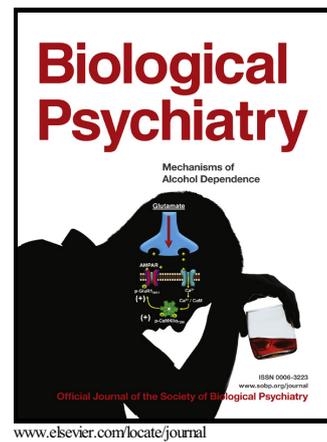


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Left-right asymmetry of maturation rates in human embryonic neural development

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Abstract

Background

Left-right asymmetry is a fundamental organizing feature of the human brain, and neuro-psychiatric disorders such as schizophrenia sometimes involve alterations of brain asymmetry. As early as 8 weeks post conception, the majority of human fetuses move their right arms more than their left arms, but because nerve fibre tracts are still descending from the forebrain at this stage, spinal-muscular asymmetries are likely to play an important developmental role.

Methods

We used RNA sequencing to measure gene expression levels in the left and right spinal cords, and left and right hindbrains, of 18 post-mortem human embryos aged 4-8 weeks post conception. Genes showing embryonic lateralization were tested for an enrichment of signals in genome-wide association data for schizophrenia.

Results

The left side of the embryonic spinal cord was found to mature faster than the right side. Both sides transitioned from transcriptional profiles associated with cell division and proliferation at earlier stages, to neuronal differentiation and function at later stages, but the two sides were not in synchrony ($p = 2.2 \text{ E-}161$). The hindbrain showed a left-right mirrored pattern compared to the spinal cord, consistent with the well-known crossing over of function between these two structures. Genes that showed lateralization in the embryonic spinal cord were enriched for association signals with schizophrenia ($p = 4.3 \text{ E-}05$).

Conclusions

These are the earliest-stage left-right differences of human neural development ever reported. Disruption of the lateralised developmental programme may play a role in the genetic susceptibility to schizophrenia.

Introduction

Many human cognitive processes are partially lateralized towards either the left or right sides of the brain(1). Identifying genes involved in brain asymmetry is important because it can shed light on developmental processes that fine-tune neural circuits for their high-level functions(2, 3), and because various psychiatric and cognitive disorders can involve alterations of brain asymmetries(4, 5). At 8 weeks post conception, or roughly embryonic Carnegie Stage (CS) 23, 85% of fetuses move their right arms more than their left arms, a proportion strikingly similar to the adult rate of right-handedness(6). Furthermore, fetal hand motor asymmetry at 13 weeks post conception predicts handedness at age twelve years(7). Left–right anatomical differences of the cerebral hemispheres have been detected by ultrasound scanning as early as 18 weeks post conception(8). In infants, the right hemisphere has been reported to develop functionally and anatomically earlier than the left in various respects, including that left-hemisphere language-related brain regions develop more slowly than their contralateral counterparts (9-11). These observations indicate a broadly lateralized program of motor and cognitive development that begins *in utero* in our species, and suggest that a general model can involve different rates of maturation on the two sides of the central nervous system.

Gene expression profiling from the left and right cerebral cortex in post-mortem human fetuses has revealed little, or only tentative, evidence for laterality of mRNA expression *in utero*(12-

15). One study, though, suggested that in 12-17-week-old fetuses the right cerebral cortex matures faster than the left, in terms of gene expression profiles (16). However, the corticospinal tract, which descends from the motor and somatosensory cerebral cortices to the spinal cord, only reaches the point of left-right crossover in the inferior hindbrain at 8 weeks post conception - the age of observed lateralisation of arm movements-, but does not yet extend into the spinal cord(17). Thus, lateralization of motor behavior at this stage is unlikely to reflect 'top-down' asymmetry projected from the cerebral cortex. Instead, a 'bottom-up' model is that molecular asymmetries already arise in the developing spinal cord prior to 8 weeks post conception. Developing vertebrate spinal cords show spontaneous electrophysiological activity which is affected by the formation of chemical synaptic networks(18), such that any left-right molecular differences might be reflected in patterns of spontaneous limb movements. In this study, we used RNA sequencing to measure gene expression profiles in the left and right spinal cords and hindbrains of human embryos aged between 4 to 8 weeks post-conception, in order to gain new insights into left-right differentiation of the human central nervous system within the earliest period of development yet analyzed in this way.

People with schizophrenia have an elevated rate of left-handedness(19), and have shown alterations of various aspects of brain asymmetry in some, but not all, studies(20-26). Recent genome-wide association analysis in tens of thousands of participants has implicated individual genes and genetic networks involved in embryonic and fetal brain development in susceptibility to schizophrenia, bipolar disorder and depression(27-29). Studies of patient-derived neural progenitor cells have also indicated disruptions of early developmental processes in schizophrenia(30). We therefore hypothesized that genes showing early developmental lateralization in the spinal cord or hindbrain might also show an enrichment of association signals within GWAS results for schizophrenia. Although spinal cord abnormalities are not a known feature of schizophrenia, if subtle embryonic asymmetries within the spinal cord are important for patterning broader asymmetries of the brain later in development, then it is possible that disruptions of this early program might have consequences for disorder susceptibility.

