistics
fMRI full sample size	$n = 1,544$ (53% female)
Age, y (range)	14.41 (12.56–16.04)
Affective go/no go task ($n = 1,333$)	
Positive stimuli	12.3 (8.09)
Negative stimuli	14.0 (7.42)
SWM ($n = 1,506$)	
Between search error	19.3 (14.0)
Delay discounting task ($n = 1,518$)	
Small reward	0.0381 (0.0516)
Medium reward	0.0276 (0.0404)
Large reward	0.0176 (0.0342)
SDQ ($n = 1,534$)	
Hyperactivity (parent-rated)	2.86 (2.56)
ESPAD ($n = 1,495$)	
Lifetime alcohol consumption	17.2 (34.3)
TCI-R ($n = 1,521$)	
Impulsiveness	26.74 (4.80)

Statistics are mean (SD) unless noted otherwise. ESPAD, European School Survey Project on Alcohol and Drugs; SDQ, Strengths and Difficulties Questionnaire; SWM, spatial working memory; TCI-R: Temperament and Character Inventory, Revised Version. Distributions of variables were shown in Fig. S7.

cases vs. controls was only observed in boys ($R = -0.22$; $P = 7.20 \times 10^{-3}$; $df = 146$). We observed a monotonically decreased mean activation in node 1 with higher hyperactivity, providing no evidence for a U-shaped model between hyperactivity and BOLD response (20) (Fig. S34). There was a highly significant association between impulsivity (as assessed with the Cloninger's Temperament and Character Inventory, Revised Version) (Table 1) (21) and parent-rated hyperactivity ($P = 3.44 \times 10^{-11}$; $df = 1,523$), but only a modest explanation of variance ($R^2 = 0.029$).

Genome-Wide Association Study of Reward Sensitivity. The involvement of the striatum in reward anticipation is well-established, and striatal node 1 was associated with both neuropsychological indicators of dysfunctional reward processing and behavioral symptoms of hyperactivity. Therefore, we carried out a genome-wide association study (GWAS) of node 1 BOLD response during reward anticipation in the IMAGEN sample ($n = 1,403$). We detected a signal in the sixth intron of the *vacuolar protein sorting-associated protein 4A* (*VPS4A*) gene locus, the C/T SNP rs16958736. The major C allele was associated with decreased activation in the striatal node 1 ($R = 0.14$; $P = 1.30 \times 10^{-7}$) (Fig. 2A and Fig. S44). Although the *VPS4A* signal does not reach the commonly used threshold for genome-wide significance ($P = 5.00 \times 10^{-8}$), it remains significant if corrected for the number of independent tests (22), where the 0.05 significance threshold was detected at $P = 1.71 \times 10^{-7}$. It is, thus, a strongly suggestive candidate. A similar but weaker association was also observed in the small win vs. no win contrast of anticipation phase ($R = 0.06$; $P = 0.0366$). *VPS4A* encodes an ATPase involved in trafficking of G protein-coupled receptors, including dopamine receptors (23). *VPS4A* genotypes did not alter the direction of the correlation between BOLD response in node 1 and hyperactivity (Fig. S3B). The stability of the association of *VPS4A* with node 1 was supported by both the consistent directional associations across recruitment sites (seven of eight; $R = 0.13$; $P = 3.26 \times 10^{-6}$ from metaanalysis) and normally distributed t statistics from bootstrapping analyses ($R = 0.14$; $P = 1.53 \times 10^{-7}$; mean t statistic) (Fig. 2B, SI Materials and Methods, and Fig. S4B). To assess the genetic information of the entire *VPS4A* locus, we conducted a haplotype analysis, and *VPS4A* was associated with the striatal node 1 (Table S5) ($\eta^2 = 0.02$; $P = 1.58 \times 10^{-4}$; omnibus test) as a single haplotype block (SI Materials and Methods and Fig. S5). This association was driven by a positive association of haplotype 4 ($R = 0.12$; frequency = 0.033; $P = 1.33 \times 10^{-5}$; $df = 1,392$), and it was mainly observed in

boys ($R = 0.14$; $P = 2.29 \times 10^{-4}$; $df = 653$) and observed less in girls ($R = 0.09$; $P = 0.019$; $df = 730$).

