MOLECULAR CORRELATE OF MOUSE EXECUTIVE FUNCTION. TOP-DOWN AND BOTTOM-UP COMPLEMENTATIONS BY PRESYNAPTIC VERTEBRATE BRAIN-SPECIFIC \textit{Ntn}G \textit{ENE} PARALOGS

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ABSTRACT

Executive function (EF) is a regulatory construct of learning and general cognitive abilities. Genetic variations underlying the architecture of cognitive phenotypes are likely to affect EF and associated behaviors. Mice lacking one of \textit{Ntng} gene paralogs, encoding the vertebrate brain-specific presynaptic Netrin-G proteins, exhibit prominent deficits in the EF control. Brain areas responsible for gating the bottom-up and top-down information flows differentially express \textit{Ntng1} and \textit{Ntng2}, distinguishing neuronal circuits involved in perception and cognition. As a result, high and low cognitive demand tasks (HCD and LCD, respectively) modulate \textit{Ntng1} and \textit{Ntng2} associations either with attention and impulsivity (AI) or working memory (WM), in a complementary manner. During the LCD \textit{Ntng2}-supported neuronal gating of AI and WM dominates over the \textit{Ntng1}-associated circuits. This is reversed during the HCD, when the EF requires a larger contribution of cognitive control, supported by \textit{Ntng1}, over the \textit{Ntng2} pathways. Since human \textit{NTNG} orthologs have been reported to affect human IQ (\textsuperscript{1}), and an array of neurological disorders (\textsuperscript{2}), we believe that mouse \textit{Ntng} gene paralogs serve an analogous role but influencing brain executive functioning.
INTRODUCTION

Executive function (EF) is a heterogeneous construct that can be viewed as a set of processes executively supervising cognitive behaviors (3). EF is an umbrella term for working memory (WM), attention and impulsivity (AI), and response inhibition, and is thought to account for the variance in cognitive performance (4). WM, due to its storage and processing components, is viewed as a bimodal flexible system of a limited capacity. Since WM maintains current information and simultaneously supports its execution, as a latent factor underlying intelligence (5), it has been termed as “the central executive” (6) attention-controlling system dependent on consciousness (7). However an awareness-independent model has been also proposed (8,9). General learning (Ln) ability depends on attention and WM interaction (10) as well as perception, the causal and informational ground for the higher cognitive functions (11). Perception guides our thinking about and acting upon the world and serves as an input to cognition, via a short-term memory mediated interactions (12). A possible mechanism linking perception and cognition would be attention (13).

Perception (bottom-up) and cognition (top-down) have been historically viewed as independently operating encapsulating domains. Such embodiment has paved a ground for the view that perceptual experiences can be influenced by cognitive state (for references see 14), consequently elaborated into the brain predictive coding approach currently dominating cognitive neuroscience (15), and positing that attention is a property of brain computation network (16). However this has been challenged by the opposite opinion that “cognition does not affect perception” (17). Regardless whether or not such a cognitive-sensory dichotomy exists, herein we view perception and cognition as two main information streams the EF exerts its actions upon, possibly through active association.

We have previously described the function of two vertebrate-specific brain-expressed presynaptic gene paralogs, NTNG1 and NTNG2, complementary affecting verbal comprehension and WM in human subjects, that underwent an accelerated evolution in primates and extinct hominins (1). This pair of genes has been also implicated in the phenomena of antagonistic pleiotropy, a trade-off between the evolution-driven cognitive function elaboration and an array of concomitant neuropathologies, rendering the human brain phenotypically fragile (2). Ntngs also complementary diversify the mouse behavior (18).
Despite the fact that EF abrogation is a major determinant of problem behavior and disability in neuropsychiatric disorders (19), the genetics underlying EF remains elusive with no causative vector agents (e.g. genes) have yet been reported. Herein we show that NTNG paralogs affecting human IQ also affect mouse learning and brain executive functioning.

RESULTS

Randomizing mouse genotypes in a search for genotype-phenotype interactions. We used a non-parametric data analysis approach for two behavioral paradigms: 5-choice serial reaction time task (5-CSRTT, 20), and radial arm maze (RAM), to measure selective attention and impulsivity (AI), and spatial working memory (WM), respectively, in Ntng1/− and Ntng2/− mice. We calculated mouse genotype-independent ranking (as for a mixed population), and the rank variance (as a proportion of variance explained, PVE) for each behavioral parameter and a global rank for each paradigm. This allowed us to avoid common in a behavior-reporting literature a genotype-attributed single parameter reporting bias via the data “cherry-picking” satisfying common standards of statistical significance tests (e.g. 18, see Supplementary Figures 1 (SF1) and 2 (SF2) for the same data being processed in a traditional way). This also permitted us to compare the observed phenotypes between both paradigms for the genetically independent groups of mice, simultaneously searching for potential interactions among them. We were able to follow the dynamics of the behavioral heterogeneity and to deduce inferences between the mouse phenotypic and genotypic traits interaction affecting executive function (EF).

Affected AI for both Ntng paralogs, and WM for the Ntng2 gene, modulated by the cognitive demand. 5-CSRTT data (ST1-1) show that Ntng1/− population of mice is characterised by a large span of the ranks variance (PVE>90%) occupying not only bottom 4 but also top 4 rank positions and outcompeting their wild type littermates (Fig.1(A-D)-1). Ntng1 ablation generates mice with both strong proficit and deficit of AI, extending beyond a single affected parameter estimate (Fig.1C,G), but with the averaged rank per a genotype undistinguishable of that of their wild type littermates, and more than 90% of the variance attributable to Ntng1/− genotype (Fig.1A-1). A higher cognitive demand task phase (HCD) reduces the rank variance down to 76% but at the expense of a lower rank (Fig.1E-1). During the low cognitive demand task phase (LCD), opposite to Ntng1/− mice, Ntng2/− subjects’ rank is twice lower comparing to their genetically unmodified
littermates with the rank variances almost identical (Fig.1A-2) but later changed during the HCD (Fig.1E-2).

Robustness of the WM deficit upon Ntng2 depletion in mice is the most prominently evidenced by the bottom 4 mouse ranks with 12/13 out of 16 being occupied by the knockout mice (Fig.2C-2,G-2) and by low behavioral consistency across the sessions and parameters cross-correlations (Fig.2H-2) during the HCD. At the same time, the absence of Ntng1 in mice affected only the LCD sessions performance (Fig.2D-1) but did not render them behaviorally distinguishable from the wild type littermates during the HCD (Fig.2H-1).

