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Genetic Contribution to Variation in Cognitive Function: An fMRI Study in Twins

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manner. The rapidity with which these shoal-forming waves spread once the initial conditions are satisfied is indicative of the advantage the group has over the isolated individual in transferring information over great distances. Our observations also provide ecosystem-scale evidence that a critical population density triggers rapid transition from disordered to highly synchronized behavior, and small groups of leaders often play crucial roles in affecting the actions of much larger groups, as has recently been predicted in general theoretical investigations (19, 21, 27–29), simulations, and laboratory experiments (26, 27) about animal group behavior (20, 30, 31). These findings provide information essential to the conservation of marine ecosystems that vast oceanic fish shoals inhabit.

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Materials and methods

Figs. S1 to S6

References

Movie S1

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Genetic Contribution to Variation in Cognitive Function: An fMRI Study in Twins

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Little is known about the genetic contribution to individual differences in neural networks subserving cognition function. In this functional magnetic resonance imaging (fMRI) twin study, we found a significant genetic influence on brain activation in neural networks supporting digit working memory tasks. Participants activating frontal-parietal networks responded faster than individuals relying more on language-related brain networks. There were genetic influences on brain activation in language-relevant brain circuits that were atypical for numerical working memory tasks as such. This suggests that differences in cognition might be related to brain activation patterns that differ qualitatively among individuals.

The direct link between genes, brain, and behavior can be difficult to establish (1). Structural and functional investigations in the human and mouse brain suggest that some genes are expressed in highly specific brain regions, whereas other genes have more global effects (1–4). The

total heritability of individual differences can be examined with twin studies (5–8). Functional magnetic resonance imaging (fMRI) studies investigating specific brain regions assumed to subservise some cognitive function did not demonstrate high heritability of brain activity (9–14). Genetic influences on brain activation in areas that typically subservise a cognitive function might be modest because these areas will be activated similarly among humans. By contrast, brain regions activated in some individuals only might be better candidates for genetic analysis. Thus, genetic effects should be tested cortex-wide.

Structural and functional brain investigations suggest that brain areas that are similarly activated among humans may be embedded in larger brain networks that vary among individuals (1, 4), possibly causing individual differences in cognition. An attractive candidate for the study of genetic influ-

ences on brain networks is working memory for digits under arithmetic distraction. Heritability estimates for behavioral measures in this task are high (15), and stable individual differences in the spatial organization of function-carrying areas were shown (16). A distractor task causes an interruption of verbal rehearsal, leading to rapid forgetting (17). The decay model of working memory (18, 19) states that numbers can be retained without explicit verbal rehearsal, but it does not specify neural correlates of these memory processes. The triple-code model (20) claims that number processing and arithmetic require both magnitude and language-related number representations in inferior parietal cortex, angular gyrus, and perisylvian cortex. Individuals holding numbers in memory in a language-related or magnitude code suffer from code interference when executing arithmetic tasks. Employing early motor coding routes protects memory traces from distraction (21), which corresponds to the importance of finger representations for number processing also in adulthood (22).

For genetic fMRI studies, appropriate brain alignment, sufficient individual differences, reliability, and statistical power are of core importance (6, 23–25). We used an extended twin design consisting of male monozygotic (MZ) twins with an additional nontwin brother, where every brother is related to both twins. We examined reaction times (RT) as a measure of proficiency and blood oxygen level-dependent (BOLD) response as a measure of relative brain (de)activation (26) during two identical scanning sessions in all participants. These two observations of the phenotypes of interest were entered into a genetic structural equation model (SEM) that estimates additive genetic effects corrected for measurement error (23). Heritability h^2 was expressed as the percentage of reliable variance accounted for

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by additive genetic factors. With 10 families, sufficient power (0.82) is provided to detect ($\alpha = 0.05$) a heritability of 80% or more after correction for measurement error (accounting for one-sixth of the variance) in a bivariate design (23–25).

In the digit memory tasks (15), participants had to verify (recognition phase) whether a single digit was contained in a previously memorized digit set (encoding phase) that consisted of either two (DTM2) or four (DTM4) digits. The tasks differed in the distraction phase, which consisted of either simple arithmetic interference (additions and subtractions) or of object categorization (fruits, vegetables, kitchen utensils, and tools). The latter was employed with a memory load of four digits (DTC4), because two digits did not provoke sufficient individual differences (25).

