

The TWIN-E Project in Emotional Wellbeing: Study Protocol and Preliminary Heritability Results Across Four MRI and DTI Measures

Justine M. Gatt,^{1,2} Mayuresh S. Korgaonkar,¹ Peter R. Schofield,^{3,4} Anthony Harris,^{1,2} C. Richard Clark,⁵ Karen L. Oakley,^{1,2} Kaushik Ram,^{1,2} Hope Michaelson,¹ Sarsha Yap,¹ Melinda Stanners,⁵ Vikki Wise,⁵ and Leanne M. Williams^{1,2}

¹Brain Dynamics Centre, Westmead Millennium Institute, University of Sydney Medical School-Westmead, Westmead Hospital, Australia

²Discipline of Psychiatry, University of Sydney Medical School-Westmead, Westmead Hospital, Australia

³Neuroscience Research Australia, Barker Street, Randwick, Australia

⁴School of Medical Sciences, University of NSW, Sydney, Australia

⁵Brain Health Clinics and School of Psychology, Flinders University, Adelaide, Australia

Despite the significant advancements being made in the neurogenetics for mental health, the identification and validation of potential endophenotype markers of risk and resilience remain to be confirmed. The TWIN-E study (The Twin study in Wellbeing using Integrative Neuroscience of Emotion) aims to validate endophenotype markers of mental health across cognitive, brain, and autonomic measures by testing the heritability, clinical plausibility, and reliability of each of these measures in a large adult twin cohort. The specific gene and environmental mechanisms that moderate prospective links between endophenotype markers and the final outcome of wellbeing will also be identified. TWIN-E is a national prospective study with three phases: I) baseline testing on a battery of online questionnaires and cognitive tasks, and EEG, MRI, and autonomic testing; II) 12-month follow-up testing on the online assessments; and III) randomized controlled trial of brain training. Minimum target numbers include 1,500 male/female twins (18–65 years) for the online assessments (Phase I and II), 300 twins for the EEG testing component, and 244 twins for the MRI testing component. For Phase III, each twin out of the pair will be randomized to either the treatment or waitlist control group to test the effects of brain training on mental health over a 30-day period, and to confirm the gene–environment and endophenotype contributions to treatment response. Preliminary heritability results are provided for the first 50% of the MRI subgroup ($n = 142$) for the grey matter volume, thickness, and surface area measures, and white matter diffuse tensor imaging fractional anisotropy.

■ **Keywords:** resilience, mental health, endophenotypes, genetics, stress, imaging

From the leaps being made in genetics and brain science we know that there is not a one-to-one mapping between genetic risk and behaviors of good or poor mental health. Instead, an interaction of our genes and environmental experiences, and how they impact brain circuitry, enables the flexibility and resilience of good mental health, or produces the overt signs and symptoms of mental illness. In order to quantify these interactions, we need to identify ‘endophenotype’ markers. These are biologically based markers that are intermediate between genes and overt behavior. This is the equivalent of, for example, in cardiology where tests of coronary artery patency are a link between risk factors and overt symptoms of myocardial infarction.

To define an endophenotype, several key criteria such as heritability, reliability, and clinical plausibility must be established (Gottesman & Gould, 2003; Hasler et al., 2004). We know that the heritability of common mental disorders is high — typically > 40% (Burmeister et al., 2008) — but we do not know the heritability of endophenotypes

RECEIVED 22 September 2011; ACCEPTED 3 November 2011.

ADDRESS FOR CORRESPONDENCE: Dr Justine Gatt, Brain Dynamics Centre, Westmead Millennium Institute, University of Sydney Medical School-Westmead, Westmead Hospital, Westmead NSW 2145, Australia. E-mail: justine.gatt@sydney.edu.au

predicting these disorders. We also do not know which genetic variants contribute to risk for poor mental health versus resilience. Reliability is reflected in stability over time, and clinical plausibility is about being conceptually related to mental health and illness. Twin research is essential to establishing these criteria. Recent studies have started to recognize the need to understand neurobiological links with genetic function, and potential endophenotypes across different brain or autonomic modalities (e.g., de Zubicaray et al., 2008; Kremen et al., 2010; Kochunov et al., 2011). The ‘TWIN-E’ (the Twin study in Wellbeing using Integrative Neuroscience of Emotion) study has been designed to achieve this with 1,500 twins in the prediction of risk and resilience for emotional brain health over time. It assesses genetics, both risk and resilience factors to mental illness, together with measures of behavior, cognition, emotion, psychophysiology, autonomic activity, and brain imaging to predict the spectrum of wellbeing through to psychopathology.

Linking genetics with brain measures requires going beyond the usual approaches used in neurological and psychological research. It needs an integrative theoretical framework. Methodologically, it requires a standardized approach that enables these multiple measures to be acquired in a systematic and time-effective way in the same participants. In the TWIN-E study, we draw on the standardized methods used to establish the Brain Resource International Database (Gordon et al., 2008; Gordon & Williams, 2010 [Data available freely for research purposes via the BRAINnet Foundation, www.BRAINnet.net]).

The TWIN-E study is couched in a framework that integrates common theoretical concepts across disciplines and measures. We highlight four core brain processes that have plausibility for understanding mental health; emotional, feeling, thinking, and self-regulatory functions (Gordon & Williams, 2010; Williams et al., 2008). This framework offers an integrative way to test hypotheses across areas of mental health, consistent with the broader initiatives in brain science and psychiatry (e.g., Williams et al., 2010b, c). These include the shift to a brain-based diagnostic classification system for DSM and the NIMH strategy for identifying biological constructs that underpin classification: referred to as the Research Domain Criteria (RDoC) project (Insel et al., 2009, 2010). The RDoC project aims to create classifications of functional domains derived from research that spans genes, (electro)physiology, neural circuits, and behavior. The ultimate goal is to change the way clinical groups are defined; for instance, shifting from a traditional clinical symptomatology classification system to one that is based on the integration of information across these levels of measurement. Endophenotypes provide a way forward for identifying and differentiating complex phenotypic symptomatology characteristic of mental disorders. They capture the underlying neurogenetic mechanisms contributing to disease, which are unaccounted for in the initial deriva-

tions of the DSM categories. TWIN-E focuses in particular on how these functions contribute to the experience of emotional health. We focus on emotional health defined as traits of risk and resilience on a continuum. Our reasoning is that endophenotypes that predict poor emotional health are risk factors for psychopathology, given that loss of emotional regulation is a hallmark of most mental disorders. Endophenotypes predicting positive emotional wellbeing therefore define what makes us resilient.

Our aims are to:

1. Establish the heritability of candidate endophenotypes for trait markers that put us at ‘risk’ for mental illness versus factors that determine ‘resilience’, tested in the same twin sample. These include cognitive performance on emotion identification tasks, psychophysiological activity during emotion tasks, heart rate variability, and cortico-limbic brain circuitry.
2. Evaluate the clinical plausibility of these endophenotypes. We will test if these endophenotypes contribute to confirmed associations between risk–resilience markers of emotional health (e.g., emotion regulation strategies, emotional resilience, and negative biases), and life outcomes or ‘wellbeing’ (e.g., mental health status, quality and satisfaction with life, and work productivity). By testing twin pairs, we will be able to derive the additive genetic component that underlies such associations.
3. Identify the genetic and environmental variants that contribute to the expression of these endophenotypes and moderate their impact on emotional health and life outcomes. Specific candidate genes known to modulate emotional brain function will be targeted. We will focus on early life trauma, based on evidence for its role in modifying genetic effects (Gatt et al., 2009, 2010a), but also extend to other factors including parenting style, health practices, and positive life events.
4. Evaluate the reliability of endophenotypes, defined by their predictive utility over 12 months. Under this aim, we will operationalize what endophenotypes predict risk for poor emotional health and poor life outcomes versus those that predict increased resilience and optimal emotional health.
5. Evaluate what aspects of risk and resilience are modifiable using brain training exercises delivered over a 1-month period. We will test whether brain training is effective in modifying emotional health, and whether the endophenotypes can predict treatment response.

In this paper, we report on the first outcomes addressing Aim 1. We present preliminary heritability estimates for cortico-limbic brain circuitry and cortical connectivity from brain imaging measures acquired for the first 50% of the sample (142 twins). These measures were from structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI), yielding data on both grey matter

(volume, cortical thickness, and surface area) and white matter (DTI fractional anisotropy [FA], index of white matter connectivity). Heritability estimates give the first insights into the variation attributed to genetics versus environment in these measures in this sample. We drew on previous brain imaging evidence for a strong genetic contribution accounting for total grey matter volume (h^2 : 60–82%; Baaré et al., 2001; Geschwind et al., 2002; Posthuma et al., 2002; Winkler et al., 2010; Wright et al., 2002), and the cortical lobes, especially frontal (56–83%; Geschwind et al., 2002; Hulshoff Pol et al., 2006; including orbitofrontal, 54%, Wright et al., 2002) and occipital (69–85%; Geschwind et al., 2002; Hulshoff Pol et al., 2006) involved in emotion regulation and perception, respectively. Moderate genetic contributions have also been observed for the relatively smaller cortices involved in processing and experiencing emotion (e.g., temporal lobe, 59%; parietal lobe, 47%; Geschwind et al., 2002). The limbic and paralimbic (including anterior cingulate) structures show a similarly moderate amount of genetic contribution (e.g., hippocampus, 40–64%; amygdala, 55–80%, posterior cingulate gyrus, 51%; Hulshoff Pol et al., 2006; Kremen et al., 2010; Sullivan et al., 2001; Wright et al., 2002). Fewer studies have focused on the heritability of cortical thickness and surface area, but they suggest a strong genetic contribution for these measures; estimates for total cortical thickness range from 69% to 81%, and for total surface area, from 71% to 89% (Panizzon et al., 2009; Winkler et al., 2010). While the size of the contribution is similar to that for grey matter volume, the genetic influences on cortical thickness and surface area appear to be unrelated. In contrast to these volumetric measures, DTI assesses the myelination and axonal growth of white matter that interconnects cortical regions. Of the few DTI twin studies of white matter to date, adult samples report a wide range of heritability estimates (34–66%) for the FA measure (Chiang et al., 2011; Kochunov et al., 2010), while null results have been seen in young 9-year-old twins (Brouwer et al., 2010). Reflecting this background, our study considers each of these brain imaging measures of both grey and white matter; grey matter volume, cortical thickness, surface area, and DTI (FA). To our knowledge, no previous study has estimated the contributions due to genetics versus environment, or their contribution, in these four measures, in the same sample of twins.

The present results will form the basis for subsequent analyses focusing on replication, stability over time, specific genotype–environment contributions and relationships with self-report, cognitive, and physiological measures in twins. Our goal is to use these combined outcomes from this study to achieve new insights into the biological basis of what puts us at risk for poor mental health, versus what keeps us resilient, with the ultimate goal of providing an evidence base for prevention strategies. Other important aspects of the endophenotype concept include association to disease and co-segregation of the endophe-

notype and disease within families (Gottesman & Gould, 2003). These criteria will be addressed in our collaborative studies in patients and family members with mental illness, which adopt the same standardized measures as adopted in TWIN-E. Association with disease will be assessed in our current clinical trials focusing on patients with depression (Williams et al., 2011), anxiety disorder (Williams et al., 2007), or attention deficit hyperactivity disorder (Williams et al., 2010c). Effects of co-segregation will be assessed in a separate study focusing on family members of clinical patients with depression or anxiety.

Study Organization

TWIN-E is funded by the Australian Research Council (ARC) Linkage grant LP0883621, with Brain Resource Ltd as Industry Partner. The chief investigators listed on this project are Professor Leanne M. Williams (University of Sydney), Professor Peter R. Schofield (Neuroscience Research Australia and University of New South Wales), Professor C. Richard Clark (Flinders University and Brain Health Clinics, South Australia), Associate Professor Anthony Harris (University of Sydney), and Doctor Justine M. Gatt (University of Sydney). Doctor Gatt is also the APDI (Australian Postdoctoral Industry) Research Fellow linked to this grant, and TWIN-E Project Coordinator.

The study is coordinated and managed from the Brain Dynamics Centre, University of Sydney site. Participant recruitment is performed in collaboration with the Australian Twin Registry, Melbourne, Australia. The two EEG testing sites are the Brain Dynamics Centre, University of Sydney, NSW, Australia; and the Flinders University, Adelaide, SA, Australia. MRI testing is conducted at Westmead Hospital, Sydney, Australia. DNA samples are prepared at Genetic Repositories Australia, located at Neuroscience Research Australia, Sydney, Australia.

Study Design

This is a multisite, cross-sequential, randomized-control trial of 1,500 healthy same-sex 18–65-year-old monozygotic (MZ) and dizygotic (DZ) twins, conducted in Australia. Following recruitment, the study incorporates three phases. Phase I and II are a baseline and longitudinal follow-up of the cross-sectional sample across four age decades, and constitute the ‘original’ study funded by the ARC Linkage grant. Phase III is a new ‘appended’ component involving a randomized-control trial and is currently supported in kind from our industry partner, Brain Resource Ltd.