**Proficit and deficit in learning associated with the Ntng+/− genotypes.** The complementary segregation of Ntng+/− gene paralogs-associated behavioral phenotypes within the distinct modules of EF (Fig.3; ST1 and ST2) has prompted us to analyse the operant conditioning learning (ST1-1 and ST1-2 (Sc_spatial1, Ln)) by mice, assuming that AI and WM may interact. And indeed, Ntng1+/− mice outperform their genetically unmodified littermates learning faster during the LCD (Fig.4(A,B)-1, LCD) but are unable to sustainably cope with the growing cognitive demand (Fig.4(A,B)-1, HCD). At the same time, Ntng2+/− mice display a prominent deficit of Ln (Fig.4(A,B)-2, LCD), which is becoming stronger with the growing demand to succeed (Fig.4(A,B)-2, HCD). In overall, the pattern of Ln behavior caused by the genetic ablation of either of Ntngs completely matches that of WM testing on the RAM (Fig.2), summarised in Fig.3. The contribution of AI to the Ln deficit is demonstrated by the rank correlations of Ln vs. AI (from Fig.1) which is stronger during the LCD for both Ntng genetically ablated mouse populations (Fig.4C-1,2).

**Complementary expression of Ntng paralogs in the brain and their interaction.** The robust phenotype of the abrogated EF for both Ntng gene paralogs affecting either AI or WM, or both, is supported by the predominant expression of both genes within the information processing brain areas, complementary sequestering them either within bottom-up (for Ntng1) and top-down (for Ntng2) neuronal pathways (Fig.5A-C). The presented hierarchy for the Ntng paralogs brain distribution is supported by two times lower level of the Ntng2 expression under Ntng1+/− background (Fig.5D-2), with no effect on Ntng1 expression when Ntng2 is absent (Fig.5D-1) in the life-long cognitively trained in senile mice (randomly selected from ST-1 and ST-2).

**Genotype prediction based on the phenotype input, the rank.** To search for
inferences of the genes perturbations on behavioral output we have calculated the probability clustering for each genotype based only on the ranking data input, in genotype-blind manner (Fig.6A). The obtained pattern corroborates the relationship between genotypes and associated with them phenotypes with affected EF closely resembling the experimental data (Figs.1-3).

**Mouse behavioral phenotypic proximity assessment.** To calculate a phenotypic distance between the genotypes comprising a single mixed population we used the obtained ranks and plotted them against the related PVE for each behavioral parameter, generating two linear plots (Fig.6B), each representing a single contributing genotype. This let us further to calculate the phenotypic distance (using the classical Euclidean geometry) between the genotypes as the shortest distance between two parallel lines. The obtained geometrical plots are in a full agreement with the experimentally observed behaviors (Figs.1-3) but additionally pinpoint a contribution of each individual parameter sometimes located outside of the main cluster with others, e.g. PreP for the Ntng1\(^{-/-}\) (Fig.6B-1, AI-LCD), OE for the Ntng1\(^{-/-}\) (Fig.6B-2, AI-HCD), and CN for the Ntng2\(^{-/-}\) (Fig.6B-2, WM-LCD). Next, using the Ln rank and its PVE from Fig.4 as \((x,y)\) coordinates we have assessed the phenotypic proximity of the Ntng1\(^{-/-}\) and Ntng2\(^{-/-}\) mouse AI and WM phenotypes to the Ln deficit.

**Task learning (Ln) ability as an outcome of AI and MW interactions.** With the assumption that a shorter distance from the Ln coordinates to the genotype-specific linear plot generates higher likelihood that the given genotype contributes to the Ln associated behavior, we were able to build a relationship graph among the Ln, AI and WM interactions modulated by the cognitive demand (Fig.7A). The dynamics of the Ntng gene paralogs hierarchy interaction is presented on Fig.7B, calculated by the reciprocal plug-in of the rank and its PVE for one gene paralog into the linear plot for the other one (ST7).

**DISCUSSION**

**Inferring genotype-phenotype relationships for the Ntng paralogs ablation caused EF perturbations phenotypes.** The hierarchy of WM and selective attention interplay has been always a point of fierce debates (21). In the present study we look at this interaction through the prism of mouse operant conditioning learning ability, perturbated by either of Ntng gene paralogs ablation (Fig.4). Since it is known that averaging animal behavior across individual subjects (as shown in
SF1-1 and SF1-2) may smear out control variables (22), we used rank instead of classical data mean (Figs.1-3) approach and the rank variance (proportion of variance explained, PVE) per genotype, as a measure of difference (23:p.16), to assess the behavioral variability caused by the genetic variations interacting with the experimental demand. To proof any existing inferences between the behavior of Ntng\(^{-/-}\) mice and the ablated gene, we used mouse rank as a randomized dependent variable of the mixed population noting that any non-randomized variables would be only correlational (24). That is, we have presented the mouse behavioral rank distribution as a function of genotype, when one of the Ntng paralogs has been genetically inactivated. At the same time, we have tried to elaborate on the statement that the structure of genotype–phenotype map is the matter and not the variance components of the population itself (25). The open question in such genotype-phenotype interaction paradigm is to what degree a genetic variability is capacitive enough to explain the phenotypic variance and the strength of such interaction causality. More specifically, how far the behavioral (whole organism) variability (under the pressure of the growing cognitive demand) represents the neuronal (cellular) variability caused by a gene knockout exerted perturbations.

**Cognitive phenotypes of Ntng\(^{-/-}\) mice.** Vertebrate brain-specific presynaptically expressed Ntng1\(^{-/-}\) and Ntng2\(^{-/-}\) mice do not exhibit gross anatomical or developmental abnormalities (26) rendering them unique models to study brain cognitive functioning in the absence of known “house-keeping” functional distortions, avoiding gene-manipulations-exerted non-causal confounders. Noteworthy the resemblance of Ntng1\(^{-/-}\) and Ntng2\(^{-/-}\) mice behavioral phenotypes with the human schizophrenia subjects behavioral etiology (characterised by the EF control pathologies), both genes have been reportedly associated with (1,2). Two different populations of mice were used for two different behavioral paradigms to avoid the phenomena of learning transfer between the behavioral tests, and, at the same time, to check for the genotype induced phenotypic stability across the different paradigms but sharing the principal underlying component of WM testing. And indeed, slow operant conditioning learning (5-CSRTT) for Ntng2\(^{-/-}\) mice has been recorded (Fig.4A,B-2) and is explainable by the dysfunction of procedural (working) memory strongly affecting the RAM performance (Fig.2A-H-2).