Haplotype Analysis of *VPS4A* with Hyperactivity. Haplotype 4 of *VPS4A* was significantly associated with hyperactivity in boys ($R = 0.08$; $P = 0.0216$; $df = 935$), but there was no association of rs16958736, indicating that, although the *VPS4A* gene is involved in the regulation of reward sensitivity and hyperactivity, this SNP is likely to be a marker for an undetected causal genetic variation. Because there is no functional neuroimaging sample of comparable magnitude with the MID task, we were forced to restrict replication to *VPS4A* and hyperactivity in two independent samples (Table S6). In the Saguenay sample (24) of 481 adolescent boys (SI Materials and Methods), we found a significant association of *VPS4A* with the ADHD symptoms in both the overall haplotypes ($\eta^2 = 0.04$; $P = 0.0239$; omnibus test) and haplotype 4 ($R = 0.09$; $P_{\text{one tailed}} = 0.0200$; $df = 478$). In the Avon Longitudinal Study of Parents and Children sample (25) (SI Materials and Methods), we confirmed the association of *VPS4A* haplotypes with hyperactivity in 2,550 13-y-old boys ($\eta^2 = 0.01$; $P = 0.0271$; omnibus test) but not for haplotype 4.

Gene Manipulation in *Drosophila*. *Vps4* is the highly conserved, sole ortholog of two mammalian *VPS4* genes (26), and the fly protein has 74% identity and 86% similarity to human *VPS4A*. We neuronally overexpressed *Drosophila Vps4* and found that these flies were hypoactive ($P < 0.01$; Cohen's $d = 1.02$; $t = 5.68$; $df = 31$), whereas flies with neuronal *Vps4* knockdown showed significant

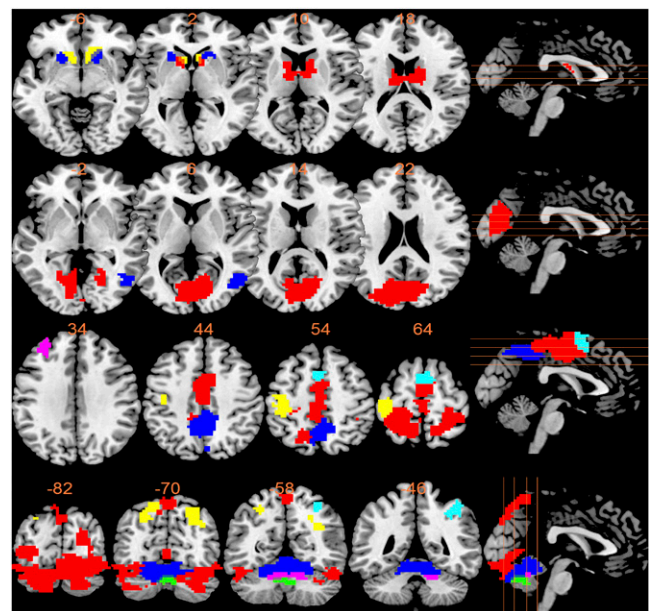


Fig. 1. Illustration of fMRI clusters/nodes. (Row 1) Node 1: module 7, caudate (red); module 15, putamen (blue); and module 21, nucleus accumbens (yellow), with multislicing axial view at Montreal Neurological Institute (MNI) coordinate z equal to $-6, 2, 10$, and 18 . (Row 2) Node 2: module 3, visual area V1 and V2 (red) and module 19, the right parietal/temporal/occipital area (blue), with multislicing axial view at MNI coordinate z equal to $-2, 6, 14$, and 22 . (Row 3) Node 3: module 2, primary somatosensory and motor areas (red); module 6, anterior precuneus (blue); module 10, left precentral and postcentral gyrus (yellow); module 17, dorsorostral supplementary motor area (light blue); and module 29, left dorsolateral prefrontal cortex (purple), with multislicing axial view at MNI coordinate z equal to $34, 44, 54$, and 64 . (Row 4) Node 4: module 1, visual area V3 and V4 (red); module 4, cerebellum anterior lobe and declive of posterior lobe (blue); module 5, superior parietal lobe (yellow); module 11, right supramarginal gyrus (light blue); module 16, arbor vitae (purple); and module 22, cerebellum vermis (green), with multislicing sagittal view at MNI coordinate y equal to $-82, -70, -58$, and -46 .