Phase I involves three parts (Figure 1). All participants undertake Part 1 (target minimum $N = 1,500$ individuals or 750 twin pairs), which involves the completion of the computerized assessments WebQ and WebNeuro via the Internet at home, and the provision of a saliva sample for DNA testing. Part 2 involves a subset of twins (target minimum $n = 300$ individuals or 150 twin pairs; 20%

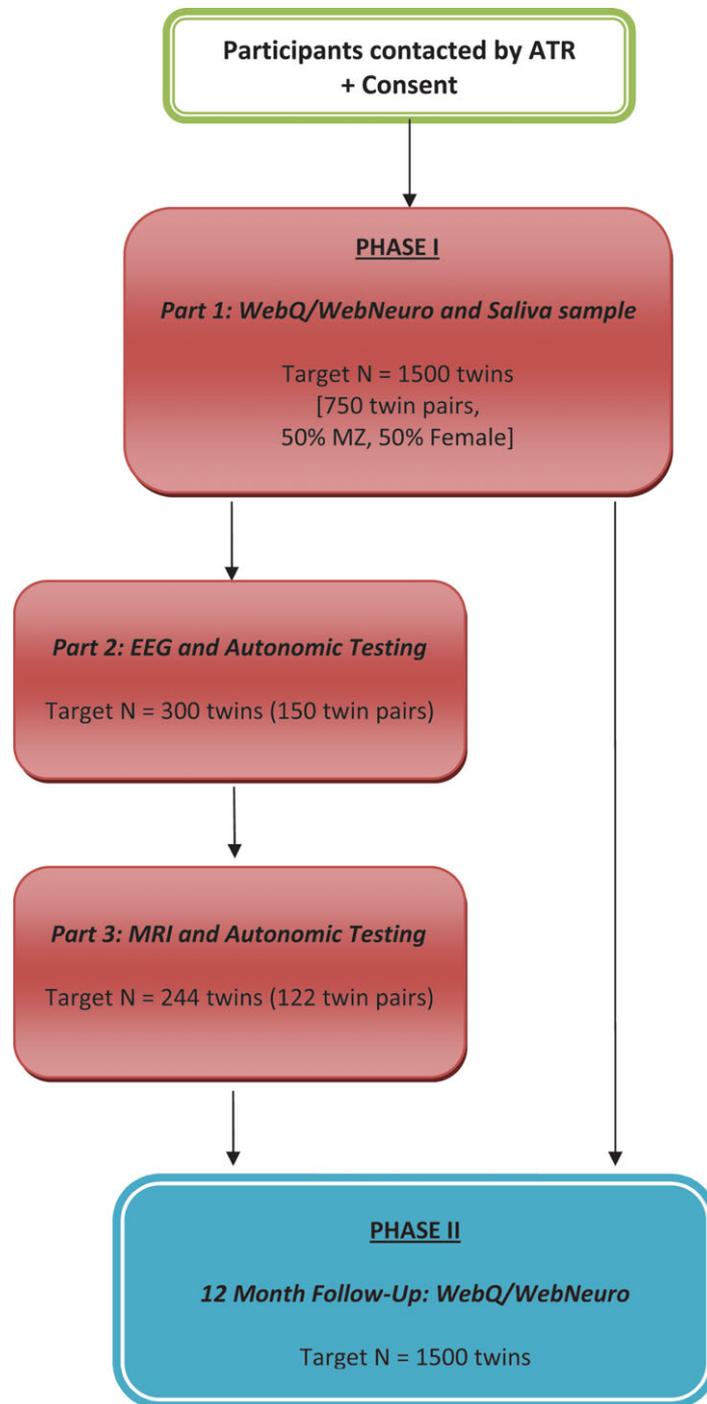


FIGURE 1

TWIN-E Study Design: Phase I (Baseline) and Phase II (12-month follow-up). *Study Compliance:* A reminder email is sent to participants if the assessment remains uncompleted for 2 weeks, followed by telephone reminder calls at 2-week intervals until the web assessment is complete. Reminders are terminated following the sixth contact and the participant is then considered a 'passive non-responder'. *Participant reimbursement:* Participants are reimbursed with a one-page summary report of preliminary heritability outcomes for cognitive performance upon completion of Phase I, Part 1; and \$50 travel costs per EEG and MRI session for Phase I, Parts 2 and 3.

of total) who reside in Sydney or Adelaide, and who will be asked to complete electrophysiological and autonomic testing at the Brain Dynamics Centre, NSW, or Flinders University, South Australia. Part 3 involves inviting those

participants who complete their EEG at the Brain Dynamics Centre to also undertake structural (sMRI) and functional MRI (fMRI) testing (target minimum $n = 244$ individuals or 122 twin pairs; 16% of total). Phase I test-

ing began in June, 2009, and will be completed by March, 2012.

Phase II of the study is the longitudinal component and involves inviting all participants to again complete the WebQ and WebNeuro via the Internet 12 months after their initial tests (Figure 1). Phase II testing began in June, 2010, and is due for completion by March, 2013.

Phase III is an embedded sub-study. It is a randomized-controlled trial of web-delivered training for brain health and wellbeing (Figure 2). All twin pairs who complete Phase II are invited into Phase III. They are then randomly allocated to either a treatment or waitlist-control group to assess the effects of training on emotional health following a recommended 30-day program. The training program is called 'MyBrainSolutions' and is designed to target emotional functions, stress, and cognition (O'Connor et al., 2010). Phase III testing began in August, 2010, and is due for completion by May, 2013.

Participants

Ethical Considerations

Ethics approval has been obtained from the Human Research Ethics Committees of both the University of Sydney (03-2009/11430) and Flinders University (FCREC #08/09). Each participant provides written informed consent prior to participation.

Inclusion and Exclusion Criteria

We are aiming to test 1,500 participants (750 twin pairs) across Australia for the baseline cohort, comprising 50:50 MZ to DZ pairs and 50:50 males to females. The age range of 18–65 years was selected to include the age-of-onset for most common psychiatric disorders, and to match the existing clinical and control cohorts from the Brain Resource International Database (www.brainnet.net). Children and adolescents, and adults over the age of 65, were excluded to minimize extreme effects of age (i.e., turbulent developmental changes during childhood/adolescence and typical deteriorations in health that occur during older age).

Eligible participants are same-sex, healthy, adult twin pairs, with English as primary language, and of European ancestry to avoid population stratification effects in genetic analyses. Exclusion criteria include: lifetime/current psychiatric illness (based on diagnostic self-report¹); stroke or neurological disorder; genetic disorder; brain injury (causing loss of consciousness for more than 10 minutes); chronic and serious medical conditions (e.g., cancer or heart disease); blood-borne illnesses (e.g., HIV, hepatitis); drug/alcohol substance abuse; and sensory impairments to hearing, hand movement, or vision (not corrected by glasses/lenses). For those twins involved in the MRI-testing component, several other safety criteria are checked and confirmed prior to testing, including prior surgeries, body piercings, tattoos or permanent eyeliner, magnetic dentures, the presence of metal clips, implants, stents, rods or screws,

or foreign metal fragments and shrapnel. In addition, participants are automatically excluded from the MRI session if they are pregnant or breast-feeding, or weigh over 150 kilograms.

Recruitment

Recruitment of twin pairs is organized via the Australian Twin Registry (ATR; full approval received 3 October, 2008). The ATR is a national volunteer register of twins interested in contributing to research studies, funded by an Australian NHMRC Enabling Grant. It currently contains around 33,000 twin pairs (equivalent to approximately 10% of all twins in Australia), with 52% MZ twin pairs and 48% DZ twin pairs.

For this study, ATR randomly selected those twin pairs that suited the criteria stipulated using an 'approach wizard'. Sixteen criteria sets were specified to ensure an even coverage of participants, including variation on zygosity (MZ vs. DZ), sex (male:female vs. female:female) and age bands (18–30, 31–40, 41–50, and 51–65 years). Upon selection, ATR approached the twin pairs with a mailed package that included information about the study, a response form and a screening questionnaire (including questions on ethnicity, primary language, mental/physical health, and Internet access). Up to four rounds of phone follow-up were attempted to non-responders. The participant details of interested twin pairs were forwarded to the Project Coordinator via a secure FTP server on a weekly basis. Participants were then mailed further information to initiate their participation in the study, including participant information sheets and consent forms, a saliva kit and instructions to complete the web assessments over the next week. This recruitment process was initiated in April, 2009.

Measures & Procedures

The methodology used in the current study is standardized across testing sites and has been previously validated in several normal and clinical populations (Paul et al., 2005b, 2007; Silverstein et al., 2007; Williams et al., 2005), and is available in the Brain Resource International Database (Gordon, 2003; Gordon et al., 2008; Gordon & Williams, 2010).

Genetics

Genomic DNA was extracted from saliva samples collected using the Oragene DNA self-collection kit (DNA Genotek, Ottawa, Ontario, Canada) using a fully automated extraction procedure (Autopure LS, Qiagen Inc., Maryland, USA). The yield and purity of each DNA sample was assessed by UV spectrophotometry prior to genetic analyses.

Initial genetic analyses of single nucleotide polymorphism (SNP) and other genetic markers will be undertaken in candidate genes of widespread interest and which form part of the hypothesis-driven components of this project. Genes to be targeted will involve those known to modulate

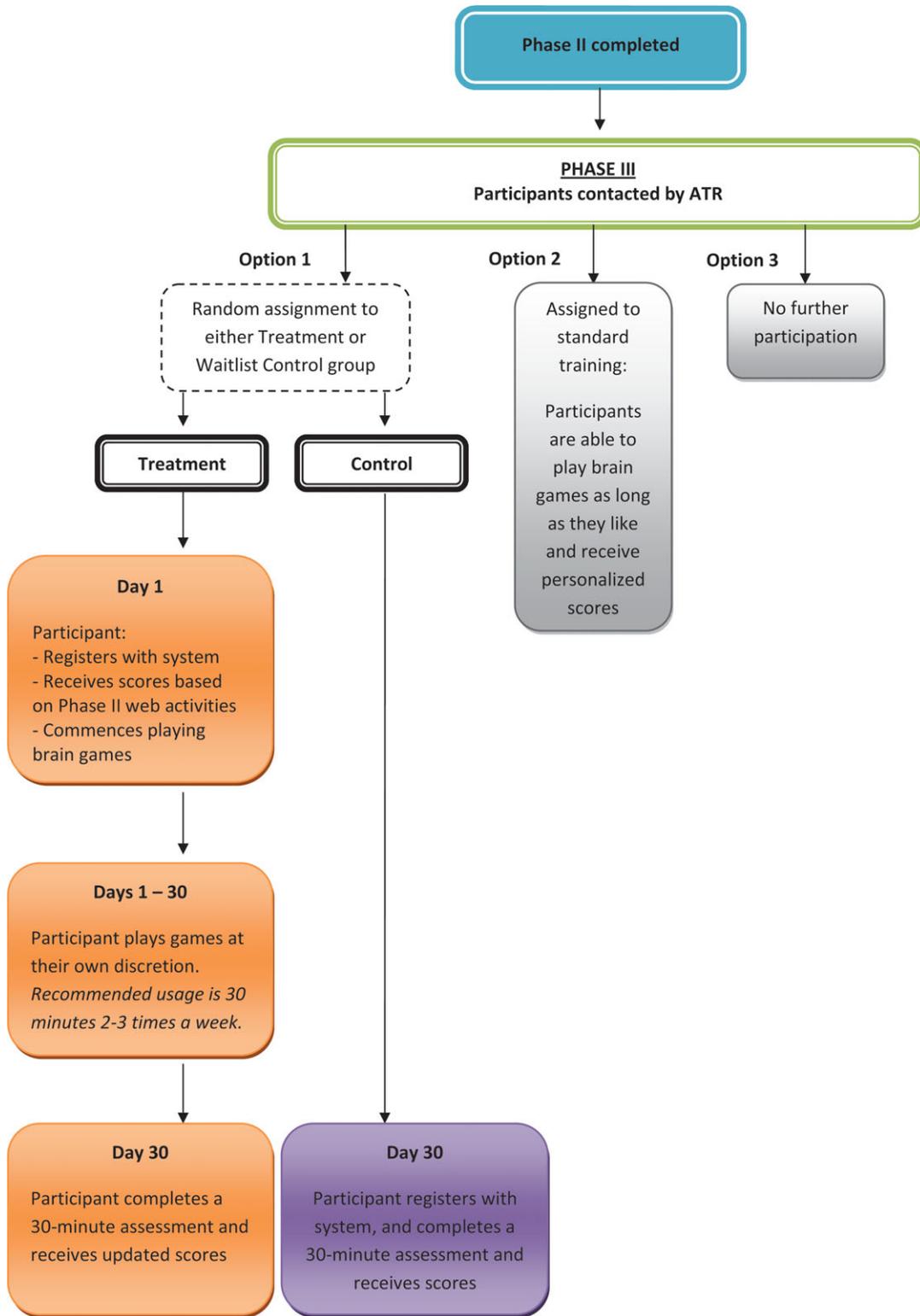


FIGURE 2

TWIN-E Study Design: Phase III (Brain Training). *Study Compliance:* A reminder email is sent to treatment and control participants if they have not registered with the MyBrainSolutions online platform within 2 weeks, followed by telephone reminder calls at 2-week intervals until registration. Reminders are terminated following the third contact and the participant is then considered a 'passive non-responder'. Once registered, treatment participants receive three automated email/SMS reminders sent on Days 25, 29, and 30 to complete the assessment on Day 30. The researchers attempt one final contact 2 weeks following Day 30, for incomplete assessments. *Participant reimbursement:* Participants are reimbursed with their personalized 'brain profile' scores and open access to an online cognitive training portal upon completion of Phase III.

neurotransmitters, neuropeptides, and relevant hormones integral to emotional brain function and stress responses. These include the serotonin transporter (*SLC6A3*) linked promoter polymorphism (5-HTTLPR) ‘short’ versus ‘long’ allele; serotonin receptors 1A (*HTR1A* rs6295) C vs G alleles, 2A (*HTR2A* rs6313) T vs C alleles, and 3A (*HTR3A* rs1062613) C vs T alleles; monoamine oxidase A (*MAOA* promoter VNTR repeat) ‘low’ versus ‘high’ activity alleles; catechol-O-methyl transferase (*COMT* Val158Met rs4680) Val versus Met alleles; and brain derived neurotrophic factor (*BDNF* Val66Met rs6265) Val versus Met alleles (Gatt et al., 2007, 2008, 2009, 2010a, b; Schofield et al., 2009; Wacker & Gatt, 2010; Williams et al., 2008, 2009a, 2010a). Allele frequencies are calculated to ensure that the samples are in Hardy-Weinberg equilibrium.

