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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Imaging and software analysis was done using the Zeiss confocal microscope LSM700 using and the respective Zeiss Zen microscopy software or the Eclipse Ti-E inverted Microscope and the NIS-Elements Microscope Imaging Software from Nikon. The FACS sorting was done using the BD FACS-TM Sortware, Version 1.2.0.142.
Data analysis	Data analysis was done with Matlab. For the detailed custom codes and algorithms used see https://github.com/linnarsson-lab/DH_paper_public_code Clustering analysis scripts with explanation: analysis_5_bsv7.m – initial analysis of all cells neurons_only_bsv7.m backSpinSplit_v7_hannah.m – backspinV2 algorithm gaba_only_bsv7_noIEG_new.m – clustering of gaba cells only glut_only_bsv7_noIEG.m – clustering of glut cells only glut_rearr_bsv7_noIEG.m – script for plotting heatmap (markertable) gaba_rearr_bsv7_noIEG.m – script for plotting heatmap (markertable) merge_glut_gaba_v1.m – merging data after separate gaba/glut clustering plot_cluster_num_mol_genes_v1.m – plot number of genes/molecules plot_dendrogram_and_junction_tables_v2.m – analysis of splits on dendrogram plot_tsne_neurons_monod_v3.m – make tsne plots of neurons

save_final_cells_to_GEO_v1.m – save final dataset for GEO submittion

Text files with the classification saved. These files are read by the different clustering and plotting scripts.
lev1_markertable_allcells_backspinv7_lev5_20-Jun-2016.txt
classif_all_noIEG_bsv7_lev5_22-Jul-2016_excl.txt
classif_gaba_noIEG_bsv7_lev5_22-Jul-2016.txt
classif_gaba_noIEG_bsv7_lev5_22-Jul-2016_excl.txt
classif_glut_noIEG_bs7_lev5_22-Jul-2016_excl.txt
classif_hannah_markertable_neurons_backspinv2_lev7_21-Jun-2016.txt

Image analysis Codes with short explanation:
RNAscope_quant_dorsalhorn_v4.m – function that run the quantification of RNAscope spots
run_analysis_ARC_v1.m – scripts that run analysis for all ARC staining images
run_analysis_all_v1.m – scripts that run analysis for all cell types validation staining images
ARC_celltype_combination_count_left_right_v1.m
collect_RNAscope_counts_all_ARC_v2.m – script to collect counting results from many section analyzed
collect_RNAscope_counts_all_GABA_v2.m – script to collect counting results from many section analyzed
collect_RNAscope_counts_all_Glut_v2.m – script to collect counting results from many section analyzed
get_ref_points_coordinate_ARC_v1.m – script to record reference points for alignment of multiple dorsal horn sections into a reference section
get_ref_points_coordinate_GABA_v1.m – script to record reference points for alignment of multiple dorsal horn sections into a reference section
get_ref_points_coordinate_Glut_v1.m – script to record reference points for alignment of multiple dorsal horn sections into a reference section

Scripts for the different plots presented in the figures:
plot_ARC_density_vary_th_layer_distribution_v3.m
plot_ARC_vary_th_layer_distribution_v3.m
plot_GABA_Glut_celltype_layer_distribution_v2.m
plot_GABA_Glut_overlay_density_v1.m
plot_GABA_Glut_overlay_density_v2_from_ARC.m
plot_blobs_celltypes_layer_dist_v1.m
plot_glut_overlay_layer_specific_v1.m
plot_onepage_ARC_celltype_layer_distribution_v3.m
plot_onepage_GABA_Glut_celltype_layer_distribution_v3.m
plot_onepage_overlay_GABA_Glut_celltype_layer_distribution_v2.m

Scripts for setting the layer masks for LSC and CSC:
record_layer_mask_CSC_v1.m
save_layer_masks_CSCref_v2.m
save_layer_masks_LSCref_v1.m
save_ref_sections_LSC_CSC.m