Methods and Materials

Differential expression analysis

RNA sequencing was performed separately for left and right spinal cords, and left and right hindbrains, dissected from eighteen post mortem human embryos aged between 4 and 8 weeks post conception at the times of their terminations (Carnegie stages 13 to 23; Supplementary table S1), which had been donated to the UK's Human Developmental Biology Resource following voluntary medical abortions. Details of sample collection, dissection, preparation, RNA sequencing, and data quality control can be found in the supplementary information. In Rstudio (v99.9.9), gene mRNA expression data were normalized and transformed into log₂ cpm (counts per million). Genes were filtered to retain only those for which at least three libraries had at least five reads per gene, separately for each brain structure. Linear models with observational-level weights were fitted to obtain average expression values for each gene on each side (left or right), separately for spinal cord and hindbrain, and moderated t-statistics were used to assess differential expression between sides using the Bioconductor limma package (version 3.22.7) with *voom*(31). This design allowed paired analysis of the left and the right sample from the same embryo for each tissue. In multidimensional scaling (MDS) analysis of the gene expression data, no dimensions were found to relate to collection batch or time from clinic until freezing, and the number of pairs of samples was relatively low to support the inclusion of covariate effects (beyond the individual-level effect that was already accounted for by paired-sample testing). Therefore we did not consider these factors in the differential expression analysis.

Many genes showed a pattern of increasing or decreasing expression with age, as assessed across the different embryos of varying ages. A linear slope was fitted through bilateral expression as log₂ (cpm) on CS for each gene. The slope coefficient of each gene was plotted against the left-right

differential expression t-value, and the Pearson correlation between the slope coefficients and t-values was computed (SPSS v20.0.0.1).

Gene ontology enrichment

The Gene Ontology (GO) assigns genes to around 4000 gene-sets representing Biological Processes, in a loosely hierarchical structure(32). To search for Biological Process gene-sets that were enriched on the left- or right sides of each structure, we used GSEA, the Gene-Set Enrichment Analysis tool from the Broad Institute, build 00044 (January 2016), combined with Molecular Signatures Database (MsigDB) gene-sets (c5.bp.5.1; <http://www.go2msig.org/cgi-bin/prebuilt.cgi>), curated genes updated April 2015 (33, 34). Analysis was performed using the 'preranked' mode, provided in the software. For this analysis genes were ranked by lateralization t-value from positive (higher expression on the right) to negative (higher expression on the left) t-values. This way positive enrichment in the GSEA results would indicate that members of a particular gene-set, on average, show relatively higher expression on the right side of the tissue, and negative enrichment would indicate relatively higher expression on the left side. GSEA reports a family-wise error rate (FWER) which is a conservative measure to correct for multiple testing³⁶. To identify gene-sets that were lateralised in both spinal cord and hindbrain, we selected gene-sets that had absolute NES (normalised enrichment score, as calculated by GSEA software) exceeding 2.0 in both tissues. To cluster the GO gene-sets into larger units, in order to indicate the broad types of processes implicated, REVIGO (<http://revigo.irb.hr/>) was used(35). See Supplementary text for more details.

Transcription factor target enrichment

To identify Transcription Factors (TFs) that play important roles in lateralisation of spinal cord and hindbrain, we performed enrichment analysis for TF targets in our left-right differential expression data. Gene-sets, provided by MsigDB, were characterised by having a particular target motif near their transcription start site. Gene-set collection c3.tft.5.0 from MsigDB, updated June 2015, contained 615 gene-sets. Enrichment analysis was performed as described for Gene Ontology gene-sets (above). See Supplementary text for more details.

Overlap with schizophrenia GWAS results

We downloaded the publicly available association statistics from the Psychiatric Genomics Consortium (PGC) schizophrenia genome-wide association study of up to 36,989 cases and 113,075 controls (<https://www.med.unc.edu/pgc/results-and-downloads>; SCZ2)(29). We tested the hypothesis that genes which showed lateralised expression in the embryonic spinal cord or hindbrain were enriched for association signals with schizophrenia, by running the PASCAL (Pathway scoring algorithm) software (36). For this, we defined one gene-set comprising the 681 genes that showed lateralisation (towards either the right or the left) in the embryonic spinal cord with an absolute t-value > 2.0 for the left-right comparison, and another gene-set for the 816 genes that showed lateralization with absolute t-value > 2.0 in the embryonic hindbrain. Further gene sets, also defined from the transcriptomic data, were tested in order to better understand the factors driving the results obtained with the first two sets (see Results). Briefly: PASCAL uses per-SNP association statistics, as derived from a GWAS study, to compute one association score for every known gene. Information on linkage disequilibrium between SNPs is included in this step. The score for a given gene therefore indicates how strongly genetic variation within, or in linkage disequilibrium with, that gene is associated with schizophrenia. These GWAS-based gene-scores are subsequently used to compute gene-set enrichment within defined sets of genes (here as defined from the transcriptomic data). The enrichment analysis tests whether the genes in a given set have, on average, higher GWAS-based gene-scores than the other genes in the genome. No cut-off was made on the GWAS-based gene scores, so that for all genes the degree of association with schizophrenia was taken into account. We used the option 'max' in PASCAL for evaluating the gene-scores, combined with the rank-based option 'chi' for evaluating gene-set enrichment, and otherwise default settings.