The capacity to undertake additional genotyping exists, either as single or multiple SNPs using primer extension followed by mass spectrometry analysis on the Sequenom MassARRAY system (Sequenom, San Diego, CA) or by insertion/deletion electrophoretic size separation assays. A whole-genome association study, using either the Illumina or Affymetrix platforms or an examination of copy number variation, could also be undertaken in the future.

Questionnaire & Cognitive Assessments

For Phase I (baseline) and Phase II (12-month follow-up), participants were required to complete the WebQ and WebNeuro assessments upon receiving their instructions by mail from the researchers. The standardized assessment takes approximately 60 minutes to complete, and is a self-administered, web-based test battery (Silverstein et al., 2007).

WebQ Assessments. The WebQ online assessment is a test battery of self-report questionnaires assessing the following domains (see Tables 1 & 2 for detail):

Twin status (zygosity): At Phase I, participants complete a Zygosity Questionnaire to assess zygosity in terms of MZ, DZ, or IZ (indeterminate zygosity) status (Table 1). For those pairs with indeterminate zygosity, we plan to determine zygosity via DNA testing.

Health screen: At Phase I and II, participants complete a global screen for mental and physical health status, including the Medical History Questionnaire (which includes items adapted from the Mental Status Examination; Trzepacz and Baker, 1993) and the Somatic and Psychological Health Report (SPHERE) (Hickie et al., 1998) (Table 1).

Emotional health status (risk and resilience): At Phase I and II, participants complete several self-report measures of emotional health spanning the domains of Feeling and Self Regulation. To assess Feelings, the Depression Anxiety and Stress (DASS-42) (Lovibond, 1998; Lovibond & Lovibond, 1995) was administered, and, at Phase II only, the Modified Differential Emotions Scale (mDES) (Fredrickson et al., 2003) (Table 1). To assess Self Regulation, the Brain

Resource Inventory of Social Cognitions (BRISC) (Gordon et al., 2008; Williams et al., 2008), NEO Five-Factor Inventory (NEO-FFI) (McCrae & Costa, 2004), Emotion Regulation Questionnaire (ERQ) (Gross & John, 2003), and the Internal Control Index (ICI) (Duttweiler, 1984) were administered, and, at Phase II only, the Ego-Resilience Scale (ER89) (Block & Kremen, 1996).

Life outcome measures (wellbeing): Measures of functional life outcomes included the World Health Organization Quality of Life — brief format (WHOQoL-brev) scale (WHO, 1998; Murphy et al., 2000), the Satisfaction With Life Scale (SWLS) (Diener, 1985), and the World Health Organization Health and Work Performance Questionnaire (HPQ) (Kessler et al., 2003), at Phase I and II (see Table 1).

Environmental factors: At Phase I, participants complete several assessments of childhood and adulthood experiences including the Traumatic Life Events scale, the Early Life Stress Questionnaire (ELSQ) (Cohen et al., 2006), and the Measure of Parental Style (MOPS) (Parker et al., 1997), together with other assessments of health behaviors (e.g., general lifestyle, diet, exercise, sleep). At Phase II, the health behavior questionnaires were repeated and a new measure assessing positive and negative Daily Life Events that had occurred in the year between Phase I and Phase II was also administered (see Table 2).

WebNeuro Assessments. At Phase I and II, participants also complete a number of cognitive tasks that assess emotion and thinking processes via the WebNeuro platform, which is an online testing platform using a standard keyboard and mouse or laptop pad (Table 3). Standardized task instructions are presented visually on the screen. Norms, reliability and construct validity have been established (BRISC and WebNeuro Assessment Manual, 2010; Mathersul et al., 2009; Williams et al., 2009b).

Cognitive measures for emotion: The Emotion Identification task and the Emotion Recognition task assess explicit and implicit Emotion sub-processes, respectively (BRISC and WebNeuro Assessment Manual, 2010; Mathersul et al., 2009; Williams, et al., 2009b).

Cognitive measures for thinking: Six sub-processes of cognitive function were assessed by at least one task: Response Speed, Impulsivity, Attention-Concentration, Information Processing Efficiency, Memory, and Executive Functioning (Mathersul et al., 2009; BRISC and WebNeuro Assessment Manual, 2010).

Electrophysiological Measures

During Phase I, electrophysiological testing is conducted using standardized hardware and software to acquire electroencephalogram (EEG), event-related potentials (ERPs) elicited by resting and activation tasks, with concurrent measures of autonomic function, including heart rate function, eye blinks, and skin conductance. Participants are required to complete six tasks during the

TABLE 1
WebQ Self-Report: Twin Status, Health Screen, Emotional Health Status and Life Outcomes

Domain	Measure	Description
Twin Status	Zygosity	A 22-item Zygosity Questionnaire was used to confirm MZ and DZ status using items adapted from previous zygosity questionnaires (Magnus et al., 1983; Goldsmith, 1991). Five new items were developed and included in the questionnaire to assess past rearing and current living arrangements (i.e., whether twins reside together).
Health Screen	Medical History	The 17-item Medical History Questionnaire assesses the medical history of an individual and their family members. It assesses specific disorders and disabilities such as neurological and psychological disorders, developmental or learning disorders, hearing loss and color blindness, serious illnesses requiring hospitalization, and physical illnesses such as stroke or arthritis. This questionnaire is part of the standardized BRID Web Questionnaire, and includes items adapted from the Mental Status Examination (Trzepacz & Baker, 1993).
	Mental Health Status	The 35-item Somatic and Psychological Health Report (SPHERE) (Hickie et al., 1998) was used to identify potential mental health problems. This questionnaire assesses symptoms such as feelings of sadness, trouble concentrating, restlessness, anxiousness, and unexplained fatigue and insomnia, and has been used as a screen for depression and anxiety disorders.
Emotional Health Status ('Risk' and 'Resilience')	Depression, Anxiety, Stress	The 42-item Depression Anxiety Stress Scale (DASS-42) yields measures of depression, anxiety, and stress for the past week (Lovibond, 1998; Lovibond & Lovibond, 1995). Participants rate each item on a 4-point scale ranging from <i>not at all</i> to <i>most of the time</i> .
	Positive and Negative Feelings	The 20-item Modified Differential Emotions Scale (mDES) assesses the presence of positive and negative feelings for the past week (Fredrickson et al., 2003). Participants rate each item on a 5-point scale ranging from <i>never</i> to <i>most of the time</i> .
	BRISC Self Regulation	The 45-item Brain Resource Inventory of Social Cognitions (BRISC) assesses the three self-regulatory processes of Negativity Bias, Emotional Resilience, and Social Skills (Gordon et al., 2008; Williams et al., 2008).
	Personality Traits	The 60-item NEO Five-Factor Inventory (NEO-FFI) assesses the five major personality traits of Neuroticism, Extraversion, Openness to experience, Agreeableness and Conscientiousness (McCrae & Costa, 2004). The items are scored on a 5-point scale ranging from <i>strongly disagree</i> to <i>strongly agree</i> .
	Emotion Regulation	The 10-item Emotion Regulation Questionnaire (ERQ) assesses emotion regulation and management processes in terms of both internal emotional experiences and external emotional expression (Gross & John, 2003). The items are scored on a 7-point scale ranging from <i>strongly disagree</i> to <i>strongly agree</i> . Participants can be scored on two subscales, Reappraisal and Suppression.
	Locus of Control	The 28-item Internal Control Index (ICI) measures the degree to which one believes they can control events that affect them (Duttweiler, 1984). The items are scored on a 5-point scale ranging from <i>rarely</i> to <i>usually</i> . Participants can be ascribed a total score on the ICI to reflect Total Internal Control, but also can be scored on two subscales, Self-Confidence and Autonomous Behavior.
Life Outcomes ('Wellbeing')	Ego Resilience	The 14-item Ego-Resiliency scale (ER-89) assesses trait or psychological resilience (Block & Kremen, 1996). The ER-89 is scored on a 4-point scale ranging from <i>does not apply at all</i> to <i>applies very strongly</i> .
	Quality of life	The 26-item World Health Organization Quality Of Life Scale (WHOQoL-brev) (WHO, 1998; Murphy et al., 2000) was used to assess overall perceptions of quality of life and health. Four sub-domains can also be assessed from the scale, including Physical Health, Psychological Health, Social Relationships, and Environment.
	Satisfaction with Life	The 5-item Satisfaction with Life Scale (SWLS) (Diener et al., 1985) was used to assess overall perceptions of life satisfaction relative to their own circumstances and standards.
	Work Productivity	The 12-item World Health Organisation Health and Work Performance Questionnaire (HPQ) (Kessler et al., 2003) was used to assess work productivity in terms of both absenteeism (hours absent from work) and presenteeism (performance level).

Note: MZ = monozygous, DZ = dizygous.

electrophysiological testing, which take approximately 1 hour to complete (Table 4).

Before starting the electrophysiological tasks, participants are asked to complete some demographic questions (e.g., age, sex, education), and questions relating to how they are feeling on the day of participation relative to their usual disposition. For the test session, participants are seated in front of a computer screen, in a dimly lit sound-attenuated room. EEG and ERPs are recorded from 26 scalp electrode sites (Fz, Fp1, Fp2, F3, F4, F7, F8, Cz, C3, C4, FC3, FCz, FC4, T3, T4, T5, T6, Pz, P3, P4, CPz, CP3, CP4, Oz, O1, and O2) using a QuikCap and NuAmps system, according to the International 10–20 system (Jasper, 1958). Recordings are relative to the virtual ground, but referenced offline to the linked mastoids (A1 and A2). Horizontal and

vertical eye movements are recorded via electrodes placed 1.5 cm lateral to the outer canthus of each eye (horizontal), 3 mm above the middle of the left eyebrow and 1.5 cm below the centre of the bottom left eyelid (vertical). Impedance is kept at no more than 5 kΩ. Continuous EEG and EOG (electro-oculographic) data is collected via the NuAmps system (Scan 4.3), with low pass attenuation of 40 dB per decade above 100 Hz, and at a sampling rate of 500 Hz. Data are corrected for eye movement offline using previously established procedures (Gratton et al., 1983).

Autonomic data is recorded simultaneously and continuously with the EEG and ERP recordings. Heart rate is recorded as an electrocardiogram (ECG) through an electrode placed at the radial pulse on the left inner wrist.

TABLE 2
WebQ Self-Report: Environmental Factors

Domain	Measure	Description
Environmental Factors	General Lifestyle	The 8-item Lifestyle Questionnaire assesses average patterns in daily lifestyle activities, including average frequency of drug, alcohol and nicotine use, hours sleep per night, and amount of time per week spent on personal leisure activities such as reading, playing mentally challenging games and puzzles, and physical activity. This questionnaire is part of the standardized BRID Web Questionnaire.
	Nutrition	The 6-item Nutrition Questionnaire assesses daily dietary patterns. It assesses the regularity in consumption of lean meat/fish, fresh produce, fast foods, and dietary supplements. This questionnaire is part of the standardized BRID Web Questionnaire.
	Social Activities	The 5-item Social Activities Questionnaire assesses frequency of recreational and social activities over the average week. It assesses frequency of involvement in activities outside of work (e.g., dance, music and languages), frequency of convening with family members and friends, attendance at sporting activities, community events, and volunteer work. This questionnaire is part of the standardized BRID Web Questionnaire.
	Sleep	The 16-item Sleep Questionnaire assesses overall quality of sleep over the past month, and was adapted from the apnea sleep index derived from sleep survey studies (Maislin et al., 1995). It determines disturbances in sleep from airway difficulties, difficulties in falling asleep, morning headaches and fatigue, falling asleep during activities, excessive sleeping, post sleep paralysis and unexplained fatigue. The symptoms can be sorted into four indices of sleep apnea, sleep quality, daytime sleepiness, and narcolepsy.
	Parenting Style	The 15-item Measure of Parental Style (MOPS) scale (Parker et al., 1997) was used to assess the individual's judgment on the behavior of each of their parents or caregivers during the first 16 years of their life. Items can be sorted into three subscales of Indifference, Abuse, and Overcontrol for mothers and fathers separately.
	Traumatic Life Events	The 2-item Traumatic Life Events checklist was devised to assess the experience of psychological traumas that had a lasting impression. The first item assessed whether or not such a trauma was experienced. The second item assessed the nature of this experience from a possible eight responses including verbal/physical abuse or neglect as a child/teenager or adult, sexual mistreatment as a child/teenager or adult, natural disaster, death of a loved one, or an event not specified. Participants were also given the option not to specify the nature of the event.
	Early Life Stress	The 19-item Early Life Stress Scale (Cohen et al., 2006) assesses traumatic and stressful childhood experiences. The ELS was developed for use in an international cohort (McFarlane et al., 2005; Paul et al., 2005a), and is based on the Child Abuse and Trauma Scale (Sanders & Becker-Laussen, 1995), which correlates with adult outcome and psychopathology. The scale includes items shown to be traumatic or extremely stressful in past studies, such as birth complications, natural disasters and warfare, familial abuse and violence, life threatening injuries and illnesses, and loss of family members. Participants endorsed whether specific events had occurred during their childhood (0–17 years), and if so, at what age.
	Daily Life Events	The 50-item Daily Life Event scale assesses significant events that have occurred over the past year, and includes some items adapted from the Social Readjustment Rating Scale (Holmes & Rahe, 1967). It includes major areas of life such as changes in interpersonal relationships (e.g., marriage, divorce), family life (e.g., health or behavior of family members), work (e.g., financial conditions, career changes), lifestyle (e.g., social activities, eating and sleeping habits), and traumatic events (e.g., serious injury or experience). For endorsed items, participants rate the impact of the event on their lives using a 7-point scale of <i>extremely negative</i> to <i>extremely positive</i> .