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available as raw data in GEO GSE103840, as analyzed data in Supplementary Table 1, and browsing summary information for individual gene is available at linnarssonlab.org/dorsalhorn. Any other relevant raw data is available from the authors upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes. Our sample sizes are similar to previous publications in the field (Zeisel et al. 2015) and were determined iteratively, to ensure that multiple animals contributed to each cluster. Reproducibility has been evaluated and is illustrated in Supplementary Fig.2. We can show that at least 7 animals contributes cells to each cluster. Using a random forest classifier approach which was trained on 80% of the cells and testing its performance on the remaining 20% of the cells we are able to show that cells repeatedly cluster within the expected cluster.
Data exclusions	Cells were excluded from single cell RNA-seq analysis if they were suspected doublets, had a molecule count below 3000 or lack of distinct expression profile. This is detailed in the Methods section "Clustering Analysis".
Replication	Single-cell analysis was carried out in several rounds of sampling, including first from wildtype mice then from transgenic mice (Vgat-Tomato mice and Vglut2-EGFP mice). Here, findings were reliably reproduced in that all cell clusters contain cells from the different samplings. Batch effects were not observed. Cell clusters determined by sequencing results were validated by in situ staining of mRNA, replicated in 2 different regions (cervical and lumbar) in 2 different animals/region. Colocalization of Arc positive cells with marker genes was replicated in 3 different animals with 3 lumbar sections from each animal.
Randomization	No randomization was performed since no experimental groups were used.
Blinding	No blinding was used, clustering was performed in an unbiased manner as described in Zeisel et al 2015.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Antibodies

Antibodies used	GAD65/67 1:500; rabbit; abcam, ab11070; ChAT 1:500; goat; Merck Millipore, AB144P CGRP 1:250; guinea pig; Peninsula/Bachem, T-5053; The respective secondary antibodies were all produced in donkey, used in a concentration 1:1000 and purchased from Invitrogen (anti-rb-Alexa488, A21206; anti-goat-Alexa555, A21432; anti-guinea pig-Alexa647, A21447.
Validation	Gad56/67: Suitable for: IHC-P, IHC-Fr, WB, ELISA using embryo and central nervous system tissue from Mouse, Rat, Chicken, Cat, Human, Zebrafish, Macaque monkey, Ref: Fu Q et al. MHC-I promotes apoptosis of GABAergic interneurons in the spinal dorsal horn and contributes to cancer induced bone pain. Exp Neurol 286:12-20 (2016). ChaT: Suitable for: IHC-P, IHC-Fr, WB, ELISA using Mouse, Rat, Chicken, Cat, Human, Macaque monkey, Zebrafish tissue) Ref: Riedel, A et al. Vesicular glutamate transporter 3-immunoreactive pericellular baskets ensheath a distinct population of neurons in the lateral septum. Journal of chemical neuroanatomy 36 177-90 2008 CGRP: suitable for IHC and ICC using canine, mouse, rat tissue.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	Laboratory C57Bl6N as well as transgenic mice (VgatCreTdTomato (C57Bl6J) and VglutGFP (C57Bl6N)) between the age of 3-11 weeks were used. Animals for the Sequencing study included males and females. For the in situ validation and functional experiments only male animals were used. The tracing study was done with female mice. For a detailed description see the respective section in Online Materials.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cell susension has been prepared from Spinal cord tissue of mice
Instrument	The sorting was perfomed using a BD Influx System (Serial number X646500Q8001)
Software	The FACS sorting was done using the BD FACS-TM Sortware, Version 1.2.0.142.
Cell population abundance	Cell suspensions from the VgatTdTomato and the VglutEGFP animals were sorted by FACS (Fluorescence-activated cell sorting). In total 165578 eGFP and 172309 Tomato positive events (cells) were sorted. Sorted cell suspension was immediately used to load Fluidigm C1 sequencing chip
Gating strategy	Cells were represented by a cloud of events in a SSC/Fluorescent-Intensity blot. The nozzle size used was 200µm with a sheath pressure of 3.0 PSI. As gating strategy we used for both, the VgatTdTomato and the VglutEGFP suspension, a high fluorescence intensity (85/29 [561]-tomato or 530/40 [488]-eGFP) and a low SSC (side scatter) value.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.