Data accessibility

RNA sequencing data have been deposited in the Gene Expression Omnibus under accession number GSE84231.

Results

Asymmetrical gene expression

We carried out transcriptomic analysis by RNA sequencing of the left and right spinal cords, and left and right hindbrains, of eighteen normally-developing human embryos aged from 4 to 8 weeks post conception at the times of their terminations (CS13-23), which had been voluntarily donated by women after they chose to end their pregnancies (Supplementary Table S1). The sequenced libraries had a median number of 47.6 E06 (s.d. 11.9 E06) clean reads. The numbers of genes included in the analyses after filtering were 13,852 genes in spinal cord and 13,730 genes in hindbrain. Average gene expression, as log₂ cpm (counts per million) was 4.86, s.d. 2.05 for the spinal cord, and 4.87, s.d. 2.04 for the hindbrain. In multidimensional scaling analysis of the transcriptomic data (Supplementary Figure S1), the first dimension reflected embryonic age, while the second dimension reflected the hindbrain-spinal cord distinction, and the paired left-right samples from a given embryo mapped closely together.

Left-right differential expression per gene was then assessed, separately within each structure, using a paired sample design and by combining data from all embryos. Overall, lateralization of gene expression was subtle, with no individual genes showing significant left-right differences in expression levels at a false discovery rate <0.01, after correcting over all genes tested. MDS analysis separately within spinal cord and hindbrain also did not identify any of the top ten dimensions as being associated with the left-right distinction (not shown). Many genes showed dramatic, bilateral decreases in spinal cord expression from CS13 to CS23, while other genes showed strong, bilateral increases in expression over this period (Fig. 1E, F for illustrative examples). We assigned every gene a linear slope for its bilateral expression change in the spinal cord from CS13 to CS23, and found a correlation ($r=-0.471$, $p = 2.2E-161$) between this slope and the left-right lateralization of genes (Fig. 1C). Genes whose spinal cord expression levels decreased bilaterally

from CS13 to CS23 also tended to show lower expression on the left side. While genes whose spinal cord expression levels increased bilaterally from CS13 to CS23 tended to show higher expression on the left side. Therefore the global developmental changes in the transcriptome over the embryonic period CS13-CS23, which both sides of the spinal cord followed, were at an advanced state in the left spinal cord compared to the right spinal cord (Fig. 1A).

In Gene Ontology analysis of spinal cord data, the left side showed, relative to the right side, higher expression of genes involved in glutamate receptor signalling and neurotransmitter transport (Fig. 2, Supplementary Figure S2A, Supplementary Table S2), which also tended to be genes whose average expression increased bilaterally from CS13 to CS23, consistent with an increase in neuronal differentiation and functional maturation over this period, which was advanced on the left. In contrast, the left spinal cord showed lower expression than the right for genes involved in diverse intracellular processes including mRNA metabolism, DNA strand elongation, chromosome segregation and protein translation (Fig. 2, Supplementary Figure S2B, Supplementary Table S2), and these gene-sets decreased bilaterally from CS13 to CS23, such that again the left side was relatively advanced. These transcriptional profiles indicate a right-side delay, in the spinal cord, of phasing out cellular division/proliferation processes and transitioning to neuronal differentiation and functional maturation (Fig. 1A). Further quantification of the left-right asynchrony, with respect to the most lateralized gene sets, can be found in Supplementary Figure S4.

The hindbrain showed essentially a left-right mirrored, but weaker, pattern compared to the spinal cord, as regards the relation of lateralization to bilateral changes in gene expression levels from CS13 to CS23 (Figures 1-3, Supplementary Figure S2C,D, Supplementary Figure S3, Supplementary Tables S3, S5). The correlation between lateralization and bilateral developmental change in expression, as seen in figures 1C and 1D, was significantly weaker in the hindbrain than the spinal cord (Fisher r-to-z transformation to compare correlations,; $z=20.23$, $p = 5.33 \text{ E-}91$). Per-gene comparisons of hindbrain and spinal cord laterality and developmental changes in expression can be found in Supplementary Figures S5 and S6.