ECG recordings are referenced offline to recordings taken from the right clavicle and the seventh cervical vertebra. Respiration is recorded via a belt placed around the participant's chest. Respiration and ECG are recorded with low pass attenuation of 40 dB per decade above 100 Hz, and at a sampling rate of 500 Hz. Both are recorded in reference to the virtual ground. Electrodermal activity (EDA) measures skin conductance via electrodes attached to the medial phalanges of the second and third digits of the left hand with the aid of a transducer (Grass, SCA1). A constant voltage of 0.5 V excites the electrodes, and the current proportional to conductance is converted to voltage, digitized at a sampling interval of 2 ms via NuAmps. Eyeblinks are measured via electromyography (EMG), recorded from an electrode placed on the left orbis ocularis muscle near the eye, located approximately 1.5 mm laterally from the electrode placed below the eye, and 2–3 mm upwards towards the horizontal eye electrode.

Instructions are delivered to participants via the computer screen and binaurally through headphones. Parti-

cipants respond to instructions via a button box, with four keys (up, down, left, and right). A mouse is used during the final task.

Brain Imaging Measures

During Phase I, all MRI testing is performed on a 3.0 Tesla GE Signa HDx scanner (GE Healthcare, Milwaukee, WI) using an eight-channel head coil. Participants are required to complete five functional scans, three structural scans, and a phase map scan, which takes approximately 75 minutes to complete (Table 5).

Prior to commencing the MRI session, each participant completes an MRI safety questionnaire screened for any contraindications for MRI, which is reviewed by a radiographer before entering the scanner room. The participant lies supine on the MRI table and is fitted with the head coil. The participant is provided with ear plugs with headphones to attenuate the scanner noise, goggles to view the tasks, and two button boxes to record responses during the task. Participants requiring vision corrections are provided

TABLE 3
WebNeuro Cognitive Performance: Emotion and Thinking Tasks

Domain	Sub-Domain	Task	Measure
Emotion	Emotion Identification	Explicit Emotion Identification	For each emotional stimuli ^a : Errors Reaction time
	Emotion Recognition	Implicit Emotion Recognition	For each emotional stimuli ^a : Errors Reaction time
Thinking	Response Speed	Motor Tapping	Number of taps Variability of pause between taps
	Impulsivity	Go–NoGo	Reaction time Variability of reaction time False ‘alarm’ errors
		Continuous Performance Test	Reaction time False ‘alarm’ errors False ‘miss’ errors
	Information Processing Efficiency	Switching of Attention	Completion time (digits + letters) Average connection time (digits + letters) Errors (digits + letters)
		Verbal Interference	Part 2–Part 1 Errors Part 2–Part 1 Reaction time
	Memory	Choice Reaction Time	Reaction time
		Digit Span	Recall span Trials correct Total immediate recall Trials 1–4
Memory Recognition		Learning rate Trials 1–4 Delayed recall Trial 7	
Executive Function	Maze	Completion time Path learning time Overrun errors Total errors	

Note: ^a Emotional stimuli include faces expressing fear, anger, sadness, disgust, happiness, and neutral expressions.

TABLE 4
Electrophysiological and Autonomic Testing: Resting and Activation Tasks

Domain	Task	EEG/Autonomic Measures	Task Description
Resting condition	Eyes Open/Closed	EEG Asymmetry & Power Average Heart Rate Heart Rate Variability	EEG is recorded for 2 minutes while participants fixate on a red dot on screen (Eyes Open), and then for 2 minutes while participants sit with their eyes closed (Eyes Closed).
Activation tasks	Oddball	P300 ERP Average Heart Rate Heart Rate Variability	Series of 300 tones presented at 75 db (each 50 ms, ISI = 1 second). Participant is instructed to press a button in response to high-pitched tones (1000 Hz), ignore low-pitched tones (500 Hz).
	Continuous Performance Test (CPT)	P450 ERP Average Heart Rate Heart Rate Variability	Series of 125 letters (B, C, D, or G) presented sequentially (each 200 ms, ISI = 2.5 seconds). Participant is instructed to press a button when the same letter appears twice in a row.
	Novelty	P300 ERP Average Heart Rate Heart Rate Variability	Series of 20 blue and green checkerboard stimuli presented briefly (200 ms) and infrequently, unexpectedly, and at random intervals within the Continuous Performance Test (ISI = 2 seconds). No response required.
	Go–No Go	N200 ERP Average Heart Rate Heart Rate Variability	168 stimuli presented sequentially (200 ms each, ISI = 2 seconds). Participant is instructed to press a button as quickly as possible for Go stimuli (in green) and withhold press for NoGo stimuli (in red).
	Emotion (masked ‘nonconscious’ implicit, Then unmasked ‘conscious’ explicit)	P120–VPP ERP Average Heart Rate Heart Rate Variability	Series of 192 facial expression stimuli depicting fear, anger, disgust, sadness, happiness or neutral under nonconscious (10 ms, 150 ms mask, ISI = 1040 ms) and conscious (500 ms, ISI = 700 ms) conditions. Stimulus-onset asynchrony in both conditions 1200 ms. Active viewing.
	Startle ‘noise burst’	Average Skin Conductance Eye blink	Series of 20 acoustic startle stimuli (white noise burst at 105 db, 50 ms duration, ISI = 10–15 seconds). The startle eye blink is measured (muscle contraction of the eye blink reflex as measured by the electromyogram).

Note: ERP = event-related potentials.

MR-compatible lenses placed within the goggles. The functional tasks are driven by a local computer placed outside the scanner room, which also records participant behavioral responses and autonomic data in a single data file for each task. To match the start of scanning sequence to the

fMRI task, each task is programmed to be triggered by a starting pulse sent from the scanner. Similar to the EEG, autonomic data (heart rate and electrodermal activity) is acquired simultaneously during the functional scans. Heart rate is measured using a peripheral pulse oximeter clipped

TABLE 5
Magnetic Resonance Imaging and Autonomic Testing: Structural and Functional Scans

Domain	Task/Scan type	MRI/Autonomic Measures	Scan Protocol/Task Description
Structural scans	T1	Grey/white matter volume Cortical thickness Cortical surface area	<u>Scan Protocol:</u> 3D T1 weighted high resolution SPGR MRI scan, TR = 8.3 ms, TE = 3.2 ms, Flip angle = 11°, TI = 500 ms, NEX = 1, Matrix = 256×256, resolution = 1 mm x 1 mm, 180 contiguous 1 mm sagittal slices.
	DTI	Fractional Anisotropy (FA), mean (MD), axial (AD), radial (RD) diffusivity measures for white matter regions and fiber tracts	<u>Scan Protocol:</u> Spin echo DTI echo planar imaging scan, 42 directions, b value = 1250, TR = 17,000 ms, TE = 95 ms, NEX = 1, Matrix = 128×128, resolution = 1.72 mm x 1.72 mm, 70 contiguous 2.5 mm axial/oblique slices covering whole brain.
	PD/T2	Proton Density T2 structural scan for clinical evaluation	<u>Scan Protocol:</u> 2D Fast Spin echo scan, TR = 4,000 ms, TE1 = 6.4 ms, TE2 = 102 ms, NEX = 1, Matrix = 256×256, resolution = 0.86 mm x 0.86 mm, ETL = 16, 70 contiguous 2.5 mm axial/oblique slices covering whole brain.
Functional scans	Oddball	Changes in fMRI BOLD signal Average Heart Rate Average Skin Conductance	<u>Scan Protocol:</u> Echo planar imaging scan, TR = 2,500 ms, TE = 27.5 ms, Flip angle = 90°, NEX = 1, Matrix = 64×64, resolution = 3.75 mm x 3.75 mm, 40 contiguous 3.5 mm axial/oblique slices covering whole brain in each volume, total 120 volumes. <u>Task Description:</u> 20 target (1000 Hz) & 100 nontarget (50 Hz) tones presented one at a time at 75 db (50 ms each, ISI = 2.4 seconds). <u>Scan Protocol:</u> See Oddball protocol
	Continuous Performance Test (CPT)	Changes in fMRI BOLD signal Average Heart Rate Average Skin Conductance	<u>Task Description:</u> 120 stimuli are presented (B, C, D, or G letters, for 200 ms each, ISI = 2.3 sec). 60 were yellow letters to be held in working memory (no consecutive repetitions), 20 were 1-back sustained attention stimuli (consecutive repetitions of a letter in yellow) and 40 were perceptual baseline stimuli in white letters. Participants were measured for the number of errors or omissions. <u>Scan Protocol:</u> See Oddball protocol
	Go–NoGo	Changes in fMRI BOLD signal Average Heart Rate Average Skin Conductance	<u>Task Description:</u> 180 Go stimuli (word ‘press’ in green) & NoGo stimuli (word ‘press’ in red) each presented sequentially (500 ms each, ISI = .75 seconds). NoGo stimuli were not repeated more than three times in a row. Participants were measured for target detection rate, response time, errors of commission, and errors of omission. <u>Scan Protocol:</u> See Oddball protocol
	Emotion (masked ‘nonconscious’, then unmasked ‘conscious’)	Changes in fMRI BOLD signal Average Heart Rate Average Skin Conductance	<u>Task Description:</u> 240 3D evoked standardized facial expressions that depicted fear, anger, disgust, sadness, happiness, or neutral were presented for 500 ms (ISI = .75 seconds). Stimuli were grouped into 8 faces expressing the same emotion and were repeated 5 times in a pseudorandom order. Participants were assessed for explicit emotion processing for the ‘Masked’ condition. The same set of facial expressions was presented for the ‘Unmasked’ condition; however, backward masking (expression presented for 10 ms immediately followed by a neutral face for 190 ms) was used to prevent conscious awareness of facial expression. <u>Scan Protocol:</u> Dual echo MRI scan, TE1 = 10 ms, TE2 = 12.5 ms, TR = 700 ms, Flip angle = 15°, NEX = 1, Matrix = 128×128, 40 contiguous 3.5 mm axial/oblique slices.
Additional Scan	Phase Map	Map magnetic field inhomogeneities for EPI distortion correction	<u>Scan Protocol:</u> Dual echo MRI scan, TE1 = 10 ms, TE2 = 12.5 ms, TR = 700 ms, Flip angle = 15°, NEX = 1, Matrix = 128×128, 40 contiguous 3.5 mm axial/oblique slices.

Note: DTI = diffusion tensor imaging, fMRI = functional magnetic resonance imaging, BOLD = blood-oxygen-level-dependent, EPI = echo planar imaging.

on the big toe of the right foot of the participant. Physiological measurements of average skin conductance are acquired using an electrodermal skin conductance system. The skin conductance response (SCR) detects unambiguous changes in arousal levels, traditionally defined as > 0.05 microSiemens above baseline (Williams et al., 2001). SCR is measured with a pair of silver chloride electrodes with 0.05 M sodium chloride gel placed on digits II & III of the left hand (Williams et al., 2001).

Brain Training

Upon completion of Phase II, twin pairs are approached by the ATR to seek interest in participating in Phase III. During this phase, participants are provided the opportunity to receive their own personal scores on the WebQ/WebNeuro assessments from Phase II of the study, and to have access to a web-based brain training portal, called MyBrainSolu-

tions (www.MyBrainSolutions.com). Scores are generated for the domains of Emotion, Thinking, Feeling and Self Regulation as portrayed by the Integrate model (O’Connor et al., 2010). These four scores are then used to compute an individualized brain profile (16 potential profiles derived from the various combinations of these four scores). Notably, none of the profiles is worded negatively, which might imply some sort of deficit, but rather are worded positively, regardless of the participant’s scores.

Various combinations of brain training are recommended, personalized to the individual and the brain profile, and to optimize and sustain existing skills, although participants may also select from any of the other games available. Such training regimes include activities that promote emotional function (e.g., focusing on positive facial, verbal, or nonverbal body cues), thinking skills (e.g., to boost attention, memory, and executive functioning skills),

regulation of feelings (e.g., strategies to minimize stress and tune into positive feelings), and self-regulation (e.g., learn skills to enhance the ability to manage emotions and feelings, and persevering to meet long-term challenges and goals). The training games are available for download to a mobile device to promote maintenance of training. The effectiveness of the training for regulating emotional health, and its effect on life outcomes like productivity, has been established in a cohort of over 4,000 employees (e.g., O'Connor et al., 2010). Convergent evidence comes from training programs focused on cognitive functions that are effective in boosting scores in as little as 17 training days, with effects maintained anywhere from 3 months to 5 years and transferrable to other skill sets and daily functioning (Jaeggi et al., 2008; Mahncke et al., 2006; Willis et al., 2006). This phase was designed to investigate the impact of activities developed to enhance the key information processes of emotion, thinking, feeling, and self-regulation, and subsequent effects on emotional health and outcomes over time. We will be able to identify the endophenotype measures that predict treatment response. By comparing twin pairs, we will also determine what is most heritable versus modifiable by environment in the effects of training.