**Behavior consistency assessment using rank.** We have also characterised the behavior of mice as a heterogeneously
randomized population through the assessment of rank consistency across the sessions and relative to other parameters (Figs.1-2D,H). Parameters cross-correlation coefficients ($r^2$, x axis) indicate a probability value of how much the rank of a mouse for a certain parameter contributes to the global (total) ranking comprised of all four parameters. If a mouse fails to keep its performance consistent either over the multiple sessions or a parameter, its rank is instantly occupied either by the same or by a littermate of a different genotype, and such event would be dynamically reflected in the $r^2$. But ranks changes and their permutations may not necessary have any dramatic consequences in the total rank calculations as soon the rank fluctuations are taking place within the same genotype-specific variance boundaries. But they are more reflective of a behavior inconsistency of an individual mouse reflected in the sum of the correlation variances per spatial or session ($y$ axis).

**WM deficit driven optimal strategy deprivation for the Ntng2-/- mice.** The global spatial WM deficit for the Ntng2-/- mice has been found robustly expressed across the three RAM parameters (Fig.2A-H-2) except for CN (arm choice number during the first 8 arm entries). This parameter represents a strategy development (during LCD) and its optimisation (during HCD) for the maximum reward collection efficiency, akin predictive type behavior of the likelihood of potential success. The fact that the Ntng2-/- mice outperform their wt littermates in CN (but during the LCD only, Fig.2C-2) reflects the chosen strategy (or a complete lack of any) of a pure random choice of a baited arm to visit, corroborating the global WM deficit (inability for strategic thinking) for the knockouts (evident from the other parameters) but with an opposite valence.

**Paralogs brain expression supporting the behavioral phenotypes.** The phenotypic complementarities among the Ntng1-/- and Ntng2-/- mice, associated either with the abrogated AI or WM, or both (Fig.3), are supported by the complementary brain expression pattern for these gene paralogs (Fig.5A). If Ntng1 is expressed mostly in the primary somatosensory gating areas (e.g. OB, thalamus and hypothalamus nuclei, midbrain and medulla, Fig.5A,B-1), Ntng2 dominates within the cortex (with the skewed expression saturation towards the lateral cortex), hippocampus (HPC), amygdala and claustrum, endopiriform and reticular nuclei, Fig5A-2), pointing the gene role of parsing top-down signals. If the sensory perception, as an entry point into the attentional state, is determined by the strength of the subcortical thalamus-
PFC (pre-frontal cortex) pathways (27), the reciprocal interactions between mPFC and HPC are pivotal for the WM functioning (28,29), with the HPC known to encode perceptual representations into memories through the correct attentional states (30). Complementing this, thalamocortical projections are vital for mediating sensation, perception, and consciousness (31-33). It is assumed that WM, despite its distributed nature (34), consists of an executive component spread over the frontal lobes and sensory cortices and interacted by the attention (7,35).

**Brain lamina-specific enrichment and EF control contribution by the Ntng paralogs.** The emergence of a six-layered neocortex is a known hallmark of the mammalian brain specialization devoted to the EF control (36,37). Both Ntng gene paralogs are extensively expressed and mutually sequestered among the separate layers of the cortex (Fig.5C). Ntng1 is predominantly located in layers 4/5 (Fig.5C-1), probably supporting the arrival of the bottom-up signals (38), while Ntng2 is located in the superficial layers 2/3 and deeper layers 5/6 (Fig.5C-2), reported as a source of top-down inputs in attention and WM demanding tasks (39). Besides that, Ntng2 has been also marked as a gene classifier for the granule neurons enriched in the cortex layer 6 (40). In overall, the complementary patterning of the Ntng gene paralogs expression supports the laminar-specific distribution of the attention-directed modalities.

**Evidence for the cognitive control taking over the perceptual load.** Analysing AI and WM interactions during the task learning (Fig.7A), we have revealed that HCD recruits more Ntng1 (bottom-up) expressing circuitry comparing to LCD, both by WM and AI, reciprocally replacing the preceding Ntng2 (top-down) contribution. This potentially points to an augmented peripheral sensory control by upregulating the bottom-up information stream. How to explain such intricacy? Attention exploits a conserved circuitry motif predating the neocortex emergence (41) and WM probably exapts the motor control of forward action modeling also elaborated since ancient times (42). The archaic origin of both modalities limits the fundamental brain resource and constrains information processing, forcing trade-offs among the objects of targeted attention through the top-down control and, possibly, causing a competition between the sensory inputs (43,44) by driving attention at representations in sensory areas where the latter gains entry into WM (7). A model has been proposed that selective attention control is directly linked to the executive control part of the WM system (45) corroborating the statement that attention...
and WM should no longer be regarded as two separate concepts, see (46) for references. The top-down control of primary sensory processing by higher cortical areas (through the recurrent inputs) has an essential role in sensory perception, as we have just demonstrated. The pervasive penetration of the cognitive control, supported by Ntng2, affects the sensory inputs, provided by the Ntng1 expression.

**An IQ for mice?** The EF control variance attributes to the cognitive performance variance and does not exist independently of general intelligence (47) as a critical determinant of human cognition (48). It is no wonder that, in our hands, the genes affecting WM and attention in mice are the same ones affecting IQ in humans (1) and also associated with a variety of devastating neurological disorders (2) and representing a example of antagonistic functional pleiotropy. The open challenge is to find out to what degree, using Ntng gene paralogs as benchmarks, we would be able to conclusively draw on either domain specific or domain general cognitive abilities of mice, or any other non-human animal subjects behavioral intelligence.

Conclusively, Ntng1 participates in bottom-up, and Ntng2 in top-down brain information flows support, representing an integrative complementary agreement between perception and cognition as two interacting functions of the brain.

**CONCLUSION**

The view of Brain (and Mind) as a modular (domain) system is appealing to evolutionary thinking (49) but is strongly biased towards “the prominence of neural reductionism” (22) pervasively dominating modern neuroscience. There is no strict definition of what a cognitive domain is but it can be viewed as a product of interaction between the top-down and bottom-up underlying neuronal circuits forming bidirectional feedback loops for the executively decisive and sensory information flows, possibly controlling themselves. Genes selectively expressed within such circuits via a non-overlapping pattern represent a tantalizing target to study the cognitive domain make-up and its evolution. An ancient Ntng gene duplication (>500 million years ago, preceding the Cambrian explosion) and subsequent co-evolution within the vertebrate genomes made Ntng gene paralogs to segregate within the top-down and bottom-up evolving information paths, presumably via subfunctionalisation, under the growing ecological demand (first land/water fish met) but different epistatic environment, both gene paralogs are embedded into. Perception and cognition interplay had eventually culminated in a reflectively subjective
representation of the external world, also called consciousness, and explicitly controlled by the EF. Unrevealing molecular correlates of the domain-specific cognitive abilities would help us better understand behavior, e.g. to clearly dissect it on actions (as self-generated thoughts) and responses (cue-induced actions), as a decomposable conjunction supporting the robust functioning of the Brain holistic state.