Transcription factor target genes

Transcription factors (TFs) are proteins which regulate the expression levels of their target genes, through binding to specific DNA sequence motifs which are located in the vicinity of their target genes in the genome(9, 11). We used binding motif data for each of 615 motifs to define sets of TF target genes. We found significant enrichment, after multiple testing correction, for targets of the TF NRSF (Neuron-Restrictive Silencer Factor; coded by the gene *REST*) among genes showing leftward lateralization in the spinal cord, with a mirror effect in hindbrain (Fig 3A,B). In addition, targets for *SF1* and *TCF3* were significantly enriched among left-lateralized genes. Similarly, targets of the TF *E2F1* (E2F transcription factor 1) were significantly enriched among genes showing rightward lateralization in the spinal cord (and leftward in the hindbrain) (Fig. 3C,D), as well as targets of *TFDP1* (Supplementary Tables S4 and S5). NRSF is a repressor of neuronal gene transcription in non-neuronal cells(37, 38). Consistent with this role, its own mRNA lateralization was contralateral to that of its target set, and it decreased bilaterally in expression from CS13 to CS23 while its targets increased (Fig. 1, Fig. 3A,B). In contrast, E2F1, which had targets expressed more highly on the right, is one of the most important proteins for entry into the S phase and progression of the cell cycle, and is a promoter of proliferation(39, 40). Again our data matched the known biology: *E2F1* lateralization was ipsilateral to its targets, whose expression decreased bilaterally in concert with it from CS13 to CS23 (Fig. 3C,D). Thus we identified individual TFs which are likely to exert developmental effects on lateralized maturation rates in the spinal cord and hindbrain, again with respect to transitioning from proliferation to neuronal differentiation, in an analysis that was independent of gene ontology analysis. More details can be found in the supplementary material.

Association with schizophrenia

Within the PGC Schizophrenia phase 2 genome-wide association results, genes which were lateralised in the embryonic spinal cord with absolute t-value > 2.0 , i.e. the most strongly left and right lateralised genes, were significantly enriched for SNPs showing association with schizophrenia ($p = 4.3 \text{ E-}05$), while no such signal was seen for genes which were lateralized with absolute t-value > 2.0 in the embryonic hindbrain ($p = 0.087$). We further tested a set of 758 genes whose developmental change in expression was > 0.3 (\log_2 (cpm)/Carnegie Stage), i.e. those genes whose expression increased most strongly from CS13 to CS23, which also tended to be more highly expressed on the left (Figure 1C), and again we found an enrichment of association signals with schizophrenia, $p = 3.3\text{E-}06$. No such enrichment was found ($p = 0.994$) among the 647 genes which decreased most strongly in their expression bilaterally from CS13 to CS23 i.e. genes with \log_2 (cpm)/Carnegie Stage < -0.3 , and which also tended to be expressed at lower levels on the left (Figure 1C). This indicates that the enrichment of association signals with schizophrenia was largely driven by genes of mature neuronal function which strongly increase bilaterally in expression during nervous system development, and also tend to be more highly expressed in the left embryonic spinal cord. However, we also tested the set of 149 left-lateralized genes ($t < -2.0$) which showed \log_2 (cpm)/Carnegie Stage < 0.2 , i.e. those with only weakly increasing, or else decreasing, bilateral expression levels from CS13 to CS23, and again found an enrichment of association signals with schizophrenia ($p = 0.017$), so that it was not only genes showing a marked expression increase with developmental age that are associated with schizophrenia.

Discussion

In this study we investigated whether the human embryonic spinal cord and hindbrain would show left-right transcriptional differences, consistent with a 'bottom-up' model in which inferior regions of the CNS may lead its lateralized development. This was motivated by observations from *in*

utero ultrasound scanning, which have indicated that asymmetries of motor behaviour precede the formation of the corticospinal tract(41), and therefore possible 'top-down' influences from the cerebral cortex.

We found that, from 4 to 8 weeks post conception, i.e. CS13 to CS23, both sides of the embryonic spinal cord transition from transcriptional profiles associated with cell division and proliferation at earlier stages, to neuronal differentiation and function at later stages, but the two sides are not in synchrony with each other. The left spinal cord follows a slightly accelerated developmental trajectory compared to the right. The hindbrain showed a left-right mirrored, but weaker, pattern compared to the spinal cord. These are the earliest left-right differences in human neural development to have ever been reported.