Heritability of brain activity at the vertex level was estimated for BOLD contrasts (23–27) separately for the encoding, distraction, and recognition phase of the working memory tasks. A substantial part of response time (tables S2 and S3) and brain activity (Fig. 1) proved to be under genetic influence (red-blue color scale) (25) (more detailed images in fig. S10, A to I). Genetic effects were found bilaterally and included visual cortex, angular gyrus, intraparietal sulcus (IPS), temporo-parietal junction, motor and premotor cortex, frontal eye field, inferior frontal gyrus, cuneus, and anterior cingulate cortex (ACC), regions related to working memory functions (28). Both number interference tasks show genetic influences on larger parts of the brain. Genetic influences were smaller for the DTC4 task that includes less resource competition. Genetic influences were modulated by memory load; they were high for DTM2 during encoding and high for DTM4 during recognition.

Next, we looked for brain areas that were significantly (de)activated among most participants. Brain areas with consistent BOLD responses (transparent yellow in Fig. 1) are considered to be typically supporting a cognitive function. We found fronto-parietal activations regularly encountered in studies of working memory (28); in line with the triple-code model, there were activations in perisylvian language-related areas and in inferior parietal number magnitude estimation areas. This activation pattern was found only for the encoding and the distraction phase. Absence of consistent left hemisphere activation during recognition with previous arithmetic distraction indicates number interference, but no interference effect was found for object categorization as a distracting activity.

We focused on individual differences in brain activation patterns because heritability and brain activation maps revealed only partial overlap (Fig. 1). Relative frequency of brain activation (RFBA) maps reflect the percentage of participants with significant brain activation, and standard deviation maps illustrate variation in brain activation contrasts. Figure 2 reports these maps for DTM4 recognition (25). Genetic influences on left fronto-parietal, perisylvian and visual cortices go along with higher standard deviations and more individualized brain activation. The partial overlap between heritability and brain activation maps suggests two mechanisms for ge-

netic influences on brain activity: Genes can influence brain activation patterns that reflect systematic quantitative differences in regions characterized by between-subject consistency in a significant BOLD contrast (red-blue, overlaid with transparent yellow in Fig. 1), but genes can also affect qualitative differences in cognition through the employment of qualitatively different neural processes. The latter case might lead to absence of a significant BOLD contrast at the group level (red-blue only), but it is in line with characteristics of genetics: Regions with more intersubject variability are more likely to show genetic effects (25). If genes affect typical as well as atypical areas of brain activation subserving cognition, this raises three questions: (i) Are brain activations under genetic influence interpretable in terms of cognition? (ii) Are the two mechanisms for

genetic influences on brain activity related to behavior? (iii) May genes affect neural networks that are highly individualized in nature?

Question (i): Digits that suffered from arithmetic interference are retrieved from memory via a right-hemispheric network comprising inferior parietal and temporal areas with low memory load and inferior parietal and inferior frontal areas with high memory load. These brain regions are reminiscent of an evolutionary old memory system found in primates (29). The absence of activation in left hemispheric language and number magnitude related cortices suggests that the arithmetic interference tasks impair the number semantic and verbal aspects of digit memory, whereas the object categorization interference task does not (Fig. 1). In the distractor phase, there is a genetic influence on