Table 2. Results of the association analyses between fMRI nodes and neuropsychological tests

Outcomes (df)	Node 1	Node 2	Node 3	Node 4
AGN omission positive stimuli (1,323)	n.s.	0.0090 (−0.09)*,†	n.s.	n.s.
AGN omission negative stimuli (1,323)	n.s.	0.0014 (−0.11)*	0.0026 (−0.11)*	0.0038 (−0.10)*
SWM between search errors (1,495)	0.0001 (−0.12)*,†	0.0003 (−0.11)*	0.0007 (−0.09)*	0.0004 (−0.11)*
Delay discounting (small reward; 1,507)	0.0461 (−0.08)*,†	n.s.	n.s.	n.s.
Delay discounting (medium reward; 1,507)	0.0209 (−0.08)*,†	n.s.	n.s.	n.s.
Delay discounting (large reward; 1,507)	n.s.	n.s.	0.0190 (−0.08)*,†	n.s.

The *P* values corrected for multiple testing were calculated based on 10,000 permutation analyses. AGN, affective go/no go; n.s., no significance; SWM, spatial working memory.

*Results were provided as *P* value (partial correlation, i.e., the effect size).

†A node was classed as predominant if its *P* value after controlling for all other nodes was smaller than 0.10 (Table S3). Study sites, gender, and handedness were controlled.

hyperactivity ($P < 0.01$; Cohen's $d = 1.03$; $t = 4.51$; $df = 19$) (Fig. 2 C–E). In rodents, the *Vps4b* paralogue is associated with locomotor activity, dysregulation of the dopamine system, and altered alcohol reward sensitivity (27). Because flies do not have noradrenaline, their catecholaminergic function is restricted to dopamine. *Drosophila* dopamine receptors—including DRD1—show closest homology to both human DRD and alpha adrenergic receptors (ADRA) and are correlated with locomotor activity (28). We confirmed this by testing *Drosophila* DRD1 (*Drd1*; also known as *Dop1R1*) mutants that were hypoactive compared with control ($P < 0.05$; Cohen's $d = 0.26$; $t = 2.08$; $df = 62$) (Fig. 2 C and F). Because in *Drosophila* locomotion, *Vps4* overexpression resulted in the same phenotype as loss of DRD1 function, we were interested in a more detailed investigation of the coexpression patterns of *VPS4A* and catecholamine genes.

Coregulation Patterns Between *VPS4A* and Catecholamine Receptors in Humans and Mice. We measured coexpressions of *VPS4A* with major pre- and postsynaptic dopamine and noradrenaline receptors in frontocortical postmortem human brain data from BrainCloud ($n = 248$) (SI Materials and Methods) (29). *VPS4A* showed a negative correlation with postsynaptic activating DRD1 ($R = -0.22$; $P = 6.48 \times 10^{-4}$) and a positive correlation with presynaptic inhibitory DRD2S ($R = 0.39$; $P = 1.85 \times 10^{-10}$). Similarly, in mouse striatum ($n = 31$) (SI Materials and Methods) (30), we observed a negative correlation of *Vps4a* and *Drd1* expression ($R = -0.48$; $P = 6.80 \times 10^{-3}$) and a positive correlation of *Vps4a* and *Drd2* expression ($R = 0.53$; $P = 2.07 \times 10^{-3}$). *VPS4A* was also significantly correlated with presynaptic ADRA2C expression in both human ($R = 0.56$; $P = 1.40 \times 10^{-21}$) and mouse data ($R = 0.49$; $P = 5.70 \times 10^{-3}$).

Discussion

We describe a coordinated neural network, which is activated on response to anticipated reward. This network involves a core subcortical node in the striatum (node 1) as well as accessory cortical nodes in the visual association cortex (node 2) and somatosensory cortex (node 3). In our population-based sample, BOLD responses in these nodes were preferentially associated with either ADHD symptoms (node 1) or lifetime alcohol consumption (node 2). Increased BOLD response in node 1 was also associated with better short-term working memory and reduced delay discounting, which together with its localization, may suggest a contribution to the initiation and monitoring of goal-directed behaviors (5). This node is likely to work in concert with cortical nodes 2 and 3 (6, 7) to execute motivated, planned behaviors. BOLD response in the occipital visual node 2 was correlated with affective processing and stimulus expectancy (7). Its link with the striatal reward circuit in node 1 was associated with alcohol consumption. These findings may emphasize the joint modulation of reward-related function and dysfunction by attentional affective and motivational factors. The sensorimotor areas, such as the supplementary motor area, in node 3 were not predominantly associated with externalizing behavior, and they may be viewed as output modules that are driven by the valence and arousal of the rewarding stimuli

and instigate motor responses but also, may act back on the striatal reward node 1 (5). This hypothesis was supported by the correlation of BOLD response in node 3 with delay discounting, predominantly to large rewards. Thus, we hypothesize that node 1 may act together with nodes 2 and 3 associated with perception, cognition, and motor control to process reward. Specificity of the reward anticipation network for these different reward-related cognitive and externalizing behavioral symptoms may be provided by distinct network configurations, which are reflected in different association patterns of nodes and their connecting links. Reward-related disorders, including alcohol use disorders and ADHD, have strong comorbid relations. Comorbidity between ADHD and alcohol abuse in adults of 12.9% (31) and 61–64% in adolescence (32) suggests relatedness of the neural mechanisms underlying these disorders, which to date, are diagnosed separately and treated differently.