The transcriptional mirroring between spinal cord and hindbrain is broadly consistent with the well-established crossing over (decussation) of structure and function in the inferior hindbrain(42). As the corticospinal tract only arrives at the inferior hindbrain by CS23(17), any molecular asymmetry in descending axons would not be expected to affect the spinal cord directly, although it may affect the hindbrain by CS23. Therefore, in the spinal cord particularly, transcriptional asymmetry from CS13 to CS23 is likely to originate independently of 'top down' forebrain influences. Studies earlier than CS13 could pinpoint the developmental triggers for this lateralized program, although such samples are not usually available through voluntary termination of pregnancy. Upstream developmental triggers might occur within the precursors of the spinal cord itself, or within peripheral neural or even non-neural tissue, and may involve both genetic and environmental stimuli, such as have been found in fish and bird species (43, 44).

The early lateralization in human spinal cord and hindbrain that we have identified may influence later asymmetries of the cerebral cortex(16), possibly via the formation of asymmetrical spinal-cortical circuitry and feedback. The slower transcriptional maturation that we observed of right spinal cord, and left hindbrain, is consistent with previously reported left-hemisphere delayed development of the infant forebrain(17) and foetal cerebral cortex(16), at least with respect to the

corticospinal tract and ascending proprioceptor and touch pathways, which cross over in the hindbrain but run ipsilaterally from forebrain to hindbrain. Our data therefore support a general model in which left-right differences of maturational rates are fundamental to creating structural and functional lateralization of the human brain, as was discussed by Corballis & Morgan in 1978(45), although those authors envisioned a generally left-hemisphere maturational advantage. In terms of anatomy, the earliest developmental asymmetry of the human brain yet reported is a left-greater-than-right volumetric difference of the choroid plexuses at roughly 9 weeks post conception(46). In fact, the volume of a structure does not necessarily bear a direct relation to its maturation rate, e.g. a prolonged developmental phase of cell proliferation prior to differentiation could result in a relatively larger structure, which would be due to a kind of early developmental delay. This consideration might help reconcile some of the observations by Corballis & Morgan(45).

We found that genes showing embryonic CNS lateralization, in the spinal cord, contain variants in the population which contribute to the polygenic liability to schizophrenia, more often than the rest of the genes in the genome. Genes whose expression levels increased during the embryonic period, and also tended to be expressed slightly more strongly in the left spinal cord, played the largest role in this enrichment for schizophrenia association signals. These same genes tend to have mature neuronal functions, such as being involved in glutamatergic neurotransmission, and it is therefore possible that variants in these genes associate with schizophrenia due to later functions in the brain, and not causally via alterations of an embryonic program for CNS laterality. However, we also found that a set of left-lateralized genes in the spinal cord which show relatively low developmental increases, and even decreases, also showed enrichment for association signals with schizophrenia, suggesting that laterality itself may be involved in disorder susceptibility. Given the strong relation between lateralization and developmental change of expression, it is not possible with our data to fully disentangle these aspects.

Lateralization of CNS structure and function is found across the vertebrates(47), probably reflecting the general, dual advantages of efficiency of function within each of the two sides, and

division of labour between them. Some developmental mechanisms underlying human CNS lateralization are therefore likely to be evolutionarily ancient, even if other aspects of the elaboration of asymmetry, such as for left-hemisphere language in the typical human brain(42), are evolutionarily modern. In mice, different spinal interneuron populations derive from separate progenitor domains which have distinct transcriptional profiles, and the timing of neurogenesis also affects spinal cord neuronal diversification(42). Such high-resolution spatial and temporal analysis of human embryonic tissue is limited for practical and ethical reasons. Our data may point to a conserved set of asymmetry mechanisms which can be analysed further in animals (47). However, the extent to which these mechanisms operate in different species could vary widely. The human population-level bias of roughly 90% towards right-handedness is among the strongest known for any mammal, although this may relate to the human demands of very fine motor control, and is likely to be reinforced and amplified as usage patterns diverge between the two limbs throughout development(48). Interestingly, while our data point to a lateralized developmental program that is under strong genetic control in the typically lateralized human, it should be noted that left-handedness and other aspects of non-typical brain lateralization have been shown to be largely influenced by non-genetic factors (49, 50).

In summary: Gene expression analysis of left and right spinal cord and hindbrain in human embryos revealed differences in transcriptional maturation rates. The asymmetrical patterns within spinal cord and hindbrain were largely mirrored with respect to each other, which suggests that lateralized transcription is linked to descending and ascending neuronal tracts which cross over in the inferior hindbrain. Since descending tracts only arrive at the inferior hindbrain by CS23, embryonic lateralisation within the spinal cord is not likely to be directed from the forebrain. Variants in genes which showed leftward lateralisation in the embryonic central nervous system are involved in susceptibility to schizophrenia.

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Author Contributions

C.F. conceived the study and designed the experiments. C.G.F.de.K and G.K. analysed the data. S.L. led the dissection of the samples. J.J. and G.C. performed preliminary data analysis. S.E.F. contributed to data interpretation. C.F. and C.G.F.de.K wrote the paper.