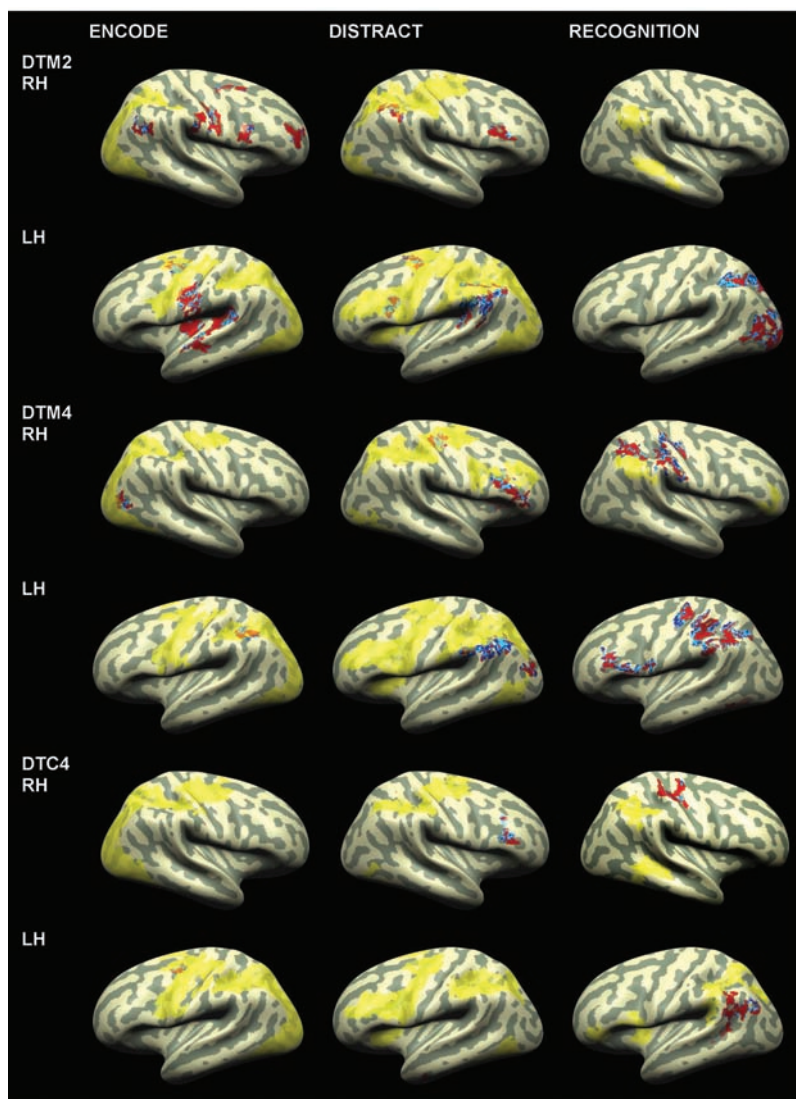


Fig. 1. Error-corrected heritability estimates (red-blue scale, based on data from two identical scanning sessions) of functional brain activations for three working memory tasks, and significant group-level brain activation contrasts against resting baseline (transparent yellow), visualized on an inflated, cortically aligned average brain, separately for left (LH) and right (RH) hemispheres with encoding, distraction, and recognition phase from left to right: DTM2 resp.; DTM4: two resp., four digits memorized with arithmetic distraction; DTC4: four digits memorized with object categorization as distraction. Red: heritability > 80% detectable with statistical power > 0.82; light blue: heritability 60 to 80% (power > 0.44); dark blue: heritability < 60% (power < 0.44).

executive functions subserved by right prefrontal areas for all three tasks (Fig. 1). It is most extended in the high load plus arithmetic distractor condition, exerting the highest load on the executive processor (18). In the left hemisphere, there is substantial heritability of activation in the angular gyrus, but only for number interference tasks. The latter finding suggests number-specific retrieval mechanisms from long-term storage (18, 20). In the rec-

ognition phase, there is a genetic influence on arithmetic distraction in the left parietal cortex, extending into the frontal cortex for high working memory load. This suggests that retrieval from the working storage (18) is under substantial genetic influence, but without significant group activation effect. This implies that conventional brain activation analyses at the group level might be less informative for genetic differences research.

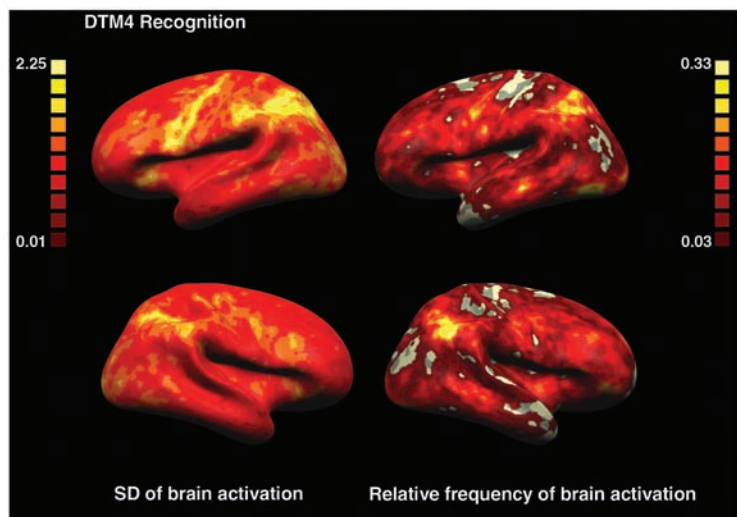


Fig. 2. Standard deviation of averaged (test and retest-run) brain activation t -contrast (cognitive activation minus resting baseline) maps (left) as well as relative frequency of brain activation maps (right) for the DTM4 recognition phase.