It is a limitation of this manuscript that some of the proposed functions of the nodes identified were partly based on reverse inference (33). Although we are aware of the potential logical fallacy associated with this method, its application was inevitable to help interpret our findings from data-driven analyses. Nevertheless, we tried to minimize the risk for inaccurate interpretations by increasing prior probability through linking task-based data with neuropsychological and behavioral measures that are directly related to the psychological construct interrogated in the task setting (i.e., reward processing) (34). In addition, reward anticipation involves a number of additional psychological functions, such as higher attention, salience attribution, valuation, and arousal, which are inherent in reward anticipation and may have contributed to the observed associations.

We found a negative correlation of hyperactivity and BOLD response in the striatal node 1, which is consistent with previous studies in both clinical (2, 20) and nonclinical samples (35). However, it has been suggested that, in population-based samples, impulsivity measures show mostly positive correlation with VS activation during reward anticipation, whereas in clinical studies of ADHD patients, often a negative correlation is observed (20). Plichta and Scheres (20) have proposed three models explaining these relations, which assume an inverted U shape, (genetic) moderators, or unrelatedness to account for the observed inconsistencies. In the IMAGEN dataset, we did not find evidence

Table 3. Results of the association analyses between fMRI nodes and psychopathological assessment

Outcomes (df)	Node 1	Node 2	Node 3
ADHD symptoms (1,523)	0.0310 (−0.07)*,†	n.s.	n.s.
Alcohol usage (1,484)	n.s.	0.0038 (−0.09)*,†	0.0432 (−0.07)*

*Results were provided as *P* value (partial correlation, i.e., the effect size).

†A node was classed as predominant if its *P* value after controlling for all other nodes was smaller than 0.10 (Table S4). Study sites, gender, and handedness were controlled.