When approached by the ATR, the participants are provided with three possible options: (i) to participate in the brain training phase and be randomly allocated to either a treatment or waitlist control group, (ii) to be provided access to the brain training web portal but not be randomized to a group (and therefore their data will not be used in the current study), or (iii) nonparticipation (see Figure 2). The details of interested twins are then forwarded to the project coordinator. When both twins agree to participate, one twin is randomized to the treatment group and the other to the waitlist control group. The treatment group is asked to register and play the games for 30 days. For optimal benefits, participants are encouraged to play the brain games for 30 minutes, two to three times a week, for 30 days; however, ultimately usage (frequency, duration, time of day) is determined by the participant (these factors will be considered in statistical analyses). Participants are then requested to complete a 30-minute web assessment following the 30-day period. Waitlist control twins are granted access to the brain training platform following this 30-day period and are requested to complete the 30-minute web assessment. The assessment includes a subset of web measures collected during Phase I and II; namely, all of the WebNeuro cognitive tasks plus the selected WebQ measures of WHOQoL-brev, SWLS, mDES, BRISC, ERQ, and ER-89.

Data Management

A rigorous data management procedure is adopted in this study to store and transport data, and to maintain data integrity (for quality control procedures, see Williams et al., 2011).

Genetics

Saliva samples are collated in Oragene[®] DNA sample collection kits (DNA Genotek Inc.) at the Brain Dynamics Centre and stored at room temperature. They are couriered every 1–2 months to Genetic Repositories Australia (GRA), an Enabling Facility supported by NHMRC Grant 401184 for DNA extraction. GRA utilizes a fully automated, large-volume nucleic acid purification system (Qiagen Autopure[®] LS) using Qiagen's Puregene chemistry and optimized protocols to purify high-quality, high-molecular-weight DNA. The Autopure LS purifies highly stable DNA suitable for archiving, and high-performance DNA for sensitive downstream applications, including PCR, restriction digestion, SNP analyses, and southern blotting. Extracted DNA was quantified fluorometrically using Quant-iT[™] PicoGreen[®] dsDNA reagent (Invitrogen Australia Pty Ltd) on an Eppendorf Mastercycler RealPlex⁴ (Eppendorf South Pacific Pty Ltd) and stored in 1.0 X TE buffer at either 4 °C (short term) or -80 °C (long term) until required for genotyping at completion of Phase I. Genotyping is undertaken using standardized assays, which allows custom genotyping of SNPs within candidate genes. Both genotypic and allelic data are scored and stored for analysis.

Questionnaire & Cognitive Assessments

WebQ and WebNeuro are completed at home on the Internet or on the laboratory computer at Sydney or Adelaide testing sites. The WebQ and WebNeuro are linked so that when the WebQ is finished, the WebNeuro will automatically commence. The web assessments are designed so that each self-reported item or cognitive response entered by the participant is logged and time stamped.

Electrophysiological Measures

Electrical brain and autonomic data are recorded onto the laboratory computer as participants complete each task. The computer registers each data point every 2 ms and writes these data with a time-stamped log file. Upon completion of the test session, the data is uploaded as an xml file to the Data Center database at Brain Resource.

The Scoring Server for EEG and autonomic data (in the form of 'Neuroscan 5' files) includes a series of artifact correction and rejection procedures. Low-frequency and high-frequency noise is removed by high-pass and low-pass filters, power line artifact by notch filters, and muscle and blink artifact by second-order blind identification and canonical correlation analysis. The Scoring Server also includes quality control software that detects five additional primary sources of artifact (using thresholds for abnormal voltage, baseline shifts, and kurtosis) for removal prior to quantifying the data. EEG, ERP, and autonomic measures are quantified by algorithms in the software that have been verified against the gold standard of manual scoring with high inter-scorer reliability (Task Force of the European Society of Cardiology, 1996).

Brain Imaging Measures

The MRI data analysis and quantification is performed using a unix-based automated analysis pipeline comprising separate sub-analysis streams for each of the functional, T1 structural, and DTI MRI scans. The fMRI data preprocessing and analysis are performed using the statistical parametric mapping (SPM5) software (www.fil.ion.ucl.ac.uk/spm) running on a MATLAB platform (MathWorks, Natick, USA). The fMRI preprocessing algorithms includes motion correction, normalization to standard MNI brain template, smoothing and high pass filtering of fMRI time series to remove respiratory and cardiac artifacts. Changes in BOLD signal for each fMRI task in the form of activation image maps and % BOLD signal values for brain regions are quantified. The T1 structural data is processed using the freesurfer analysis suite (<http://surfer.nmr.mgh.harvard.edu/>). This stream involves whole-brain parcellation of cortical and subcortical structures. Measures of volume, cortical thickness and surface area (only volume for the subcortical structures) for the brain regions are quantified. The DTI data is processed and analyzed using tools from the FMRIB Software Library (FSL) (Smith et al., 2004; <http://www.fmrib.ox.ac.uk/fsl>). DTI images are preprocessed to correct for motion and eddy current distortions. Diffusion tensor models are fitted voxel-wise to quantify measures of fractional anisotropy, and mean, axial, and radial diffusivity for white matter regions and fiber tracts. All MRI data files for each participant are compressed in a single GNUzip file for backup and archiving. All quantified data is stored in both the GNUzip image or in the form of numerical value.

Analytical Approach

The aims of this study will be examined using a series of biometrical genetics and structural equation modeling (SEM) analyses comparing MZ to DZ twin pairs using the statistical software SPSS, R, and OpenMx. In this sense, we will use the phenotypic covariance between related twin pairs to provide information on the relative importance of genetics versus environment in contributing to total phenotypic variance, as well as how gene–environment interaction or correlation may moderate such relationships. Saturated and hypothesized models will be compared using differences in maximum likelihood estimates, based on the p -value of .05. Proposed endophenotype markers include each of the tested cognitive, brain, and autonomic measures, with the phenotype of interest being emotional health status, and the secondary predictive outcome being life outcomes.

The four main aims of the study will be addressed as follows:

Aim 1: We plan to first identify and confirm the heritability of each of the proposed endophenotypes. Heritability will be confirmed using univariate ACE (additive genetic, common environment, unique environment)

and ADE (additive genetic, dominant genetic, unique environment) twin models with SEM on the whole sample (Evan et al., 2002). The moderating impact of sex and age differences on heritability estimates will also be considered.

Aim 2: We plan to establish the clinical plausibility of the endophenotypes confirmed in Aim 1. To do this, we first need to confirm the association between the phenotype, emotional health status, and life outcomes in a healthy cohort. This analysis will include factor analytical modeling to derive the specific measure(s) that best capture trait risk versus resilience. The contribution of each endophenotype to this phenotype–life outcome relationship will then be examined. Bivariate genetic modeling in the twins will be used to confirm these relationships (Loehlin, 1996), which will provide an estimate of the relative contribution of additive genetics versus unique/shared environment in common pathways.

Aim 3: We plan to identify the specific genetic and environmental factors that moderate associations between the endophenotypes and mental health outcomes. For these analyses, a series of GxE interaction or r GE correlation models will be tested against the univariate ACE twin model (Purcell, 2002). In this sense, we are testing the moderating impact of specific genes or environmental variables on the ACE components. Determination of the use of GxE or r GE models will depend on the hypothesized relationship between the gene and environmental variables, with a GxE interaction conceptualized as a variation in terms of a genetic *sensitivity* to environments, whereas the r GE correlation is conceptualized as a variation in terms of a genetic disposition towards *exposure* to different environments (Purcell, 2002). In the first instance, the environmental variable of interest will be early life stress; however, we plan to expand this parameter to the other tested environmental factors (e.g., positive life events, differences in general lifestyle) in future analyses.

Aim 4: We plan to establish the predictive utility of each of the endophenotypes in predicting poor versus good health over the 12-month period. Characteristics of a risk versus resilience profile will be devised; that is, we will confirm the cognitive, neural, and autonomic correlates of trait risk and resilience, and how these profiles predict mental health over time, taking into account moderating impacts of genes and environment. To achieve this aim, several bivariate and multivariate genetic models (Neale and Maes, 2004) will be tested to confirm (a) the common factor model that specifies which *combination* of endophenotype variables together share variance with trait risk–resilience and life outcomes, drawing from results in Aim 2, (b) the added variance that is contributed from multiple time points, and (c) the moderating influence of key genetic and environmental measures identified in Aim 3 (as well as other covariates).

Aim 5: We plan to evaluate the effects of brain training on improving emotional and life outcomes scores, and the

role of endophenotypes in predicting treatment response. We will employ a repeated-measures ANOVA design to first compare those twins randomly allocated to the treatment group with their waitlist control sibling, to assess the relative efficacy of the brain-training program on improving scores over a 30-day period. By comparing twin pairs, age and sex are matched across the groups and a significant amount of variation in environmental exposure shared between the twins during the test period will be controlled. Then, focusing on the treatment group alone, we will be able to compare treatment responders to non-responders to identify key characteristics of the training module that contributed to significant effects (e.g., frequency of usage, types of training), the baseline phenotype, and endophenotype characteristics that predict treatment response, and the specific gene–environment measures that moderate these effects.

Sample Size, Power, and Effect Size

Our minimum target numbers meet requirements for sufficient power to detect significant additive genetic effects in the total sample and EEG/MRI subsets for all three study phases. Power estimates are limited to heritability values $>.20$ for the WebQ/WebNeuro total sample, or $>.40$ for the EEG and MRI subsets within the bounds of cost constraints (Visscher, 2004). These estimates are based on optimized proportion ratios using maximum likelihood estimates (Visscher, 2004). This translates to a minimum of 321 pairs for $H^2 > .20$ at the ratio of .56 MZ twin pairs, equivalent to a minimum of 180 MZ pairs to 141 DZ pairs, which will be surpassed for the WebQ-WebNeuro sample of 750 twin pairs; and a minimum of 122 pairs for $H^2 > .40$ at the ratio of .59 MZ twin pairs, equivalent to a minimum of 72 MZ pairs to 50 DZ pairs, which will also be met for the EEG subset (150 pairs) and the MRI subset (122 pairs). To minimize comparisons with multiple variables, the overarching representative factors will be considered in analyses where possible (e.g., one measure of memory function rather than individual performance components as portrayed by the sub-domains in Table 3). Data reduction methods such as principal components analysis will be used to confirm these hierarchical structures.

Preliminary Results

Analyses

For the purposes of the current paper, we have conducted some preliminary heritability analyses in the first 50% of participants, with 142 twins (40 MZ and 31 DZ twin pairs, 58% female, age 18–61 years, mean age 37 years) for the structural brain imaging measures to demonstrate the relative heritability of different brain regions pivotal to emotional brain function. As a broad estimate of heritability, we calculated the intra-class correlation coefficient (ICC) for the MZ and DZ twin pairs, and then calculated heritabil-

ity estimates using the Holzinger's heritability equation: h^2 or $A = 2(\text{ICC}_{\text{MZ}} - \text{ICC}_{\text{DZ}})$ (Holzinger, 1929; also sometimes referred to as 'Falconer's estimate'). The estimates for common environment (C) and unique environment (E) were also calculated using derivatives of this formula: $C = \text{ICC}_{\text{MZ}} - A$; $E = 1 - \text{ICC}_{\text{MZ}}$. z scores and p -values were calculated for the difference in ICC between MZ and DZ twin pairs. This method was employed as a preliminary investigation of the data, but definitive structural equation modeling methods will be used on the complete sample. The limitation of this approach includes the possible computation of estimates larger than 1, or negative values, which may be a result of gene–environment interactions or differences in variance (Stromswold, 2001). Therefore, in our interpretation, we consider only a range of scores and relative strength of effects (i.e., 'strong' heritability/environment effects for values $.70$; larger, 'moderate' effects for values between $.40$ and $.60$; and 'small' effects for values less than $.40$). Results are portrayed illustratively, but actual heritability estimates are provided in the Supplementary Tables for completeness.

The T1 structural & DTI MRI scans were analyzed using the freesurfer analysis suite and FSL software as described in the Data Quantification section above. Cortical surface reconstruction and volumetric segmentation using the T1 images were performed for each individual participant. Both the cortical surface and subcortical structures were automatically labeled using both a subject-independent probabilistic atlas (Desikan-Killiany atlas) labeled by hand and subject-specific measured values (Fischl et al. 2004), dividing into 34 different gyral-based cortical and the main subcortical areas in each hemisphere. Grey matter volumetric, cortical thickness and surface area measures for all the cortical regions, and only volume for the subcortical regions, were extracted and analyzed. For the DTI data, the fractional anisotropy images for each participant were transformed to standard Montreal Neurological Institute (MNI) space and projected on a white matter skeleton representing the centers of all white matter tracts common to all subjects. Next, the JHU ICBM-DTI-81 WM labels atlas (Mori, 2008) was used to label sections of the WM skeleton and extract mean FA values corresponding to 46 (20 bilateral & 6 inter-hemispheric) major WM tracts across the two brain hemispheres. The technical details of both these procedures have been described in detail in a recent publication by our group (Grieve et al. 2011).