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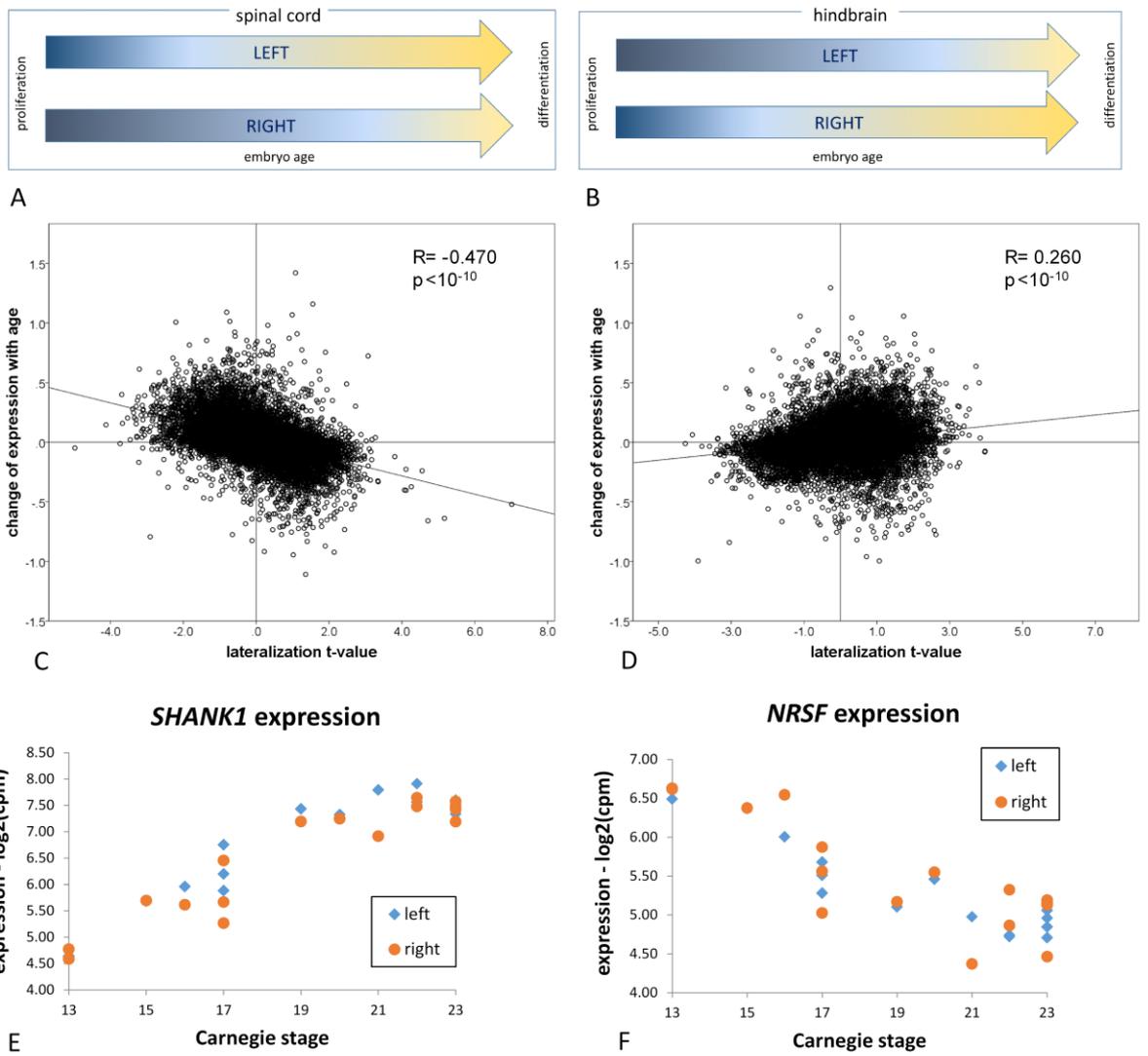