MATERIALS AND METHODS

Animals and behavioral set-ups. Animal rearing and handling experimental procedures were performed in accordance with the guidelines of RIKEN Institutional Animal Care and Experimentation Committee (ethics approval number H29-2-235(3)). Knockout animals generation and the behavioral set-ups are as described in (18) where the original behavioral datasets have been partially used by us.

Data analysis. All behavioral and transcriptional data including ranks and PVE calculations with all formulas and graphs are presented in ST1-ST5. The dynamics of the rank change for a specific parameter over the course of study and its congruence with other parameters is depicted on Figs.1-2D,H. No robustness calculations of the rank distribution pattern resistance to a sequential removal of a single behavioral subject were done; neither estimate for the minimal number of the top/bottom ranks representing the obtained pattern; it was empirically decided to be equal to the top and bottom four (Figs.1-2).

Definition of LCD and HCD. During the 5-CSRTT the cognitive demand was incremented by a shorter cue duration and longer inter-trial intervals, as specified in (18). As for the RAM, the second week of testing (sessions 8-14) was done with half-closed/half-opened doors under the gradually building cognitive demand, internally driven by the behavior optimisation strategy for the maximum likelihood of reward collection, top–down executive–attentional pressure to optimise the behavioral performance outcome, contextually similar to the operant conditioning learning (Ln) of spatial 1 of the 5-CSRTT (Fig.4).

Real-time qPCR (qRT-PCR). Primers specifically targeting beginning of each Ntng gene paralogs full-length transcripts were designed using Primer3Plus: http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi. Frozen brains RNA was isolated dissolving the mid- and frontbrain section (MFB) using RNeasy Plus Minikit (Qiagen) and the cDNA was synthesised by the QuantiTect® Reverse Transcription kit (Qiagen) using a mix of the random
hexamers and oligoT primers. cDNA synthesised from 1 ng of total RNA was used per a single qRT-PCR reaction. The lack of genomic DNA and the absence of external contaminations were confirmed by the RT-minus reactions. Neuronal-specific tubb3 transcript (β-tubulinIII) was used as an internal normaliser during the qRT-PCR co-amplifications. The $C_t$ values were collected at the threshold value of 0.4 and the arbitrary units (AU) were calculated as:

$$2^{(C_t \text{ (amplicon)} - C_t \text{ (normalizer)})} \times 10,000$$

RNA-seq cortical layers Ntng transcriptome reconstruction. The original dataset GSE27243 was generated by Belgard et al. (51) and has been reprocessed by Cufflinks and iReckon (ST5-2_cuff.xlsx and ST5-2_ireckon.zip) with the latter being used to build Ntngs expression map (ST5-2.xlsx) across the brain cortical layers (Fig.5). See Supplementary Methods for details.

Fuzzy C-Means Clustering. Represents a type of a sequential competitive learning algorithm exhibiting the stochastic approximation problem (50). Was used for the genotype predictions based on the behavioral ranks input under the genotype-blind input conditions. The details are described in the Supplementary Methods.

Statistics. Correlation coefficients ($r^2$) were obtained with Excel. One and two-way ANOVA was calculated using StatPlus (AnalystSoft Inc.). Wilcoxon rank sum test was done by Matlab (v.7.9.0 2009b) by the function ranksum.

SUPPLEMENTARY MATERIALS (SM) Contain Supplementary Methods and References, Supplementary Figures (SF1, SF2), and Supplementary Tables (ST6-7). ST1-ST5 are provided as standalone Excel files, and ST5-2_ireckon is a zip file containing compressed RNA-seq brain layers expression reads.

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COMPETING INTERESTS
Authors have no any competing interests associated with the given work.
REFERENCES

1. Prosselkov P, Hashimoto R, Polygalov D, Ohi K, Zhang Q, McHugh JT, Takeda M, and Itohara, S. (2016) Cognitive endophenotypes of modern and extinct hominins associated with NTNG gene paralogs. *Biomedical Genetics and Genomics*, 1(1): 5–13. http://doi.org/10.15761/BGG.1000103

2. Prosselkov P, Polygalov D, Zhang Q, McHugh JT and Itohara S. (2016) Cognitive domains function complementation by NTNG gene paralogs. *Biomedical Genetics and Genomics*, 1(1): 24–33. http://doi.org/10.15761/BGG.1000105

3. Mar AC, Horner AE, Nilsson SRO, Alsio J, Kent BA, Kim CH, Holmes A, Saksida LM, Bussey TJ. (2013) The touchscreen operant platform for assessing executive function in rats and mice. *Nature Protocols*, 8(10): 1985–2005. http://doi.org/10.1038/nprot.2013.123

4. Mandelman SD and Grigorenko EI (2011) "Intelligence". In *The Cambridge Handbook of Intelligence*. Edited by Sternberg RJ and Kaufmann SB. Cambridge University Press. ISBN: 052173911X.

5. Matzel LD and Kolata S. (2010) Selective attention, working memory, and animal intelligence. *Neuroscience and Biobehavioral Reviews*, 34(1): 23–30. http://doi.org/10.1016/j.neubiorev.2009.07.002

6. Baddeley A. (1992) Working Memory. *Science*, 255(5044): 556–559. http://doi.org/10.1126/science.1736359

7. Carruthers P. (2013) Evolution of working memory. *Proceedings of the National Academy of Sciences of the United States of America*, 110(S2): 10371–10378. http://doi.org/10.1073/pnas.1301195110

8. Trubutschek D, Marti S, Ojeda A, King J-R, Mi Y, Tsodyks M and Dehaene S. (2016) A theory of working memory without consciousness or sustained activity. *bioRxiv*. http://doi.org/https://doi.org/10.1101/093815
9. Soto D and Silvanto J. (2014) Reappraising the relationship between working memory and conscious awareness. *Trends in Cognitive Sciences*, 18(10): 520–525. [http://doi.org/10.1016/j.tics.2014.06.005](http://doi.org/10.1016/j.tics.2014.06.005)

10. Leong YC, Radulescu A, Daniel R, DeWoskin V and Niv Y. (2017) Dynamic Interaction between Reinforcement Learning and Attention in Multidimensional Environments. *Neuron*, 93(2): 451–463. [http://doi.org/10.1016/j.neuron.2016.12.040](http://doi.org/10.1016/j.neuron.2016.12.040)

11. Cahen A and Tacca MC. (2013) Linking perception and cognition. *Frontiers in Psychology*, 4: e144. [http://doi.org/10.3389/fpsyg.2013.00144](http://doi.org/10.3389/fpsyg.2013.00144)

12. Potter MC. (2012) Conceptual Short Term Memory in Perception and Thought. *Frontiers in Psychology*, 3: e113. [http://doi.org/10.3389/fpsyg.2012.00113](http://doi.org/10.3389/fpsyg.2012.00113)