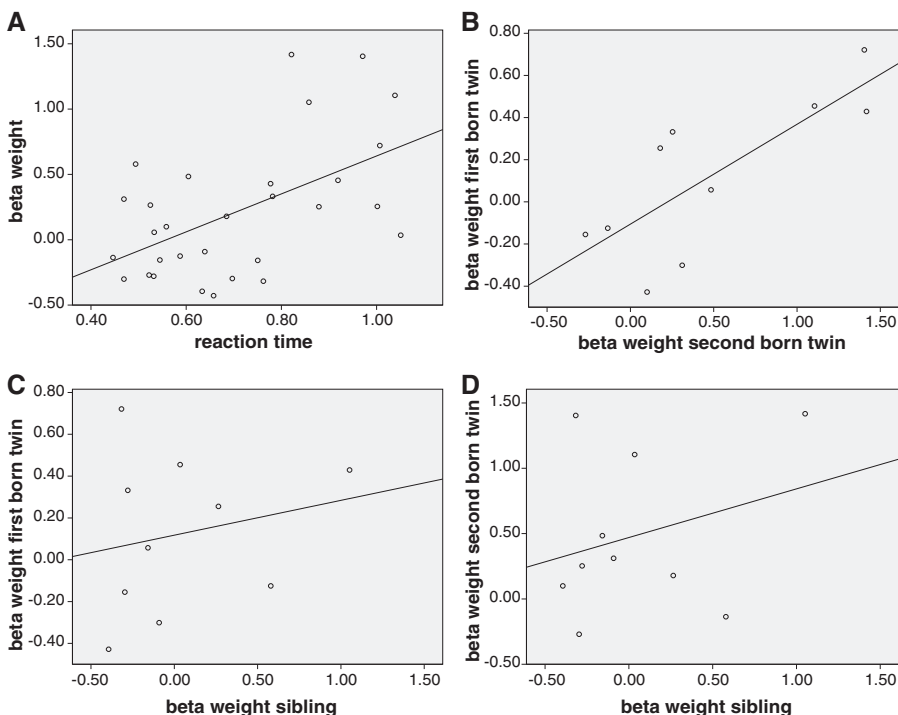


Fig. 3. Relation between observable behavior (RT) in the recognition phase and brain activation in the encoding phase of the DTM2 task in a region of interest located in Broca's area (fig. S3). (A) Relation between averaged (test and retest run) RT and brain activation contrast beta values ($r = 0.54$, $P = 0.001$, one-sided); (B to D) Twin and twin-sib correlations of brain activation contrast beta values with some participants showing deactivation and average activation about zero. This region showed a genetic influence on brain activation with $h^2 = 0.895$ (table S6A), and there was a genetic influence on RT (table S3).

Some participants showed strong activation effects outside the confines of average brain activation, indicating that this does not necessarily represent the relation between brain anatomy and working memory function (16).

Question (ii): In particular for the number interference tasks, we showed that memorizing numbers with the involvement of cortices supporting a number magnitude code (IPS) and/or a language-related code (Broca's area) suffered from code interference. This code conflict may be inferred from increased activation in anterior cingulate cortex, which was a good predictor of both response times in the distracting arithmetic and the recognition memory phase (figs. S5 and S6). In contrast, participants activating a finger representation system in anterior IPS monitored by prefrontal cortex showed no conflict and were faster for both response measures.

Broca's area and the angular gyrus were not significantly activated at the group level due to a mixed pattern of activation and deactivation. Nevertheless, for Broca's area there were genetic influences on brain activity (Fig. 3, B to D) that were related to reaction time (Fig. 3A), which in turn was genetically influenced (table S3). In conclusion, encoding processes act like a switch that affects participants' processing for the whole working memory experiment (figs. S4, S7, and S8). Atypical, but genetically influenced, brain activation topology shows its impact through neural networks that directly affect genetically influenced behavior (1, 25).

We found the most extended brain area with significant genetic influences on brain activation outside the confines of the average brain activity maps in the DTM4 recognition phase in the left hemisphere (Fig. 2). One way to demonstrate that this brain activation is not due to regional noise is to show that activations among regions covary.

Question (iii): Activation data for the DTM4 recognition phase from the left brain areas showing genetic influences on brain activation in atypical areas were subjected to SEM. Results suggested the presence of a hippocampus-guided, visual recognition network that is partly under genetic influence. Moreover, an extended network—including areas for language-related number processing as postulated in the triple-code model—was under genetic influence (fig. S9). A similar network has been identified at the anatomical level (4).

Our findings demonstrate that genetically influenced differences in brain activation patterns exist, causing qualitative differences in neurocognitive processing routes.