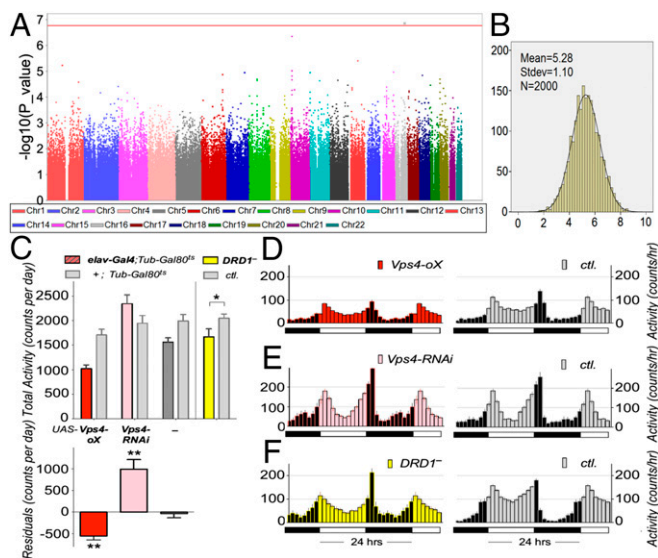


Fig. 2. Results for the *VPS4A* gene. (A) Manhattan plot of GWAS of the striatal node. The red line indicates the 5% genome-wide significance level based on the number of independent tests (22). SNP rs16958736 (Chr16: 69353587 in hg19) in the sixth intron of *VPS4A* was significant. (B) Histogram of bootstrapping results of the association between node 1 (the striatum) and SNP rs16958736; *t* statistics follow normal distribution, suggesting no hidden substructure and highly stable results. The mean *t* statistic of 5.28 (from 2,000 bootstrapping iterations) is equivalent to $P = 1.53 \times 10^{-7}$ (two-tailed test; $df = 1,393$) (SI Materials and Methods). (C) Locomotion phenotypes in *Drosophila* mutant strains. (Upper) Total daily locomotion activity of *Drosophila* expressing UAS transgenes for *Vps4* in the nervous system specifically with the *elav-Gal4* driver. In both males and females, expression of *elav-Gal4* (dark gray bar; group marked by —) reduced activity by 20%. (Lower) We, thus, corrected for this in the experimental *elav-Gal4;Tub-Gal80^{ts}* flies by dividing their total activity by 0.8, and then, we plotted the difference of the corrected experimental *elav-Gal4;Tub-Gal80^{ts}* activity from their control *+;Tub-Gal80^{ts}* activity. This figure shows that *Vps4* overexpression (*ox*) significantly decreased (Cohen's $d = 1.02$; $t = 5.68$; $df = 31$) and that knockdown (*RNAi*) significantly increased locomotion activity (Cohen's $d = 1.03$; $t = 4.51$; $df = 19$). *DRD1* mutant flies (yellow) showed hypolocomotion compared with their genetic background-matched control (Cohen's $d = 0.26$; $t = 2.08$; $df = 62$). * $P < 0.05$; ** $P < 0.01$. (D–F) Average activity plots of flies from C. Mutants (D) *Vps4* overexpression, (E) *Vps4* knockdown, and (F) *DRD1* mutant are in *Left*, and their controls are in *Right*, respectively. Note that, for genetic reasons, we had to use females for *Vps4* overexpression, which show a less diurnal activity pattern. *ctl.*, Control.

for either a U-shaped distribution (Fig. S3A) or genetic moderation by *VPS4A* genotypes (Fig. S3B) of the correlation of hyperactivity and VS activation. However, in a previous investigation, we have observed genetic moderation by monoamine oxidase A (MAOA) genotype of the interplay of inferior frontal gyrus and VS activation on ADHD symptoms (2). The third model, which assumes unrelatedness, accounts for the possibility “that the blunted VS response as observed in individuals with ADHD is related to additional disease-related factors and not trait impulsivity per se” (20). We found highly significant association of hyperactivity and trait impulsivity, but 97% of the variance of hyperactivity could not be explained by the Temperament and Character Inventory, Revised Version impulsivity measure. Although these analyses are limited by the fact that the extreme cases were selected from a population-based sample as opposed to a clinical ADHD sample, they indicate the presence of additional stochastic factors, which contribute to the emergence of ADHD symptoms. Despite their statistical significance, the effect sizes between fMRI nodes and neuropsychological tasks and behaviors were smaller than those detected in clinical samples (20). Our data were population-based and not selected for differences in hyperactivity and/or impulsivity, which would be the case for clinical ADHD case control studies. Comparing participants with high ADHD symptoms vs. controls,

we observed a doubling of the effect size. It is also important to note that behavioral constructs, such as hyperactivity, are not optimized to reflect neural processes, thus limiting the degree of correlation with measures of brain activation.

VPS4A is well-conserved across species (26) and highly expressed in the brain (ds.bioGPS.org/?dataset=GSE1133&gene=27183). It encodes a member of the ATPases associated with diverse cellular activities (AAA) family involved in the late steps of the generating endosomal multivesicular bodies (MVBs) by promoting membrane invagination and scission from the limiting endosome membrane (36). MVBs are then delivered to lysosomes for protein degradation or recycled to the plasma membrane (37). In neurons, MVBs are involved in trafficking of neurotransmitter receptors after endocytosis, including dopamine and noradrenaline G protein-coupled receptors in the striatum (23). Although we did not find an association of rs16958736 with *VPS4A* expression, we observed significant correlations of *VPS4A* expression and expressions of dopamine and noradrenaline receptors in both human frontal cortex and mouse striatum. These brain regions were investigated as exemplary given that dopaminergic and noradrenergic gene expression and extracellular responses (e.g., to alcohol treatment) are comparable between striatum and other brain areas (38, 39). Increased expression of *VPS4A* is correlated with decreased expression of postsynaptic *DRD1* and increased expression of presynaptic *DRD2* and *ADRA2C*, thus possibly altering reward sensitivity and level of activity (27, 28). The observed coregulation patterns are consistent with the result of the manipulation of *Vps4* and *Drd1* in *Drosophila*, where *Vps4* and *Drd1* have opposing effects on activity. They also conform to the predominant association of the striatal node 1 with ADHD symptoms and ADHD symptoms with *VPS4A*. Together, these data give rise to the hypothesis that *VPS4A* affects activity and reward processing by regulating brain catecholaminergic systems, including D1 and D2 dopaminergic and $\alpha 2C$ adrenergic receptors. Nevertheless, detailed molecular and physiologic studies are required to further investigate possible functional relations between *VPS4A* and signal transductions of catecholamine.