13. Brown H, Friston K and Bestmann S. (2011) Active Inference, Attention, and Motor Preparation. *Frontiers in Psychology*, 2: e218. [http://doi.org/10.3389/fpsyg.2011.00218](http://doi.org/10.3389/fpsyg.2011.00218)

14. Mroczko-Wąsowicz A. (2016) Editorial: Perception-Cognition Interface and Cross-Modal Experiences: Insights into Unified Consciousness. *Frontiers in Psychology*, 7: e1593. [http://doi.org/10.3389/fpsyg.2016.01593](http://doi.org/10.3389/fpsyg.2016.01593)

15. Clark A. (2013) Whatever next? Predictive brains, situated agents, and the future of cognitive science. *Behavioral and Brain Sciences*, 36(3): 181–204. [http://doi.org/10.1017/s0140525x12000477](http://doi.org/10.1017/s0140525x12000477)

16. Rosenberg MD, Finn ES, Scheinost D, Constable RT and Chun MM. (2017) Characterizing Attention with Predictive Network Models. *Trends in Cognitive Sciences*, 21(4): 290–302. [http://doi.org/10.1016/j.tics.2017.01.011](http://doi.org/10.1016/j.tics.2017.01.011)

17. Firestone C and Scholl CF. (2015) Cognition does not affect perception: Evaluating the evidence for “top-down” effects. *Behavioral and Brain Sciences*, 39: e229. [http://doi.org/https://doi.org/10.1017/S0140525X15000965](http://doi.org/https://doi.org/10.1017/S0140525X15000965)

18. Zhang Q, Goto H, Akiyoshi-Nishimura S, Prosselkov P, Sano C, Matsukawa H, Yaguchi K, Nakashiba T and Itohara S. (2016) Diversification of behavior and postsynaptic
properties by netrin-G presynaptic adhesion family proteins. *Molecular Brain, 9*(1): e6.  
http://doi.org/10.1186/s13041-016-0187-5

19. Royall DR, Lauterbach EC, Cummings JL, Reeve A, Rummans TA, Kaufer DI, LaFrance CW, Coffey CE. (2002) Executive Control Function: A Review of Its Promise and Challenges for Clinical Research. *The Journal of Neuropsychiatry and Clinical Neurosciences, 14*(4): 377–405. http://doi.org/10.1176/jnp.14.4.377

20. Bari A, Dalley JW and Robbins TW (2008) The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nature Protocols, 3* (5), 759–767. http://doi.org/10.1038/nprot.2008.41

21. Abrahamse E, Majerus S, Fias W and Dijck J-P. (2015) Editorial: Turning the Mind’s Eye Inward: The Interplay Between Selective Attention and Working Memory. *Frontiers in Human Neuroscience, 9*: (e616). http://doi.org/10.3389/fnhum.2015.00616

22. Gomez-Marín A and Mainen ZF. (2016) Expanding perspectives on cognition in humans, animals, and machines. *Current Opinion in Neurobiology, 37*: 85–91. http://doi.org/10.1016/j.conb.2016.01.011

23. Laudański LM. (2013) Between Certainty and Uncertainty: Statistics and Probability in Five Units with Notes on Historical Origins and Illustrative Numerical Examples (Intelligent Systems Reference Library) Springer. ISBN-13: 978-3642256967.

24. Jazayeri M and Afraz A (2017) Navigating the Neural Space in Search of the Neural Code. *Neuron, 93*(5), 1003–1014. http://doi.org/10.1016/j.neuron.2017.02.019

25. Paixão T and Barton NH. (2016) The effect of gene interactions on the long-term response to selection. *Proceedings of the National Academy of Sciences, 113*(16): 4422–4427. http://doi.org/10.1073/pnas.1518830113

26. Nishimura-Akiyoshi S, Niimi K, Nakashiba T and Itohara S. (2007) Axonal netrin-Gs transneuronally determine lamina-specific subdendritic segments. *Proceedings of the National Academy of Sciences of the United States of America, 104*(37): 14801–14806. http://doi.org/10.1073/pnas.0706919104
27. Wimmer RD, Schmitt LI, Davidson TJ, Nakajima M, Deisseroth K, Halassa MM. (2015) Thalamic control of sensory selection in divided attention. *Nature*, 526(7575): 705–709. [http://doi.org/10.1038/nature15398](http://doi.org/10.1038/nature15398)

28. Jin J and Maren S. (2015) Prefrontal-Hippocampal Interactions in Memory and Emotion. *Frontiers in Systems Neuroscience*, 9: e170. [http://doi.org/10.3389/fnsys.2015.00170](http://doi.org/10.3389/fnsys.2015.00170)

29. Spellman T, Rigotti M, Ahmari SE, Fusi S, Gogos JA, Gordon JA. (2015) Hippocampal–prefrontal input supports spatial encoding in working memory. *Nature*, 522(7556): 309–314. [http://doi.org/10.1038/nature14445](http://doi.org/10.1038/nature14445)

30. Aly M and Turk-Browne NB. (2016) Attention promotes episodic encoding by stabilizing hippocampal representations. *Proceedings of the National Academy of Sciences of the United States of America*, 113(4): E420-429. [http://doi.org/10.1073/pnas.1518931113](http://doi.org/10.1073/pnas.1518931113)

31. Riga D, Matos MR, Glas A, Smit AB, Spijker S and Oever MCV. (2014) Optogenetic dissection of medial prefrontal cortex circuitry. *Frontiers in Systems Neuroscience*, 8: E230. [http://doi.org/10.3389/fnsys.2014.00230](http://doi.org/10.3389/fnsys.2014.00230)

32. John ER. (2002) The neurophysics of consciousness. *Brain Research Reviews*, 39(1): 1–28. [http://dx.doi.org/10.1016/S0165-0173(02)00142-X](http://dx.doi.org/10.1016/S0165-0173(02)00142-X)

33. Alitto HJ and Usrey WM. (2003) Corticothalamic feedback and sensory processing. *Current Opinion in Neurobiol.*, 13(4): 440–445. [http://10.1016/S0959-4388(03)00096-5](http://10.1016/S0959-4388(03)00096-5)

34. Christophel TB, Klink PC, Spitzer B, Roelfsema PR and Haynes J-D. (2017) The Distributed Nature of Working Memory. *Trends in Cognitive Sciences*, 21(2): 111–124. [http://doi.org/10.1016/j.tics.2016.12.007](http://doi.org/10.1016/j.tics.2016.12.007)

35. Postle BR. (2006) Working memory as an emergent property of the mind and brain. *Neuroscience*, 139(1): 23–38. [http://doi.org/10.1016/j.neuroscience.2005.06.005](http://doi.org/10.1016/j.neuroscience.2005.06.005)