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Changes in Temperature Preferences and Energy Homeostasis in Dystroglycan Mutants

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Temperature affects the physiology, behavior, and evolution of organisms. We conducted mutagenesis and screens for mutants with altered temperature preference in *Drosophila melanogaster* and identified a cryophilic (cold-seeking) mutant, named *atsugari* (*atu*). Reduced expression of the *Drosophila* ortholog of dystroglycan (DmDG) induced tolerance to cold as well as preference for the low temperature. A sustained increase in mitochondrial oxidative metabolism caused by the reduced expression of DmDG accounted for the cryophilic phenotype of the *atu* mutant. Although most ectothermic animals do not use metabolically produced heat to regulate body temperature, our results indicate that their thermoregulatory behavior is closely linked to rates of mitochondrial oxidative metabolism and that a mutation in a single gene can induce a sustained change in energy homeostasis and the thermal responses.

Earth has experienced cooling and warming cycles, and organisms exposed to these climate changes either were exterminated or adapted to survive (1, 2). Animals have thermoregulatory systems to adapt their physiological functions, such as energy utilization, growth, reproduction, and locomotion, in response to the wide range of changes in ambient temperature (3–5). Although mobile animals commonly select a preferred temperature, the biochemical and metabolic processes that underlie the temperature preference remain poorly understood (5–7).

We isolated several mutants with aberrant temperature preferences; these included warm-seeking

mutants, temperature-insensitive mutants, and the cryophilic mutant, designated as *atsugari* (*atu*), described here. On a linear thermal gradient ranging from 12° to 35°C, the third-instar larvae

of wild-type *Drosophila* (Canton S) that had grown at 25°C showed a strong temperature preference that peaked at 22°C (Fig. 1, A and C). The *atu* mutant larvae had a preference peak at 18°C (Fig. 1, B and D). The behavioral traits of the *atu* mutant, including assays of olfactory, visual, and locomotory functions, were normal (fig. S1). To exclude the potential effects of the genetic background on the *atu* mutation, we outcrossed the *atu* mutant with the isogenic line *w¹¹¹⁸* and generated P-element excision strains. The *atu* mutant larvae again exhibited low-temperature preference after outcrossing, and a revertant line with precise P-element excision had a normal temperature preference that peaked at 22°C (Fig. 1E).

We cloned the genomic DNA that flanked the P element in the *atu* mutant. A P element had been inserted 251 base pairs (bp) downstream of the transcription initiation site in the first exon of the *Drosophila* ortholog of the mammalian gene for dystroglycan (*DmDG*) (8) (fig. S2, A and B). The inserted P element reduced the expression of the DmDG transcript to 15% of that in wild-type larvae (Fig. 1H). The reduced expression of DmDG in the *atu* mutant was confirmed with polyclonal antibodies to DmDG (Fig. 1I). Immunohistolog-

Table 1. Reversal of the *atu* cryophilic phenotype by the transgenic expression of DmDG and phenocopying by an RNA interference-mediated suppression of DmDG in the wild-type larvae. Comparisons among multiple groups were evaluated by two-way analyses of variance (ANOVAs) followed by the Tukey-Kramer post hoc tests. In each group, values not sharing the same superscript letter are significantly different ($P < 0.05$). Effects of the cell-specific transgenic expression in the neurons of the antennomaxillary complex (19) (figs. S4 and S5) were also examined. The numerical analyses of data are shown in table S1. μ and σ^2 denote population mean and population variance, respectively.

Flies	Preferred temperature μ (°C), σ^2	
	μ	σ^2
<i>DmDG-overexpression experiment</i>		
<i>atu</i>	$\mu = 18.8^a$	$\sigma^2 = 3.5^2$
Control <i>atu:UAS-DmDG</i> line	$\mu = 18.5^a$	$\sigma^2 = 3.5^2$
Control <i>atu:actin5C-GAL4</i> line	$\mu = 18.4^a$	$\sigma^2 = 4.5^2$
Ubiquitous-DmDG transgenic <i>atu</i> line	$\mu = 21.1^b$	$\sigma^2 = 3.8^2$
Accessory cell–DmDG transgenic <i>atu</i> line	$\mu = 18.2^a$	$\sigma^2 = 3.0^2$
Soma sheath cell–DmDG transgenic <i>atu</i> line	$\mu = 18.2^a$	$\sigma^2 = 3.0^2$
<i>DmDG-depletion experiment</i>		
<i>w¹¹¹⁸</i>	$\mu = 22.3^a$	$\sigma^2 = 2.8^2$
Control <i>actin5C-GAL4</i> line	$\mu = 22.0^a$	$\sigma^2 = 3.3^2$
Control <i>UAS-dsRNA</i> line	$\mu = 21.0^b$	$\sigma^2 = 3.5^2$
DmDG-depleted line	$\mu = 19.9^c$	$\sigma^2 = 3.7^2$

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