Our approach to neurobehaviorally characterize reward anticipation can potentially be extended to other behavioral domains and help contribute to a reclassification of mental disorders (40). It may facilitate the identification of causal neural mechanisms important for the identification of previously unidentified targets and repurposing of existing drugs as well as the establishment of neurobehaviorally informed end points for clinical trials (41).

Materials and Methods

Details are in SI Materials and Methods.

MID Task. During the anticipation phase of the MID task, cues are shown to indicate that no reward, a small reward, or a large reward might be won during the trial. Participants are then shown a target stimulus and asked to respond to the target to gain the reward. We analyzed the contrast comparing the BOLD signal during anticipation of large (or small) rewards with the BOLD signal during anticipation of no rewards; the only difference between the two conditions was the presented target stimuli.

WVCNA. The R package WGCNA (42) was implemented to perform the WVCNA of the contrasts of the MID task. The final dataset involved 1,544 participants and 92,119 voxels after removing null data and outliers. Using the scale-free topology criterion, the soft threshold parameter was set to eight (Fig. S6A). The stabilities of generated modules were assessed through bootstrapping (Fig. S6B).

GWAS and Haplotype Analysis. The Efficient Mixed Model Association Expedited was implemented to perform the GWAS for the eigenvoxels of selected fMRI modules (genetics.cs.ucla.edu/emmax/). Haplotype blocks were generated and illustrated through the software Haploview (<https://sourceforge.net/projects/haploview/>) using the solid spine of the linkage disequilibrium method with parameter 0.80. Haploview was also used to generate the Manhattan plot of GWAS results from Efficient Mixed Model Association Expedited (Fig. 2A). The haplotype phases were estimated through software PLINK (pku.mgh.harvard.edu/~purcell/plink/).

Drosophila Experiments. Locomotion activity was measured in the *Drosophila* Activity Monitor System (Trikinetics) at 25 °C in 12:12-h light–dark cycles for 3 d. Activity was measured in 30-min bins and aggregated into 1-h bins (Fig. 2 D–F). Males were used where possible, but for *Vps4* overexpression, we assayed females (because both *Vps4[EP]* and *elav-Gal4* are on the X chromosome), which show less marked crepuscular behavior than males (i.e., do not take a siesta) as previously reported (43). Experimental (*elav-Gal4;Tub-Gal80[ts];UAS-transgene*) and control (*+/Tub-Gal80[ts];UAS-transgene*) flies were in the same hybrid genetic background (*Berlin/transgene*). The DRD1 receptor loss-of-function mutant used was allele *f02676*, and it was outcrossed to *w Berlin* (44).

Ethical Approval. The IMAGEN Study was approved by local ethics research committees at each research site: King's College London, University of Nottingham, Trinity College Dublin, University of Heidelberg, Technische Universität Dresden, Commissariat à l'Energie Atomique et aux Energies Alternatives, and University Medical Center. Informed consent was sought from all participants and a parent/guardian of each participant.