36. Smaers JB, Gómez-Robles A, Parks AN and Sherwood CC. (2017) Exceptional Evolutionary Expansion of Prefrontal Cortex in Great Apes and Humans. *Current Biology*, 27(5): 714-720. [http://doi.org/10.1016/j.cub.2017.01.020](http://doi.org/10.1016/j.cub.2017.01.020)
37. Hardung S, Epple R, Jäckel Z, Eriksson D, Uran C, Senn V, Gibor L, Yiezhar O, Diester I. (2017) A Functional Gradient in the Rodent Prefrontal Cortex Supports Behavioral Inhibition. Current Biology, 27(4): 549–555. http://doi.org/10.1016/j.cub.2016.12.052

38. Nandy AS, Nassi JJ and Reynolds JH. (2017) Laminar Organization of Attentional Modulation in Macaque Visual Area V4. Neuron, 93(1): 235–246. http://doi.org/10.1016/j.neuron.2016.11.029

39. Kerkoerle T, Self MW and Roelfsema PR. (2017) Layer-specificity in the effects of attention and working memory on activity in primary visual cortex. Nature Communications, 8: e13804. http://doi.org/10.1038/ncomms13804

40. Lake BB, Ai R, Kaeser GE, Salathia NS, YungYC, Liu R, Wildberg A, Gao D, Fung H-L, Chen S, Vijayaraghavan R, Wong J, Chen A, Sheng X, Kaper F, Shen R, Ronaghi M, Fan J-B, Wang W, Chun J, Zhang, K. (2016) Neuronal subtypes and diversity revealed by single-nucleus RNA sequencing of the human brain. Science, 352(6293): 1586–1590. http://doi.org/10.1126/science.aaf1204

41. Krauzlis RJ, Bollimunta A, Arcizet F, Wang L. (2014) Attention as an effect not a cause. Trend Cog Sci, 18(9): 457–464. http://doi.org/http://dx.doi.org/10.1016/j.tics.2014.05.008

42. Jeannerod M. (2006) Motor Cognition. Oxford University Press. ISBN: 0198569653

43. Koch C and Tsuchiya N. (2007) Attention and consciousness: two distinct brain processes. Trends in Cognitive Sciences, 11(1): 16–22. http://doi.org/10.1016/j.tics.2006.10.012

44. Boxtel JJA, Tsuchiya N and Koch C. (2010) Consciousness and attention: on sufficiency and necessity. Frontiers in Psychology, 1: e217. http://doi.org/10.3389/fpsyg.2010.00217

45. Vandierendonck A. (2014) Symbiosis of executive and selective attention in working memory. Frontiers in Hum Neurosci, 8: E588. http://doi.org/10.3389/fnhum.2014.00588

46. Quak M, London RE and Talsma D. (2015) A multisensory perspective of working memory. Frontiers in Hum Neurosci, 9: E197. http://doi.org/10.3389/fnhum.2015.00197
47. Royall DR and Palmer RF. (2014) “Executive functions” cannot be distinguished from general intelligence: two variations on a single theme within a symphony of latent variance. *Frontiers in Behav Neurosci*, 8: E369. [http://doi.org/10.3389/fnbeh.2014.00369](http://doi.org/10.3389/fnbeh.2014.00369)

48. Lucenet J and Blaye A. (2014) Age-related changes in the temporal dynamics of executive control: a study in 5- and 6-year-old children. *Frontiers in Psychology*, 5: E831. [http://doi.org/10.3389/fpsyg.2014.00831](http://doi.org/10.3389/fpsyg.2014.00831)

49. Burkart JM, Schubiger MN and Schaik CP. (2016) The evolution of general intelligence. *Behavioral and Brain Sci*, Jul 28: 1–65. [http://doi.org/10.1017/S0140525X16000959](http://doi.org/10.1017/S0140525X16000959)

50. Pal NR, Bezdek JC, and Hathaway RJ. (1996) Sequential Competitive Learning and the Fuzzy c-Means Clustering Algorithms. *Neural Networks*, 9(5): 787–796.

51. Belgard TG, Marques AC, Oliver PL, Abaan HO, Sirey TM, Hoerder-Suabedissen A, García-Moreno F, Molnár Z, Margulies EH, Ponting CP. (2011) A Transcriptomic Atlas of Mouse Neocortical Layers. *Neuron*, 71(4): 605–616. [http://doi.org/10.1016/j.neuron.2011.06.039](http://doi.org/10.1016/j.neuron.2011.06.039)