Results

For grey matter, results for volume, cortical thickness, and grey matter surface area are presented in Figure 3 and Supplementary Tables 1–3. For white matter, results for DTI fractional anisotropy are presented in Figure 4 and Supplementary Table 4. For brevity, we have reported only those regions with heritability estimates larger than $.40$ in the supplementary tables (with the exception of grey matter volume for subcortical regions, which have been included for

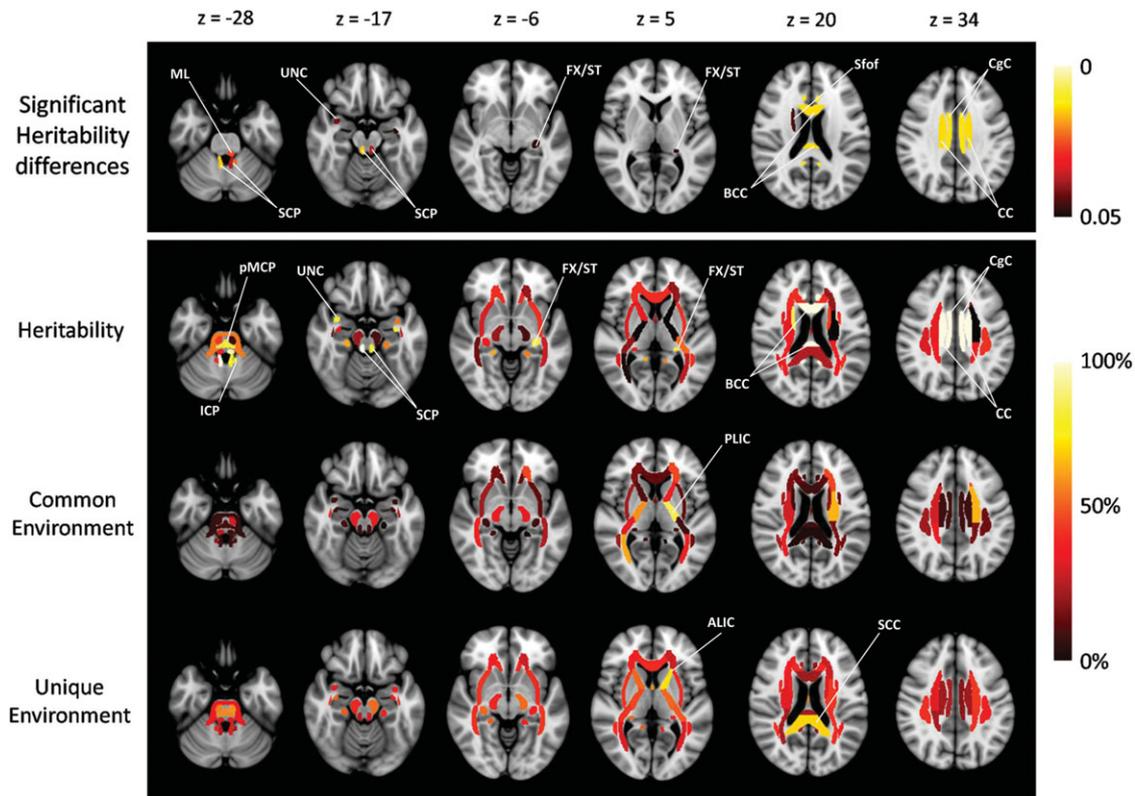


FIGURE 4

Maps of genetic influences on white matter integrity using diffuse tensor imaging (DTI) data. Regions of significant heritability effects with $p < .05$ (top panels) and the relative additive genetic (heritability), and common and unique environmental contributions to each region (bottom panels) are shown. Values of .70 or above are considered *strong*, .40–.60 as *moderate*, and values .40 or below as *small*. Regions with $> 70\%$ effects are labeled: ALIC, anterior limb of the internal capsule; BCC, body of the corpus callosum; CC, corpus callosum; CgC, cingulum bundle at cingulate cortex; FX, fornix; ICP, inferior cerebellar peduncle; ML, medial lemniscus; PLIC, posterior limb of internal capsule; pMCP, pontine crossing tract middle cerebellar peduncle; SCC, splenium corporis callosi; SCP, superior cerebellar peduncle; Sfof, superior fronto-occipital fasciculus; ST, stria terminalis; UNC, uncinate.

cortex and anterior cingulate (left caudal and right rostral) were strong, contributing to the overall pattern described above (Figure 3). In contrast, heritability for the limbic subregions was small to moderate (Table S1). Strongest heritability for grey matter volume was in the right superior parietal cortex (Figure 3, Table S1). This region has been implicated functionally in self-regulation of emotion (Beauregard et al., 2001). Other regions with strong heritability included the subregions of the frontal lobe (left paracentral, right lateral orbitofrontal), the left superior temporal sulcus and cuneus of the occipital cortex, and subregions of the right parietal cortex (superior parietal and cuneus).

Unique environment (E) accounted for most of variance in grey matter volume in a different region of the prefrontal cortex bilaterally; the frontal pole and the left frontal parorbitalis (Figure 3). The frontal pole has been associated in particular with cognitive and factual processing or reasoning, distinct from the medial prefrontal circuitry, which is involved in emotion-processing tasks (e.g., Hynes et al., 2006). Unique environment accounted similarly for most of the variance in grey matter volume for the bilateral temporal

pole, and most of the regions within the limbic/paralimbic cortex, including the bilateral entorhinal cortex and posterior cingulate, and the left rostral anterior cingulate (Figure 3). This finding complements the small-to-moderate genetic contribution for limbic structures. In contrast, common environment (C) accounted for most variance in grey matter volume for the subcortical regions, including the bilateral hippocampus, putamen and pallidum, right caudate and thalamus, left amygdala, specific aspects of the brainstem (mid posterior, central, mid anterior), and specific regions within the parietal cortex (including the left post-central and supramarginal cortex, and the right inferior parietal region) (Figure 3).

Cortical Thickness. Results for cortical thickness suggested strong heritability for the right lateral prefrontal regions and caudal anterior cingulate, and bilateral temporal and left parietal regions (Figure 3, Table S2). Contributions to genetic variance were strongest for the right lateral areas of the occipital cortex (Figure 3, Table S2).

Unique environment demonstrated strongest contributions to subregions of the limbic/paralimbic cortex,

including the bilateral entorhinal and rostral anterior cingulate, to specific right frontal regions (including the frontal pole, caudal middle frontal and medial orbitofrontal cortex), and to left temporal and bilateral banks of the superior temporal sulcus, and the left pericalcarine of the occipital cortex (Figure 3). In contrast, common environment contributed most variance to only specific bilateral regions of the frontal cortex (caudal middle frontal and superior frontal) and the left precuneus of the parietal cortex (Figure 3).

Grey Matter Surface Area. For surface area, a very similar pattern of contributions from genetics versus environment was apparent for the frontal, limbic temporal, and parietal regions, as reported for the volumetric measures (Table S3). Exceptions were stronger heritability estimates for the isthmus cingulate, precuneus of the parietal cortex, and the lingual region and pericalcarine of the occipital cortex, which had only moderate heritability for the volumetric measures. Strong heritability was also found for the right postcentral gyrus of the parietal cortex.

Like the pattern for grey matter volume and cortical thickness, unique environment accounted for most of the variance in surface area for the bilateral frontal pole, temporal pole, and subregions of the limbic/paralimbic cortex (Figure 3). Similarly, common environment contributed mostly to the same regions of the parietal cortex.

White Matter. For DTI, the FA measure of white matter, the strongest heritability was found bilaterally in the cingulum bundle adjacent to the cingulate gyrus. Strong heritability values were also found for the bilateral superior cerebellar peduncle, the left superior fronto-occipital fasciculus and uncinate fasciculus, the right medial lemniscus, inferior cerebellar peduncle and fornix (cres)/stria terminalis, and the body of corpus callosum and pontine crossing tract (Figure 4, Table S4).

In comparison to the grey matter results, the high heritability seen in grey matter measures for the medial prefrontal region was also reflected by strong heritability in the following white matter tracts connecting to the frontal cortex (Figure 4): (i) cingulum bundle (WM tract connecting fibers from the medial prefrontal/anterior cingulate cortex to the limbic hippocampus and entorhinal cortex), and (ii) uncinate fasciculus (WM tract connecting the orbitofrontal cortex to limbic hippocampus/amygdala subcortical regions). Strong heritability was also evident for tracts linked to the limbic system (the fornix/stria terminalis) and to the brainstem (superior cerebellar peduncle, inferior cerebellar peduncle, and medial lemniscus).

The greatest unique environmental contribution was estimated in the right anterior limb of the internal capsule, and the splenium of the corpus callosum, whereas common environment contributed most variance to the right posterior limb of the internal capsule (Figure 4).

Discussion

In this paper, we outline the aims and protocol for a national study of twins that integrates self-report, psychophysiological, autonomic, and brain imaging measures. Our focus is on establishing heritability across measures in the same twin sample, and using these measures to establish endophenotypes for risk for poor emotional health versus what makes us resilient. The first phase of results reported here evaluated heritability in the brain imaging data.

The study is known as the 'TWIN-E' project. Participants are being recruited nationally in collaboration with the Australian Twin Registry. Thus, study results should be widely generalisable. This study will identify endophenotypes for emotional health by combining measures of self-report, functional status, genetics, cognition, psychophysiology, and brain imaging. The innovation in design is the use of standardized testing protocols to achieve this breadth of measures in the one sample.

Our first analyses of heritability used the Holzinger's heritability equation. We evaluated three measures of grey matter and one of white matter. Across grey matter measures (volume, cortical thickness, and surface area), we found common patterns of heritability for medial prefrontal and limbic regions, but distinct patterns for other regions of the cortex. For these measures, contributions to genetic variance were strong for the medial prefrontal cortex, both orbital and caudal anterior cingulate components. Unique environment made a contribution to other areas of the cortex for each of the three grey matter measures, especially the frontal pole for each. These estimates were similar to or higher than previously reported heritability estimates for volume (Winkler et al., 2010), thickness (Kremen et al., 2010; Lenroot et al., 2009; Schmitt et al., 2008; Winkler et al., 2010) and surface area (Winkler et al., 2010). These previous studies reported on their completed (and larger) samples, so the correspondence in values speaks to the generalisability of the current sample, and the capacity for replication in the new phase of TWIN-E. The medial prefrontal region has consistently been implicated in emotional functions and their regulation in non-twin studies. In this study, we plan to delineate these functional associations in the twin sample, using cross-twin, cross-trait analyses for the medial prefrontal region and emotion regulation (and other hypothesized brain regions as identified below). We will also contribute new evidence for the genetic variants that impact the medial prefrontal cortex and its function, as well as how these variants interact with environmental factors such as early life trauma.

Another region of the cortex showing a strong contribution to genetic variance was the parietal cortex. The strength of contribution differed across grey matter measures according to the specific area of the parietal cortex. Grey matter volume and surface area contributed most strongly to genetic variance in the right superior parietal region, and cortical thickness to the left inferior parietal region. These

findings build on previous twin findings that report genetic contributions to these parietal areas, but suggest the contribution to heritability may be higher for each of these three measures (Kremen et al., 2010; Lenroot et al., 2009; Panizzon et al., 2009; Schmitt et al., 2008; Winkler et al., 2010; Wright et al., 2002). This issue needs further investigation with other heritability analysis methods in the next phase of our study. Both regions of the parietal cortex have been implicated in somatic aspects of emotion processing in previous correlation studies, and in integrating sensory input about emotion cues.

Strong genetic contributions were also observed for the right superior temporal cortex, especially for cortical thickness, but also for grey matter volume and surface area. These contributions were consistent with those observed in previous studies (Lenroot et al., 2009; Schmitt et al., 2008; Winkler et al., 2010). Previous correlation studies have implicated the superior temporal cortex in encoding significant stimuli, including the sensory features of emotion stimuli such as facial expressions.

For limbic regions implicated in emotion processing, there was a small-to-moderate contribution to genetic variance in the subcortical regions for grey matter volume. Specifically, small heritability values (<40%) were found for the right hippocampus and thalamus, and left amygdala, hippocampus, and caudate, but moderate values (40–60%) were found for the right amygdala and left thalamus. Our estimates for heritability were lower than those previously reported for the amygdala (e.g., 55–71% for right amygdala in Hulshoff et al., 2006; Kremen et al., 2010; and Wright et al., 2002, vs. 48% reported here) and for the hippocampus (e.g., 64–71% for right hippocampus in Kremen et al., 2010, and Wright et al., 2002, vs. 34% reported here), but higher for others such as the thalamus (e.g., 0% in Wright et al., 2002 vs. 58% for left thalamus reported here). Notable differences in sample characteristics are likely the reason for such discrepancies. The current study was based on 142 male and female twins, aged 18–61 years. The study by Kremen et al. was based on a much larger sample ($N = 404$), but within a restricted group of 51–59 year-old males; whereas Wright et al. reported on a much smaller sample ($N = 40$) but had a similar age/gender distribution to the current study. Our analyses in subsequent phases include confirming heritability estimates in the complete sample, and within specific age groups, which will help identify the most consistent range for heritability or environmental contribution in the limbic regions in a larger sample. In the TWIN-E study, we will also examine heritability in the functional activation and connectivity of limbic circuitry during emotional tasks, as convergent evidence for structural measures.

Together, the measures of grey matter suggest that there are common aspects to which genetic factors contribute. Overlying those common aspects, there are specific distinctive components of these measures that are heritable. Grey matter volume and surface area showed the most similar

pattern of findings, consistent with the correlations that have been observed between these measures (Winkler et al., 2010).