- Knutson B, Wimmer GE (2007) Splitting the difference: How does the brain code reward episodes? *Ann N Y Acad Sci* 1104:54–69.
- Nymberg C, et al.; IMAGEN Consortium (2013) Neural mechanisms of attention-deficit/hyperactivity disorder symptoms are stratified by MAOA genotype. *Biol Psychiatry* 74(8): 607–614.
- Peters J, et al.; IMAGEN Consortium (2011) Lower ventral striatal activation during reward anticipation in adolescent smokers. *Am J Psychiatry* 168(5):540–549.
- Stacey D, et al.; IMAGEN Consortium (2012) RASGRF2 regulates alcohol-induced reinforcement by influencing mesolimbic dopamine neuron activity and dopamine release. *Proc Natl Acad Sci USA* 109(51):21128–21133.
- Plichta MM, et al. (2013) Simultaneous EEG and fMRI reveals a causally connected subcortical-cortical network during reward anticipation. *J Neurosci* 33(36):14526–14533.
- Pleger B, Blankenburg F, Ruff CC, Driver J, Dolan RJ (2008) Reward facilitates tactile judgments and modulates hemodynamic responses in human primary somatosensory cortex. *J Neurosci* 28(33):8161–8168.
- Serences JT (2008) Value-based modulations in human visual cortex. *Neuron* 60(6): 1169–1181.
- Salimpoor VN, et al. (2013) Interactions between the nucleus accumbens and auditory cortices predict music reward value. *Science* 340(6129):216–219.
- Schultz W, Tremblay L, Hollerman JR (2000) Reward processing in primate orbitofrontal cortex and basal ganglia. *Cereb Cortex* 10(3):272–284.
- Girault JA (2012) Integrating neurotransmission in striatal medium spiny neurons. *Adv Exp Med Biol* 970:407–429.
- Ladepeche L, Yang L, Bouchet D, Groc L (2013) Regulation of dopamine D1 receptor dynamics within the postsynaptic density of hippocampal glutamate synapses. *PLoS One* 8(9):e74512.
- Schumann G, et al.; IMAGEN consortium (2010) The IMAGEN study: Reinforcement-related behaviour in normal brain function and psychopathology. *Mol Psychiatry* 15(12):1128–1139.
- Mumford JA, et al. (2010) Detecting network modules in fMRI time series: A weighted network analysis approach. *Neuroimage* 52(4):1465–1476.
- Clatworthy PL, et al. (2009) Dopamine release in dissociable striatal subregions predicts the different effects of oral methylphenidate on reversal learning and spatial working memory. *J Neurosci* 29(15):4690–4696.
- Gau SSF, Chiang HL (2013) Association between early attention-deficit/hyperactivity symptoms and current verbal and visuo-spatial short-term memory. *Res Dev Disabil* 34(1):710–720.
- Kirby KN, Maraković NN (1996) Delay-discounting probabilistic rewards: Rates decrease as amounts increase. *Psychon Bull Rev* 3(1):100–104.
- Cardinal RN, Pennicott DR, Sugrathapala CL, Robbins TW, Everitt BJ (2001) Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science* 292(5526):2499–2501.
- Goodman R (2001) Psychometric properties of the strengths and difficulties questionnaire. *J Am Acad Child Adolesc Psychiatry* 40(11):1337–1345.
- Morgan M, et al. (1999) The ESPAD study: Implications for prevention. *Drugs Educ Prev Policy* 6(2):243–256.
- Plichta MM, Scheres A (2014) Ventral-striatal responsiveness during reward anticipation in ADHD and its relation to trait impulsivity in the healthy population: A meta-analytic review of the fMRI literature. *Neurosci Biobehav Rev* 38:125–134.
- Cloninger CR, Bayon C, Svrakic DM (1998) Measurement of temperament and character in mood disorders: A model of fundamental states as personality types. *J Affect Disord* 51(1):21–32.
- Li MX, Yeung JMY, Cherny SS, Sham PC (2012) Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. *Hum Genet* 131(5):747–756.
- Bloch B, Bernard V, Dumartin B (2003) "In vivo" intraneuronal trafficking of G protein coupled receptors in the striatum: Regulation by dopaminergic and cholinergic environment. *Biol Cell* 95(7):477–488.
- Pausova Z, et al. (2007) Genes, maternal smoking, and the offspring brain and body during adolescence: Design of the Saguenay Youth Study. *Hum Brain Mapp* 28(6):502–518.
- Golding J, Pembrey M, Jones R; ALSPAC Study Team (2001) ALSPAC—the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol* 15(1):74–87.

ACKNOWLEDGMENTS. This work received support from the following sources: the European Union-funded FP6 Integrated Project IMAGEN (Reinforcement-related behaviour in normal brain function and psychopathology) LSHM-CT-2007-037286; the FP7 Projects IMAGEMEND (602450; Imaging Genetics for Mental Disorders) and Multidisciplinary Approaches to Translational Research in Conduct Syndromes (603016); Innovative Medicine Initiative Project EU-AIMS 115300-2; Medical Research Council Grants 93558 (Developmental pathways into adolescent substance abuse) and MR/N000390/1 (Consortium on Vulnerability to Externalizing Disorders and Addictions); the Swedish funding agency FORMAS (Forskningsrådet för Miljö, Areella näringar och Samhällsbyggande); the National Institute for Research Biomedical Research Centre at South London and Maudsley National Health Service Foundation Trust and King's College London; Bundesministerium für Bildung und Forschung Grants 01GS08152, 01EV0711, eMED SysAlc01ZX1311A, and Forschungsnetz AERIAL (Addiction: Early Recognition and Intervention Across the Lifespan); NIH Grant R01 MH085772-01A1 (Axon, Testosterone and Mental Health during Adolescence); and NIH Consortium Grant U54 EB020403 supported by a cross-NIH alliance that funds Big Data to Knowledge Centres of Excellence. The *Drosophila* experiments were supported by NIH Grants T32 DA007290 (to D.A.G.), R01AA019526 (to A.R.), and R21AA022404 (to A.R.).