52. Yaguchi K, Nishimura-Akiyoshi S, Kuroki S, Onodera T, Itohara S. (2014) Identification of transcriptional regulatory elements for Ntng1 and Ntng2 genes in mice. *Molecular Brain*, 7(1): 19. [http://doi.org/10.1186/1756-6606-7-19](http://doi.org/10.1186/1756-6606-7-19)
Figure 1. Attention and Impulsivity (AI) estimate and the effect of cognitive demand by the analysis of rank and its variance for Ntng1⁻/⁻ and Ntng2⁻/⁻ mice. A,E. Mouse ranks and rank PVE (proportion of variance explained) based on four parameter rank measures (SF1) as detailed in ST1-1 (for Ntng1⁻/⁻) and ST1-2 (for Ntng2⁻/⁻). The rank sorting was done in a genotype-independent manner treating all mice together. Ranking for each out of four parameters was done independently of other parameters with a final re-ranking of the ranks sum to generate the final rank (shown). In case of an equal sum of the ranks, the mice were given identical ranks. PVE was calculated as a square of within genotype rank variance divided on the sum of each genotype variances squares multiplied on 100%. B,F. Mouse rank distribution across one-to-four parameters as top 4 and bottom 4 performers. C,G. Genotype-specific placing among the mice. D,H. Behavioral consistency of mice across the sessions (y axis, sum of r² correlations of a single session ranks vs. final ranks for each mouse across the sessions) and behavioral parameter cross-correlations (x axis, the r² correlation of a parameter final ranking vs. final ranking for all 4 parameters). The gene ablation-specific phenotype severity can be assessed visually by matching each parameter-corresponding vertices of the obtained quadruples. p value represents a Wilcoxon rank sum test.
Figure 2. Working memory (WM) estimate and the effect of cognitive demand by the analysis of rank and its variance for Ntng1+/− and Ntng2+/− mice. A,E. Mouse ranks and rank PVE (proportion of variance explained) based on four parameter rank measures (SF2) as detailed in ST2-1 (for Ntng1+/−) and ST2-2 (for Ntng2+/−). The rank sorting was done in a genotype-independent manner treating all mice together. Ranking for each out of four parameters was done independently of other parameters with a final re-ranking of the ranks sum to generate the final rank (shown). In case of an equal sum of the ranks, the mice were given identical ranks. PVE was calculated as a square of within genotype rank variance divided on the sum of each genotype variances squares multiplied on 100%. B,F. Mice rank distribution across one-to-four parameters as top 4 and bottom 4 performers. C,G. Genotype-specific placing among the mice. D,H. Behavioral consistency of mice across the sessions (y axis, sum of r² correlations of a single session ranks vs. final ranks for each mouse across the sessions) and behavioral parameter cross-correlations (x axis, the r² correlation of a parameter final ranking vs. final ranking for all 4 parameters). The gene ablation-specific phenotype severity can be assessed visually by matching each parameter-corresponding vertexes of the obtained quadruples. p value represents a Wilcoxon rank sum test.
Figure 3. Summary of the EF behavioral phenotypes associated with either $\text{Ntng1}^{-/-}$ or $\text{Ntng2}^{-/-}$ gene paralogs ablation. See Fig.1 and Fig.2 for details.
Figure 4. Operant conditioning (5-CSRTT) Learning (Ln) by Ntng1-/- and Ntng2-/- mice. A. Learning curves for the operant conditioning learning (reward collection) over the training period (spatial 1 of 5-CSRTT) with averaged performance behavior for the days (1-7) and (8-14), middle panel, defined as low cognitive demand (LCD) and high cognitive demand (HCD) sessions, respectively. One and two-way ANOVA was used for the statistics. B. Ranks and PVE comparisons over the LCD and HCD. The rank sorting was done in a genotype-independent manner, similar to Fig.1 and Fig.2, but using only one parameter, success (Sc), see ST2-1 and ST2-2 (Ln). Rank statistics was by Wilcoxon rank sum test. C. Learning (Ln) vs. attention and impulsivity (AI) rank correlations (from Fig.1A-1,2).
Figure 5. Complementary expression and transcription of Ntng paralogs in the mouse brain. (A) In situ hybridization of Ntng1 (left) and Ntng2 (right) of the mouse brain. From Allen Brain Atlas, accession numbers are RP_050607_01_H05 and RP_050810_04_D08, respectively. The expression colors are inverted. (B) qRT-PCR of total mRNA for the Ntng paralogs in rough brain fractions of adult naïve male mice (7-8 months old) expressed in the arbitrary units (AU) normalized by tubb3 per 1 ng of total RNA input per reaction. OB = olfactory bulb; MFB = mid- and front brain; HBC = hindbrain and cerebellum. Data are presented as a mean±SEM, n=6 mice (ST5-1). (C) RNA-seq of Ntng mRNA expression within mouse brain cortical layers. The expression model was built based on GSE27243 dataset generated by Belgard et al. (51). See Supplementary Methods for the details of data processing, ST5-2, ST5-2_cuff and ST5-2_ireckon (zipped) for the transcriptome assembly. (D) Effect of reciprocal genetic background on Ntng paralogs expression level in MFB as detected by qRT-PCR (ST5-3). Senile (20-21 months old) life-long cognitively trained mice have been used (randomly selected from ST1-1 and ST1-2). One-way ANOVA was used for the statistics.
Figure 6. Behavioral phenotypic proximity assessment for the Ntng1\(^{-/-}\) and Ntng2\(^{-/-}\) genotypes and their wild type littermates by two approaches: genotype predictions by C-means fuzzy clustering (A), and by linear regression plots of genotype-specific rank PVE vs. rank (B). A. Genotype-phenotype relationship inference. C-means fuzzy clustering (Euclidean C-means) was done in a genotype-blind manner as described in SM. See ST6-1 and ST6-2 for the exact values of clustering probabilities. B. Descriptive proximity of the operant learning explained by the AI and WM phenotypes for Ntng\(^{-/-}\) paralogs mice. Distance between the genotypes (d, dashed line), presented geometrically by the linear equation, was calculated as d=|c\(_2\)-c\(_1\)|, where ax+by+c=0 (Euclidean geometry). Distance from the learning coordinates (Ln) to the genotype-describing line was calculated as d=|c\(_1\)-y\(_1\)+ax\(_1\)|PVE values (y coordinates) are from Fig.1A,E (AI); Fig.2A,E (WM), and Fig.4B (Ln). Data for the Ntng1\(^{-/-}\)/wt population (RAM) are likely to incorporate 7.69% error since they were not normalised to the total number of animals as for the other populations (n=26 for the given case vs. n=24 mice for all other three populations).
Figure 7. *Ntng* paralogs interaction as a molecular correlate of AI and WM modalities during learning. A. AI and WM interactions modulated by the cognitive demand during the operant conditioning learning (Ln), complementary contributed by the differentially expressed *Ntng* paralogs (see Fig.5 and (52)). B. Dynamicity of *Ntng* paralogs hierarchy interactions under the LCD and HCD contributing to AI and WM. Obtained by reciprocal plug-in of rank and PVE for one *Ntng*-associated phenotype into the linear equation for another paralog-associated phenotype. Each arrow base width (the scale bar is shown) is expressed in AU and corresponds to (1/d*100) value from Fig.6B (for Ln=X), see ST7. Out of scale arrows (AI-*Ntng*1-LCD and AI-*Ntng*2-HCD) are not shown.
Supplementary Figure 1 (SF1). Attention and Impulsivity (AI) estimate for Ntng1+/− and Ntng2+/− mice by comparison of averaged behavior for 5-CSRTT. A. Exon-intron composition of mouse Ntng1 and Ntng2 genes with the removed part for each gene paralog dash-outlined. See (18) for the details of each gene construct design. (B-E, outmost left and right) Mouse performance over the spatial (2-13) presented by four behavioral parameters. The dashed line separating spatial (2-7) and (8-13) indicates the data split on the low cognitive demand (LCD) and high cognitive demand (HCD) sessions. (B-E, middle left and right) The same data as above but averaged per LCD and HCD sessions. The data for each parameter are presented as a mean±SEM (standard error of mean) and fully provided in ST1-1 (for Ntng1+/−) and ST1-2 (for Ntng2+/−). Two-way and one-way ANOVA were used for statistics.
Supplementary Figure 2 (SF2). Working memory (WM) estimate for Ntng1<sup>-/-</sup> and Ntng2<sup>-/-</sup> mice by comparison of averaged behavior for RAM. A, Exon-intron composition of mouse Ntng1 and Ntng2 genes with the removed part for each gene paralog dash-outlined. See (18) for the details of each gene construct design. (B-E, outmost left and right) Mouse performance over the days (1-14) as presented by four behavioral parameters. The dashed line separating genes with the removed part for each gene domain. Choice number (CN)

Day (1-7) LCD Day (8-14) HCD

Distance travelled (DT)

Day (1-7) LCD Day (8-14) HCD

Total arm choices (TAC)