For the white matter measures from DTI, our findings suggest that there is a strong contribution of heritability for a number of tracts, including several that connect to the limbic system. There is only one other study to date that has reported heritability estimates for specific tracts in adults (Kochunov et al., 2010); the others only report variability across the brain (Chiang et al., 2011) or within children (Brouwer et al., 2010). The pattern of moderate heritability reported here is similar to previous estimates for the superior fronto-occipital fasciculus (frontal-parietal-occipital connections), superior longitudinal fasciculus (frontal-temporal-occipital connections), external capsule (frontal-temporal-occipital connections), and genu of the corpus callosum (cerebral hemisphere connections) (Kochunov et al., 2010). We also reported moderate-to-strong heritability for the cingulum (cingulate gyrus-hippocampal connections), whereas Kochunov et al. reported only small effects. The study of Kochunov et al. was based on a similar sample distribution to the current study (19–85 years), but in 467 participants from 49 families. We reported strong heritable effects for other regions not reported by Kochunov et al., including tracts related to the limbic system (e.g., the uncinate fasciculus and fornix/stria terminalis) and in the brainstem (e.g., superior cerebellar peduncle, inferior cerebellar peduncle, and medial lemniscus). It would be important to replicate these findings in the complete sample, including teasing out the generalizability in relation to ethnicity, age, and sex differences.

These first results highlight that there is a genetic contribution accounting for variance in both grey and white matter. Results from the later phases will further identify the specific genetic and environmental contributions to each aspect of grey matter — volume, thickness and surface area. Because cortical thickness and surface area are both functions of volume (Panizzon et al., 2009), volumetric estimates may not, by themselves, discriminate between genetic and environmentally independent traits of surface area and the correlated traits of cortical thickness (Winkler, et al., 2010). Having all three measures in the same sample allows us to fractionate them into genetically informative units before their selection as endophenotypes (Panizzon, et al., 2009). Having white matter measures from the same twins allows further fractionation, and identification of whether specific genetic polymorphisms are linked to grey matter on the one hand, and to white matter on the other.

In the next phase of TWIN-E and in future research, limitations of these initial analyses need to be addressed. These include the use of more sophisticated heritability estimation. We note that some estimates using the Holzinger method are greater than 1.0. This could be due to the interaction of gene–environment effects or to the small sample size (Stromswold, 2001). This approach is also limited

because we cannot specify or compare model parameters; these include taking into account differences in variance, or the comparison of ACE to ADE (A additive genetic, D dominant genetic, and E unique environment) or AE models. For future analyses, we plan to confirm these heritability estimates in the total sample, applying the univariate twin SEM models as described above. We also plan to delineate the behavioral function of each relevant brain region using cross-twin, cross-trait analyses, thereby building on the evidence from non-twin association studies.

The results provide the first outcomes from the TWIN-E study, and indicate it is a generalizable sample that will build on previous findings. It provides the first step towards linking brain imaging data with measures of self-report, functional status, side effects, genomics, cognition, and psychophysiology, to establish endophenotypes for emotional health and risk for mental illness.

Acknowledgments

This project is supported by an Australian Research Council (ARC)-Linkage Grant (LP0883621), with Brain Resource Ltd as industry partner. The awarded study proposal was entitled 'Gene-brain pathways in emotional brain stability and instability' (full title), or 'The Emotional Wellbeing Project' (short form). JMG held an Australian Postdoctoral Linkage Fellowship (APDI) linked to this grant. The Brain Resource Ltd. (BR) was the industry partner on the ARC Linkage Grant that funded this study, but had no further role in design or implementation of the project. BR also provided in-kind support for Phase III of the study.

The chief investigators listed on this project include Professor Leanne M. Williams (University of Sydney), Professor Peter R. Schofield (Neuroscience Research Australia and University of New South Wales), Professor C. Richard Clark (Flinders University and Brain Health Clinics, South Australia), Associate Professor Anthony Harris (University of Sydney), and Dr Justine M. Gatt (University of Sydney). Dr Gatt is also the APDI (Australian Post-Doctoral Industry) Research Fellow linked to this grant, and the TWIN-E Project Coordinator. Dr Mayuresh Korgaonkar has made recent contributions to implementing the planned analyses as a collaborator. Ms Karen Oakley and Mr Kaushik Ram are two PhD students currently linked to the project. We would also like to acknowledge the assistance of the Sydney site research associates, Ms Hope Michaelson, Ms Sarsha Yap, and Ms Alicia Wilcox (past), and the Adelaide site research associates, Ms Melinda Stanners and Ms Vikki Wise.

This research was facilitated through access to the Australian Twin Registry, a national resource supported by a National Health & Medical Research Council (NHMRC) Enabling Grant (628911). DNA was prepared by Genetic Repositories Australia, supported by an NHMRC Enabling Facility (401184).

Conflicts of Interest

The Brain Resource Ltd (BR) was the industry partner on the ARC-linkage grant which funded this study, but had no further role in design or implementation of the project. JMG holds stock options in BR, has received fees from BR for consultancies unrelated to this study, and was a post-doctoral fellow on the ARC Linkage Grant which funded this project. CRC has a small shareholding (less than 5% of issued capital) in BR and some stock options in Brain Resource Ltd. LMW is a stock-holder in BR, has stock options in BR, and has received fees from BR for consultancies unrelated to this study. HM and SY are research assistants on the ARC Linkage Grant which funded this project. MK, PRS, AH, KO, KR, MS, and VS report no potential conflicts of interest.

Endnote

- 1 ATR excluded twin pairs who confirmed the presence of a past or current psychiatric illness, and/or brain injury, neurological disorder, genetic disorder, or drug-alcohol addiction via two self-report items adapted from the complete Medical History Questionnaire administered during Phase I. For those twin pairs who participate in the study, mental and physical health status will be confirmed using more comprehensive health screens included in the web questionnaire (i.e., SPHERES and Medical History Questionnaire).

References

- Baaré, W. F. C., Hulshoff Pol, H. E., Boomsma, D. I., Posthuma, D., de Geus, E. J. C., Schnack, H. G., van Haren, N. E. M., van Oel, C. J., & Kahn, R. S. (2001). Quantitative genetic modeling of variation in human brain morphology. *Cerebral Cortex*, *11*, 816–824.
- Beauregard, M., Lévesque, J., & Bourgoin, P. (2001). Neural correlates of conscious self-regulation of emotion. *The Journal of Neuroscience*, *21*, 1–6.
- Block, J., & Kremen, A. M. (1996). IQ and ego-resiliency: Conceptual and empirical connections and separateness. *Journal of Personality and Social Psychology*, *70*, 349–361.
- BRISC and WebNeuro assessment manual* (v. 1.7). (2010). Sydney and San Francisco: Brain Resource Ltd.
- Brouwer, R. M., Mandl, R. C. W., Peper, J. S., van Baal, G. C. M., Kahn, R. S., Boomsma, D. I., & Hulshoff Pol, H. E. (2010). Heritability of DTI and MTR in nine-year-old children. *NeuroImage*, *53*, 1085–1092.
- Burmeister, M., McInnis, M. G., & Zöllner, S. (2008). Psychiatric genetics: Progress amid controversy. *Nature Reviews Genetics*, *9*, 527–540.
- Chiang, M.-C., McMahon, K. L., de Zubicaray, G. I., Martin, N. G., Hickie, I., Toga, A. W., Wright, M. J., & Thompson, P. M. (2011). Genetics of white matter development: A DTI study of 705 twins and their siblings aged 12 to 29. *NeuroImage*, *54*, 2308–2317.
- Cohen, R. A., Paul, R. H., Stroud, L. L., Gunstad, J. J., Hitsman, B. L., McCaffery, J. J., Hitsman, B., Niaura, R., Clark,

- C. R., McFarlane, A., Bryant, R., Gordon, E., & Williams, L. M. (2006). Early life stress and adult emotional experience: An international perspective. *International Journal of Psychiatry in Medicine*, 36, 35–52.
- de Zubizaray, G. I., Chiang, M.-C., McMahon, K. L., Shattuck, D. W., Toga, A. W., Martin, N. G., Wright, M. J., & Thompson, P. M. (2008). Meeting the challenges of neuroimaging genetics. *Brain Imaging and Behavior*, 2, 258–263.
- Diener, E., Emmons, R. A., Larsen, R. J., & Griffin, S. (1985). The Satisfaction with Life Scale. *Journal of Personality Assessment*, 49, 71–75.
- Duttweiler, P. C. (1984). The Internal Control Index: A newly developed measure of locus of control. *Educational and Psychological Measurement*, 44, 209–221.
- Fischl, B., van der Kouwe, A., Destrieux, C., Halgren, E., Segonne, F., Salat, D. H., Busa, E., Seidman, L. J., Goldstein, J., Kennedy, D., Caviness, V., Makris, N., Rosen, B., & Dale, A. M. (2004). Automatically parcellating the human cerebral cortex. *Cerebral Cortex*, 14, 11–22.
- Fredrickson, B., Tugade, M., Waugh, C., & Larkin, G. (2003). What good are positive emotions in crises? A prospective study of resilience and emotions following the terrorist attacks on the United States on September 11th, 2001. *Journal of Personality & Social Psychology*, 84, 365–376.
- Gatt, J. M., Clark, C. R., Kemp, A. H., Liddell, B. J., Dobson-Stone, C., Kuan, S., Schofield, P. R., & Williams, L. M. (2007). A genotype-endophenotype-phenotype path model of depressed mood: Integrating cognitive and emotional markers. *Journal of Integrative Neuroscience*, 6, 75–104.
- Gatt, J. M., Kuan, S., Dobson-Stone, C., Paul, R. H., Joffe, R. T., Kemp, A. H., Gordon, E., Schofield, P. R., & Williams, L. M. (2008). Association between BDNF Val66Met polymorphism and trait depression is mediated via resting EEG alpha band activity. *Biological Psychology*, 79, 275–284.
- Gatt, J. M., Nemeroff, C. B., Dobson-Stone, C., Kuan, S. A., Paul, R. H., Bryant, R. A., Schofield, P. R., Gordon, E., Kemp, A. H., & Williams, L. M. (2009). Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Molecular Psychiatry*, 14, 681–695.
- Gatt, J. M., Nemeroff, C. B., Schofield, P. R., Paul, R. H., Clark, C. R., Gordon, E., & Williams, L. M. (2010a). Early life stress combined with HTR3A and BDNF Val66Met genotypes impacts emotional brain and arousal correlates of risk for depression. *Biological Psychiatry*, 68, 818–824.
- Gatt, J. M., Williams, L. M., Schofield, P. R., Dobson-Stone, C., Paul, R. H., Grieve, S., Clark, C. R., Gordon, E., & Nemeroff, C. B. (2010b). Impact of the HTR3A gene with early life trauma on emotional brain networks and depressed mood. *Depression and Anxiety*, 27, 752–759.
- Geschwind, D. H., Miller, B. L., DeCarli, C., & Carmelli, D. (2002). Heritability of lobar brain volumes in twins supports genetic models of cerebral laterality and handedness. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 3176–3181.
- Goldsmith, H. H. (1991). A zygosity questionnaire for young twins: A research note. *Behavior Genetics*, 21, 257–269.
- Gordon, E. (2003). Integrative Neuroscience. *Neuropsychopharmacology*, 28, S2–8.
- Gordon, E., Barnett, K. J., Cooper, N. J., Tran, N., & Williams, L. M. (2008). An 'integrative neuroscience' platform: Application to profiles of negativity and positivity bias. *Journal of Integrative Neuroscience*, 7, 345–366.
- Gordon, E., & Williams, L. M. (2010). Personalized medicine and integrative neuroscience: Towards consensus markers for disorders of brain health. In E. Gordon & S. H. Koslow (Eds.), *Personalized medicine, healthcare and integrative neuroscience*. New York: Oxford University Press.
- Gottesman, I., & Gould, T. D. (2003). The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of Psychiatry*, 160, 636–645.
- Gratton, G., Coles, M. G. H., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalography and Clinical Neurophysiology*, 55, 468–484.
- Grieve, S. M., Korgaonkar, M. S., Clark, R. C., & Williams, L. M. (2011). Regional heterogeneity in limbic maturational changes: Evidence from integrating cortical thickness, volumetric and diffusion tensor imaging measures. *NeuroImage*, 55, 868.
- Gross, J. J., & John, O. P. (2003). Individual differences in two emotion regulation processes: Implications for affect, relationships, and well-being. *Journal of Personality and Social Psychology*, 85, 348–362.
- Hasler, G., Drevets, W. C., Manji, H. K., & Charney, D. S. (2004). Discovering endophenotypes for major depression. *Neuropsychopharmacology*, 29, 1765–1781.
- Hickie, I., Hadzi-Pavlovic, D., Scott, E., Davenport, T., Koschera, A., & Naismith, S. (1998). SPHERE: A national depression project. *Australasian Psychiatry*, 6, 248–250.
- Holmes, T. H., & Rahe, R. H. (1967). The Social Readjustment Rating Scale. *Journal of Psychosomatic Research*, 11, 213–218.
- Holzinger, K. J. (1929). The relative effect of nature and nurture influences on twin differences. *The Journal of Educational Psychology*, 20, 241–248.
- Hulshoff Pol, H. E., Schnack, H. G., Posthuma, D., Mandl, R. C. W., Baare, W. F., van Oel, C., van Haren, N. E., Collins, D. L., Evans, A. C., Amunts, K., Bürgel, U., Zilles, K., de Geus, E., Boomsma, D. I., & Kahn, R. S. (2006). Genetic contributions to human brain morphology and intelligence. *The Journal of Neuroscience*, 26, 10235–10242.
- Hynes, C. A., Baird, A. A., & Grafton, S. T. (2006). Differential role of the orbital frontal lobe in emotional versus cognitive perspective-taking. *Neuropsychologia*, 44, 374–383.
- Insel, T. R., & Cuthbert, B. N. (2009). Endophenotypes: Bridging genomic complexity and disorder heterogeneity. *Biological Psychiatry*, 66, 988–989.
- Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., Sanislow, C., & Wang, P. (2010). Research domain criteria (RDoC): Toward a new classification framework for research on mental disorders. *American Journal of Psychiatry*, 167, 748–751.
- Jaeggi, S. M., Buschkuhl, M., Jonides, J., & Perrig, W. J. (2008). Improving fluid intelligence with training on