- Beyer A, et al. (2003) Comparative sequence and expression analyses of four mammalian *Vps4* genes. *Gene* 305(1):47–59.
- McBride WJ, et al. (2012) Gene expression in the ventral tegmental area of 5 pairs of rat lines selectively bred for high or low ethanol consumption. *Pharmacol Biochem Behav* 102(2):275–285.
- Riemensperger T, et al. (2011) Behavioral consequences of dopamine deficiency in the *Drosophila* central nervous system. *Proc Natl Acad Sci USA* 108(2):834–839.
- Colantuoni C, et al. (2011) Temporal dynamics and genetic control of transcription in the human prefrontal cortex. *Nature* 478(7370):519–523.
- Korostynski M, Kaminska-Chowaniec D, Piechota M, Przewlocki R (2006) Gene expression profiling in the striatum of inbred mouse strains with distinct opioid-related phenotypes. *BMC Genomics* 7:146.
- Rodgers S, et al. (2015) Externalizing disorders and substance use: Empirically derived subtypes in a population-based sample of adults. *Soc Psychiatry Psychiatr Epidemiol* 50(1):7–17.
- Chan YF, Dennis ML, Funk RR (2008) Prevalence and comorbidity of major internalizing and externalizing problems among adolescents and adults presenting to substance abuse treatment. *J Subst Abuse Treat* 34(1):14–24.
- Poldrack RA (2006) Can cognitive processes be inferred from neuroimaging data? *Trends Cogn Sci* 10(2):59–63.
- Hutzel F (2014) Reverse inference is not a fallacy per se: Cognitive processes can be inferred from functional imaging data. *Neuroimage* 84:1061–1069.
- Stark R, et al. (2011) ADHD related behaviors are associated with brain activation in the reward system. *Neuropsychologia* 49(3):426–434.
- Babst M, Davies BA, Katzmann DJ (2011) Regulation of *Vps4* during MVB sorting and cytokinesis. *Traffic* 12(10):1298–1305.
- Babst M, Katzmann DJ, Estepa-Sabal EJ, Meerloo T, Emr SD (2002) Escrt-III: An endosome-associated heterooligomeric protein complex required for mvb sorting. *Dev Cell* 3(2):271–282.
- Easton AC, et al. (2014) Rasgrf2 controls noradrenergic involvement in the acute and subchronic effects of alcohol in the brain. *Psychopharmacology (Berl)* 231(21): 4199–4209.
- Easton AC, et al. (2014) Rasgrf2 controls dopaminergic adaptations to alcohol in mice. *Brain Res Bull* 109:143–150.
- Cuthbert BN, Insel TR (2013) Toward the future of psychiatric diagnosis: The seven pillars of RDoC. *BMC Med* 11:126.
- Schumann G, et al. (2014) Stratified medicine for mental disorders. *Eur Neuropsychopharmacol* 24(1):5–50.
- Langfelder P, Horvath S (2008) WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559.
- Isaac RE, Li C, Leedale AE, Shirras AD (2010) *Drosophila* male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female. *Proc Biol Sci* 277(1678):65–70.
- Kong EC, et al. (2010) A pair of dopamine neurons target the D1-like dopamine receptor DopR in the central complex to promote ethanol-stimulated locomotion in *Drosophila*. *PLoS One* 5(4):e9954.
- Eguiluz VM, Chialvo DR, Cecchi GA, Baliki M, Apkarian AV (2005) Scale-free brain functional networks. *Phys Rev Lett* 94(1):018102.
- Herjanic B, Reich W (1997) Development of a structured psychiatric interview for children: Agreement between child and parent on individual symptoms. *J Abnorm Child Psychol* 25(1):21–31.
- Stahre M, Naimi T, Brewer R, Holt J (2006) Measuring average alcohol consumption: The impact of including binge drinks in quantity-frequency calculations. *Addiction* 101(12):1711–1718.
- Lucas CP, et al. (2001) The DISC Predictive Scales (DPS): Efficiently screening for diagnoses. *J Am Acad Child Adolesc Psychiatry* 40(4):443–449.
- Faraone SV, et al. (2005) Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 57(11):1313–1323.
- Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63(1):182–217.
- Saunders C, Limbird LE (1999) Localization and trafficking of alpha2-adrenergic receptor subtypes in cells and tissues. *Pharmacol Ther* 84(2):193–205.