Day (1-7) LCD Day (8-14) HCD

A-1

Ntng1 KO: B-1

Day (1-7) LCD

Ntng1<sup>-/-</sup> (n=13)

LCD

HCD

Day (8-14)

Distance travelled

Choice number

Total arm choices

B-2

Ntng2 KO: C-1

Day (1-7) LCD

Day (8-14) HCD

Distance travelled

Choice number

Total arm choices

C-2

D-1

E-1

D-2

E-2

Supplementary Figure 2 (SF2). Working memory (WM) estimate for Ntng1<sup>-/-</sup> and Ntng2<sup>-/-</sup> mice by comparison of averaged behavior for RAM. A, Exon-intron composition of mouse Ntng1 and Ntng2 genes with the removed part for each gene paralog dash-outlined. See (18) for the details of each gene construct design. (B-E, outmost left and right) Mouse performance over the days (1-14) as presented by four behavioral parameters. The dashed line separating days (1-7) and (8-14) indicates the data split on the low cognitive demand (LCD) and high cognitive demand (HCD) sessions. (B-E, middle left and right) The same data as above but averaged per LCD and HCD sessions. The data for each parameter are presented as a mean±SEM (standard error of mean) and fully provided in ST2-1 (for Ntng1<sup>-/-</sup>) and ST2-2 (for Ntng2<sup>-/-</sup>). Two-way and one-way ANOVA were used for statistics.
Supplementary Table 6-2 (ST6-2). Calculated distance values for the males performance for the 5-choice serial reaction time task (5-CSRTT).

| MouseID | 10363 | 10408 | 10339 | 10338 |
|---------|-------|-------|-------|-------|
|         | 0.621849387 | 0.842990662 | 0.924750459 | 0.703899189 |

For Fig. 7A, the plugging order was reciprocally reversed (shown under the table). See ST 1, ST 2, ST 3 and ST 4 for the distance values calculations and the values describing interactions (in brackets).
SUPPLEMENTARY MATERIALS (SM)
SUPPLEMENTARY METHODS

RNA-seq data analysis of GSE27243 dataset (Fig. 5C)

FASTQ files containing raw reads were downloaded from [1] originally produced by [2]. The whole dataset consisted of two groups (dorsal and lateral cortex areas), and six samples (A-F) in each group. Each sample consisted of 2~3 pairs of FASTQ files - replicates taken from one of the six layers of dorsal and lateral part of cortex. Each replicate contained approximately 110 million of 50 base pair length paired end reads, where each member of the pair is stored in a separated FASTQ file. Raw reads were filtered by using the set of scripts provided by McDonald Lab [3]. Filtering was necessary for obtaining line-by-line matched set of reads in two files comprising each replicate. Filtered FASTQ files were aligned to a mouse reference genome (mm10) by using TopHat v.2.0.8b [4] software which calls for BowTie v.2.1.0 [5], using the following command line options:

```
--library-type fr-unstranded --microexon-search --no-coverage-search -G genes.gtf
```

Two different approaches for TopHat alignment were used. In the first approach each replicate (each pair of FASTQ files) was aligned to the reference genome individually, producing single output bam file per the replicate. Second approach employed TopHat’s ability to simultaneously align replicates to the same sample. This approach yielded a single output bam file per sample (12 files in total).

Reference genome sequence, genome indexes and GTF-file containing gene annotations (version mm10 of UCSC build) adapted for use with TopHat/Cufflinks software suite were downloaded from “iGenome” project's web site [6]. TopHat's output was passed then to Cufflinks (v.2.1.1 [ref]) using the next command line:

```
$ cufflinks --library-type fr-unstranded -g genes.gtf DCTX_layerA_accepted_hits.bam
```

Transcript models generated by the first stage of Cufflinks processing (“transcripts.gtf” files from each sample) were combined into one GTF file by using Cuffcompare software – a part of the Cufflinks suite with default options. Resulting file was then used as a reference during the second stage of Cufflinks processing, when it runs in quantification-only mode. Output files generated by Cufflinks (3 files per run, containing FPKM values for genes, isoforms and transcripts expression) were processed with custom Python script. The script combined Cufflinks results from all samples into a single file with the sample labels added and limited the output information to the following genome loci: chr3:109,500,000-
110,500,000 (for Ntng1) and chr2:2,900,0000-2,950,0000 (for Ntng2). The resulted file (ST5-2_cuff.xlsx) was then inspected and analysed manually for the presence of multiple either non-annotated transcripts, or transcripts with varying boundaries or retained after the splicing upstream or downstream introns. However the data presented on Fig.5C represent a sum of all assembled transcripts’ FPKM generated by iReckon [7] allowing for novel (non-annotated) isoforms (Prosselkov et al, unpublished) assembly and quantification (ST5-2_ireckon.zip), summarized in ST5-2.xlsx. In contrast to Cufflinks, iReckon is able to perform the reads alignments to the specified chromosomes coordinates.

**C-Means fuzzy clustering (Fig.6A)**

The genotypic distance between phenotypes (Fig.6) was assessed by fuzzy C-Means clustering algorithm [8]. For any given number of mice (n=24 or 26, in rows) and their behavioral performances (mouse rank in one of four types of parameters, m=4, in columns) the algorithm calculates cluster number (1 or 2) to which any particular mouse is likely to belong to and probability of the membership. Custom script written in R programming language (package “e1071”, function “cmeans”) was used for the actual calculation. Parameters for the cmeans function are as follows:

- Number of clusters, or initial values for cluster centers: centers=2
- Maximum number of iterations: iter.max=10,000
- Distance measure: dist=”euclidean”
- Calculation method: method=”cmeans”
- Degree of fuzzification: m=2

**SUPPLEMENTARY REFERENCES**

[1] [http://wwwfgu.anat.ox.ac.uk/~grantb/mouse_layers/](http://wwwfgu.anat.ox.ac.uk/~grantb/mouse_layers/)
[2] Belgard et al. (2011) Neuron, 71:605. doi:10.1016/j.neuron.2011.06.039
[3] [http://www.mcdonaldlab.biology.gatech.edu/bioinformatics.htm](http://www.mcdonaldlab.biology.gatech.edu/bioinformatics.htm)
[4] Trapnell et al. (2013) Genome Biology, 14: R36. doi:10.1186/gb-2013-14-4-r36
[5] Langmead et al. (2012) Nat Methods, 9: 357. doi:10.1038/nmeth.1923
[6] [http://cufflinks.cbcb.umd.edu/igenomes.html](http://cufflinks.cbcb.umd.edu/igenomes.html)
[7] Mezlini et al. (2013) Genome Res, 23: 519. doi:10.1101/gr.142232.112
[8] Nikhil et al. (1996) Neural Networks, 9: 787. doi:10.1016/0893-6080(95)00094-1