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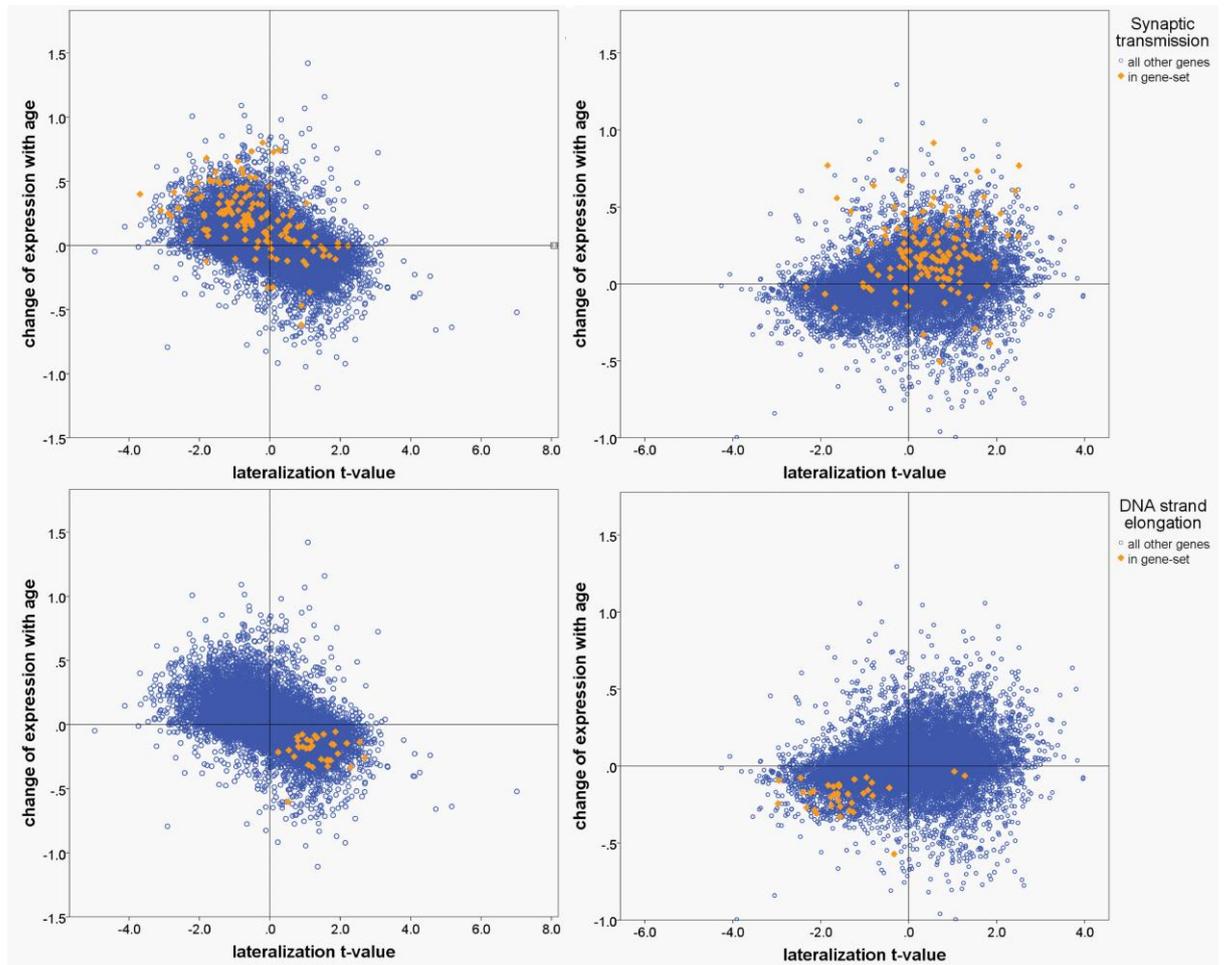
Figure legends