- working memory. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 6829–6833.
- Jasper, H. A. (1958). The ten twenty electrode system of the international federation. *Electroencephalography and Clinical Neurophysiology*, 10, 371–375.
- Kessler, R. C., Barber, C., Beck, A. L., Berglund, P. A., Cleary, P. D., McKenas, D., Pronk, N., Simon, G., Stang, P., Üstün, T. U., & Wang, P. (2003). The World Health Organization Health and Work Performance Questionnaire (HPQ). *Journal of Occupational and Environmental Medicine*, 45, 156–174.
- Kochunov, P., Glahn, D. C., Lancaster, J., Winkler, A., Karlsgodt, K., Olvera, R. L., Curran, J. E., Carless, M. A., Dyer, T. D., Almasy, L., Duggirala, R., Fox, P. T., Blangero, J. (2011). Blood pressure and cerebral white matter share common genetic factors in Mexican Americans. *Hypertension*, 57, 330–335.
- Kochunov, P., Glahn, D. C., Lancaster, J. L., Winkler, A. M., Smith, S., Thompson, P. M., Almasy, L., Duggirala, R., Fox, P. T., & Blangero, J. (2010). Genetics of microstructure of cerebral white matter using diffusion tensor imaging. *NeuroImage*, 53, 1109–1116.
- Kremen, W. S., Prom-Wormley, E., Panizzon, M. S., Eyer, L. T., Fischl, B., Neale, M. C., Franz, C. E., Lyons, M. J., Pacheco, J., Perry, M. E., Stevens, A., Schmitt, J. E., Grant, M. D., Seidman, L. J., Thermenos, H. W., Tsuang, M. T., Eisen, S. A., Dale, A. M., & Fennema-Notestine, C. (2010). Genetic and environmental influences on the size of specific brain regions in midlife: The VETSA MRI study. *NeuroImage*, 49, 1213–1223.
- Lenroot, R. K., Schmitt, J. E., Ordaz, S. J., Wallace, G. L., Neale, M. C., Lerch, J. P., Kendler, K. S., Evans, A. C., & Giedd, J. N. (2009). Differences in genetic and environmental influences on the human cerebral cortex associated with development during childhood and adolescence. *Human Brain Mapping*, 30, 163–174.
- Loehlin, J. C. (1996). The Cholesky approach: A cautionary note. *Behavior Genetics*, 26, 65–69.
- Lovibond, P. F. (1998). Long-term stability of depression, anxiety and stress syndromes. *Journal of Abnormal Psychology*, 107, 520–526.
- Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behaviour Research and Therapy*, 33, 335–343.
- Magnus, P., Bergm, K., & Nance, W. E. (1983). Predicting zygosity in Norwegian twin pairs born 1915–1960. *Clinical Genetics*, 24, 103–112.
- Mahncke, H. W., Connor, B. B., Appelman, J., Ahsanuddin, O. N., Hardy, J. L., Wood, R. A., Joyce, N. M., Boniske, T., Atkins, S. M., & Merzenich, M. M. (2006). Memory enhancement in healthy older adults using a brain plasticity-based training program: A randomized, controlled study. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 12523–12528.
- Maislin, G., Pack, A. I., Kribbs, N. B., Smith, P. L., Schwartz, A. R., Kline, L. R., Schwab, R. J., & Dingus, D. F. (1995). A survey screen for prediction of apnea. *Sleep*, 18, 158–166.
- Mathersul, D., Palmer, D., Gur, R. C., Gur, R. E., Cooper, N., Gordon, E., & Williams, L. M. (2009). Explicit identification and implicit recognition of facial emotions: II. Identifying core domains and relationships with general cognition. *Journal of Clinical and Experimental Neuropsychology*, 31, 278–291.
- McCrae, R. R., & Costa, P. T., Jr. (2004). A contemplated revision of the NEO Five-Factor Inventory. *Personality and Individual Differences*, 36, 587–596.
- McFarlane, A., Clark, C. R., Bryant, R. A., Williams, L. M., Niaura, R., Paul, R. H., Hitsman, B. L., Stroud, L., Alexander, D. M., & Gordon, E. (2005). The impact of early life stress on psychophysiological, personality and behavioral measures in 740 nonclinical subjects. *Journal of Integrative Neuroscience*, 4, 27–40.
- Mori, S., Oishi, K., Jiang, H., Jiang, L., Li, X., Akhter, K., Hua, K., Faria, A. V., Mahmood, A., Woods, R., Toga, A. W., Pike, B., Rosa Neto, P., Evans, A., Zhang, J., Huang, H., Miller, M. I., van Zijl, P. C. M., & Mazziotta, J. (2008). Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *Neuroimage*, 40, 570–582.
- Murphy, B., Herrman, H., Hawthorne, G., Pinzone, T., & Evert, H. (2000). *Australian WHOQoL instruments: User's manual and interpretation guide*. Melbourne, Australia: Australian WHOQoL Field Study Centre.
- Neale, M. C., & Maes, H. H. M. (2004). *Methodology for genetics studies of twins and families*. Dordrecht, The Netherlands: Kluwer Academic.
- O'Connor, M., Cooper, N. J., Williams, L. M., DeVarney, S., & Gordon, E. (2010). Neuroleadership and the productive brain. *Neuroleadership Journal*, 3, 37–42.
- Panizzon, M. S., Fennema-Notestine, C., Eyer, L. T., Jernigan, T. L., Prom-Wormley, E., Neale, M., Jacobson, K., Lyons, M. J., Grant, M. D., Franz, C. E., Xian, H., Tsuang, M., Fischl, B., Seidman, L., Dale, A., & Kremen, W. S. (2009). Distinct genetic influences on cortical surface area and cortical thickness. *Cerebral Cortex*, 19, 2728–2735.
- Parker, G., Roussos, J., Hadzi-Pavlovic, D., Mitchell, P., Wilhelm, P., Wilhelm, K., & Austin, M.-P. (1997). The development of a refined measure of dysfunctional parenting and assessment of its relevance in patients with affective disorders. *Psychological Medicine*, 27, 1193–1203.
- Paul, R. H., Gunstad, J., Cooper, N., Williams, L. M., Clark, C. R., Cohen, R. A., Lawrence, J. J., & Gordon, E. (2007). Cross-cultural assessment of neuropsychological performance and electrical brain function measures: Additional validation of an international brain database. *International Journal of Neuroscience*, 117, 549–68.
- Paul, R. H., Haque, O., Gunstad, J., Tate, D. F., Grieve, S. M., Hoth, K., Brickman, A. M., Cohen, R., Lange, K., Jefferson, A. L., MacGregor, K. L., & Gordon, E. (2005a). Subcortical hyperintensities impact cognitive function among a select subset of healthy elderly. *Archives of Clinical Neuropsychology*, 20, 697–704.
- Paul, R. H., Lawrence, J., Williams, L. M., Clark, C. R., Cooper, N., & Gordon, E. (2005b). Preliminary validity of

- 'IntegNeuro': A new computerized and standardized battery of neurocognitive tests. *International Journal of Neuroscience*, 115, 1549–1567.
- Posthuma, D., De Geus, E. J. C., Baaré, W. F. C., Hulshoff Pol, H. E., Kahn, R. S., & Boomsma, D. I. (2002). The association between brain volume and intelligence is of genetic origin. *Nature Neuroscience*, 5, 83–84.
- Purcell, S. (2002). Variance components models for gene-environment interaction in twin analysis. *Twin Research*, 5, 554–571.
- Sanders, B., & Becker-Lausen, E. (1995). The measurement of psychological maltreatment: Early data on the Child Abuse and Trauma Scale. *Child Abuse and Neglect*, 19, 315–323.
- Schmitt, J. E., Lenroot, R. K., Wallace, G. L., Ordaz, S., Taylor, K. N., Kabani, N., Greenstein, D., Lerch, J. P., Kendler, K. S., Neale, M. C., & Giedd, J. N. (2008). Identification of genetically mediated cortical networks: A multivariate study of pediatric twins and siblings. *Cerebral Cortex*, 18, 1737–1747.
- Schofield, P. R., Williams, L. M., Paul, R. H., Gatt, J. M., Brown, K., Luty, A., Cooper, N., Grieve, S., Dobson-Stone, C., Kuan, S. A., & Gordon, E. (2009). Disturbances in selective information processing associated with the BD-NFVal66Met polymorphism: Evidence from cognition, the P300 and fronto-hippocampal systems. *Biological Psychology*, 80, 176–188.
- Silverstein, S. M., Berten, S., Olson, P., Paul, R., Williams, L. M., Cooper, N., & Gordon, E. (2007). Development and validation of a world-wide-web-based neurocognitive assessment battery: WebNeuro. *Behavior Research Methods*, 39, 940–949.
- Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*, 23, S208–219.
- Stromswold, K. (2001). The heritability of language: A review and metaanalysis of twin, adoption, and linkage studies. *Language*, 77, 647–723.
- Sullivan, E. V., Pfefferbaum, A., Swan, G. E., & Carmelli, D. (2001). Heritability of hippocampal size in elderly twin men: Equivalent influence from genes and environment. *Hippocampus*, 11, 754–762.
- Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation*, 93, 1043–1065.
- Trzepacz, P. T., & Baker, R. (1993). The psychiatric mental status examination. Oxford: Oxford University Press.
- Visscher, P. M. (2004). Power of the classical twin design revisited. *Twin Research*, 7, 505–512.
- Wacker, J., & Gatt, J. M. (2010). Resting posterior versus frontal delta/theta EEG activity is associated with extraversion and the COMT Val158Met polymorphism. *Neuroscience Letters*, 478, 88–92.
- Williams, L. M., Gatt, J. M., Grieve, S. M., Dobson-Stone, C., Paul, R. H., Gordon, E., & Schofield, P. R. (2010a). COMT Val108/158Met polymorphism effects on emotional brain function are modulated by level of awareness and associated with negativity biases. *NeuroImage*, 53, 918–925.
- Williams, L. M., Gatt, J. M., Hatch, A., Palmer, D. M., Nagy, M., Rennie, C., Cooper, N. J., Morris, C., Grieve, S., Dobson-Stone, C., Schofield, P., Clark, C. R., Gordon, E., Arns, M., & Paul, R. H. (2008). The integrate model of emotion, thinking and self regulation: An application to the 'paradox of aging'. *Journal of Integrative Neuroscience*, 7, 367–404.
- Williams, L. M., Gatt, J. M., Schofield, P. R., Olivieri, G., Peduto, A., & Gordon, E. (2009a). 'Negativity bias' in risk for depression and anxiety: Brain-body fear circuitry correlates, 5-HTT-LPR and early life stress. *NeuroImage*, 47, 804–814.
- Williams, L. M., Kemp, A., Felmingham, K., Liddell, B., Palmer, D., Bryant, R. A. (2007). Neural biases to covert and overt signals of fear: Dissociation by trait anxiety and depression. *Journal of Cognitive Neuroscience*, 10, 1595–1608.
- Williams, L. M., Mathersul, D., Palmer, D. M., Gur, R. C., Gur, R. E., & Gordon, E. (2009b). Explicit identification and implicit recognition of facial emotions: I. Age effects in males and females across 10 decades. *Journal of Clinical and Experimental Neuropsychology*, 31, 257–277.
- Williams, L. M., Nagy, M. T., Docherty, J., & Gordon, E. (2010b). Emotion, thinking, and brain synchrony in schizophrenia: An integrative model. *Mind & Brain, The Journal of Psychiatry*, 1, 87–99.
- Williams, L. M., Phillips, M. L., Brammer, M. J., Skerrett, D., Lagopoulos, J., Rennie, C., Bahramali, H., Olivieri, G., David, A. S., Peduto, A., & Gordon, E. (2001). Arousal dissociates amygdala and hippocampal fear responses: Evidence from simultaneous fMRI and skin conductance recording. *NeuroImage*, 14, 1070–1079.
- Williams, L. M., Rush, A. J., Koslow, S. H., Wisniewski, S. R., Cooper, N. J., Nemeroff, C. B., Schatzberg, A. F., & Gordon, E. (2011). International study to predict optimized treatment for depression (iSPOT-D), a randomized clinical trial: Rationale and protocol. *Trials*, 12, 1–17.
- Williams, L. M., Tsang, T. W., Clarke, S., & Kohn, M. (2010c). An 'integrative neuroscience' perspective on ADHD: Linking cognition, emotion, brain and genetic measures with implications for clinical support. *Expert Review of Neurotherapeutics*, 10, 1607–1621.
- Willis, S. L., Tennstedt, S. L., Marsiske, M., Ball, K., Elias, J., Koepke, K. M., Morris, J. N., Rebok, G. W., Unverzagt, F. W., Stoddard, A. M., & Wright, E. (2006). Long-term effects of cognitive training on everyday functional outcomes in older adults. *Journal of the American Medical Association*, 296, 2805–2814.

- Winkler, A. M., Kochunov, P., Blangero, J., Almasy, L., Zilles, K., Fox, P. T., Duggirala, R., & Glahn, D. C. (2010). Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *NeuroImage*, 53, 1135–1146.
- World Health Organization Group. (1998). Development of the World Health Organization WHOQOL-BREF quality of life assessment. *Psychological Medicine*, 28, 551–558.
- Wright, I. C., Sham, P., Murray, R. M., Weinberger, D. R., & Bullmore, E. T. (2002). Genetic contributions to regional variability in human brain structure: Methods and preliminary results. *NeuroImage*, 17, 256–271.
-