Figure 1. Developmental changes in gene expression from CS13 to CS23. **a-b**, Schematic figures to indicate left-right differential rates of maturation in **(a)** spinal cord and **(b)** hindbrain. Both sides were found to transition from transcriptional profiles of earlier proliferation to later neuronal differentiation, but not synchronously with each other. **c-d**, The relation of per-gene bilateral changes in gene expression (Y-axis: slope of bilateral change) to differential left-right expression (X-axis: lateralization t-value), within **(c)** spinal cord and **(d)** hindbrain. R = Pearson's correlation; P: p-value for correlation. In spinal cord, genes showing higher expression on the left side (negative lateralization t) tend also to increase in expression with age (positive bilateral change), whereas those with lower expression in the left spinal cord also tend to decrease with age. In hindbrain, the patterns are left-right mirrored compared to spinal cord. **e-f**, Illustrative examples of individual genes showing large changes of expression levels from CS13-CS23 in spinal cord.

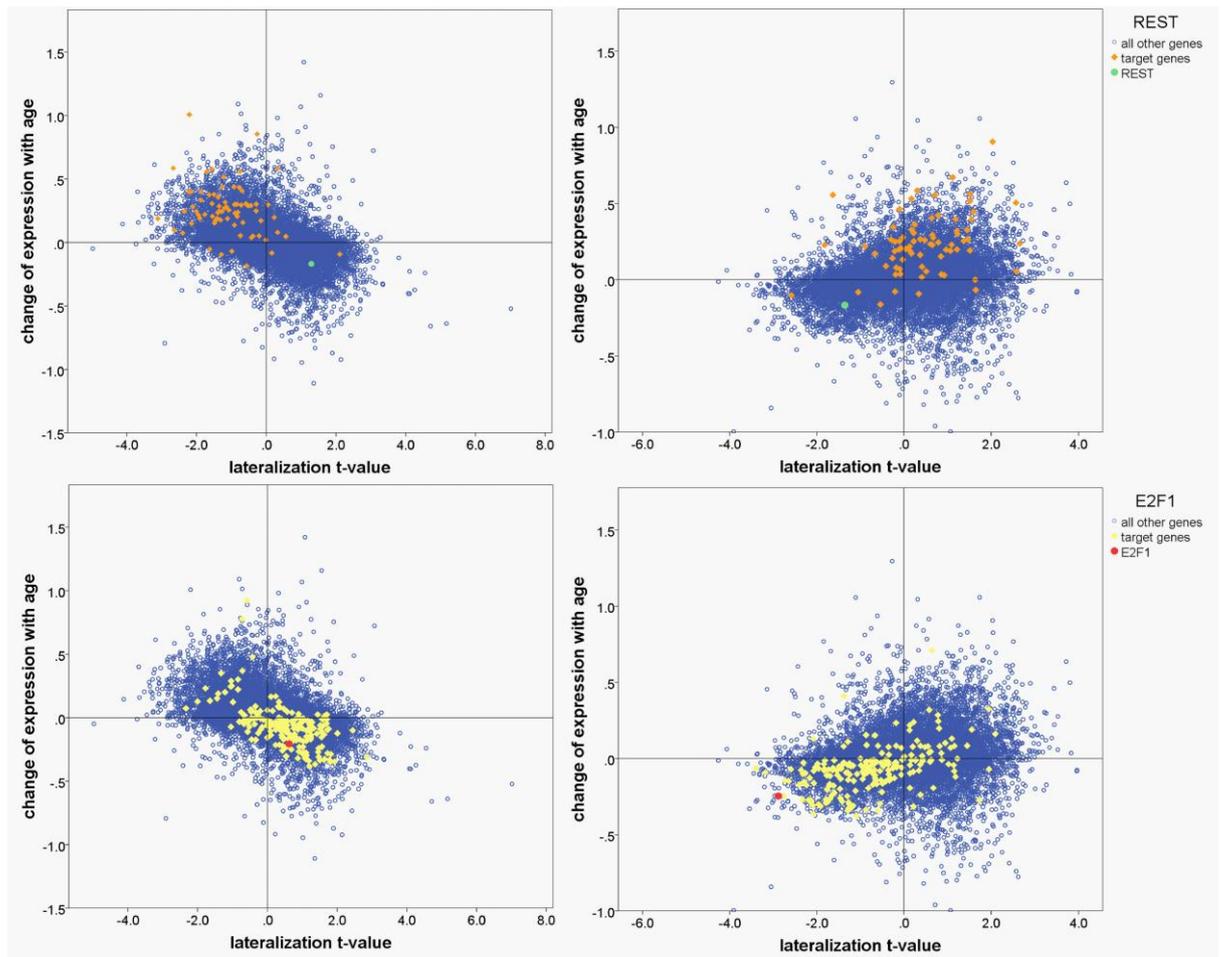
Figure 2. Examples of Gene Ontology gene-set enrichment, mirrored between spinal cord and hindbrain. X-axis: left/right differential expression t-value. Y-axis: regression slope of expression (as \log_2 (cpm)) on embryonic age (CS). Orange: genes within the gene-set. Blue: all other genes. **(a)**, Genes in gene-set 'synaptic transmission' (GO:0007268) increase in expression with age and are more highly expressed in the left spinal cord (normalized expression score (NES)=2.65; family-wise error rate (FWER)<5.0 E-04) and **(b)**, the right hindbrain (NES=2.15; FWER=0.034). **(c)**, Genes in gene-set 'DNA strand elongation' (GO:0022616) decrease in expression with age and are expressed at a lower level in left spinal cord (NES= 2.41; FWER<5.0 E-04) and **(d)**, left hindbrain (NES=2.68; FWER < 5.0 E-04).

Figure 3. Lateralization of transcription factor (TF) target gene-sets. **a-b**, Targets of the TF NRSF in **(a)** spinal cord and **(b)** hindbrain. Y-axis: slope of change in bilateral expression with embryonic age. X-axis: left/right differential expression t-value. Orange: genes with a target motif of NRSF. Green: NRSF itself. Blue: all other genes in the dataset. Genes that have the motif, in *cis*, that is targeted by the repressor NRSF are more highly expressed in the left spinal cord (normalized expression score (NES)=3.14; family-wise error rate (FWER)<5.0 E-04) and increase in expression with age. The pattern is left-right mirrored in the hindbrain. **c-d**, Targets of the TF *E2F1* in **(c)** spinal cord and **(d)** hindbrain. Axes and labels as for a-b, except that *E2F1* itself is shown in red and its targets in yellow. Genes that have the motif TTSGCGS in *cis*, which is targeted by *E2F1*, are more weakly expressed in the right hindbrain (NES=2.66; FWER<5.0 E-04) and decrease in expression with age. This lateralization is left-right mirrored in spinal cord.





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