Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/6/eaav4520/DC1

Supplementary Materials for

Awake functional MRI detects neural circuit dysfunction in a mouse model of autism

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Published 5 February 2020, *Sci. Adv.* **6**, eaav4520 (2020)
DOI: 10.1126/sciadv.aav4520

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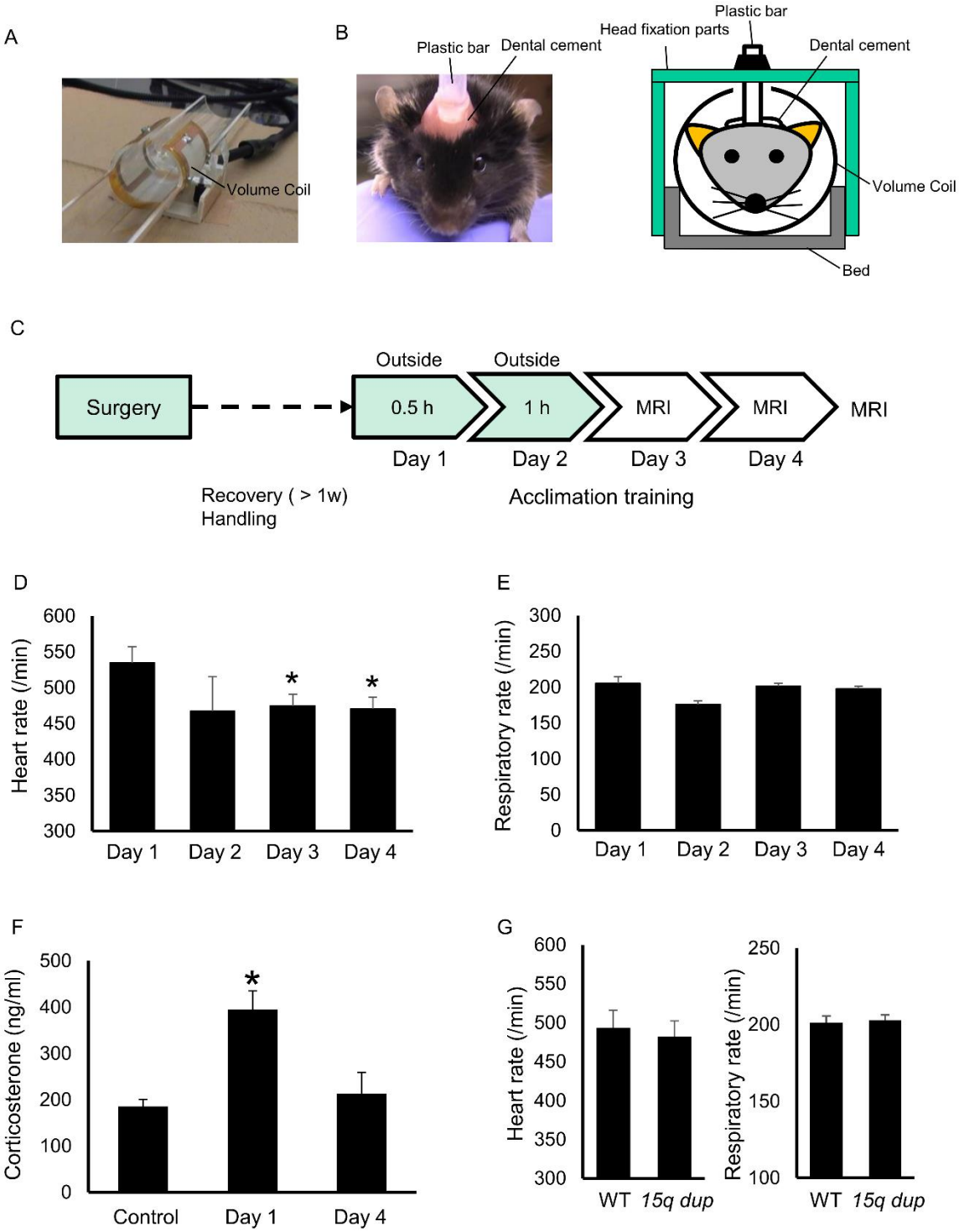

Fig. S1. Awake fMRI system and physiological parameters. (**A**) Dedicated volume coil for head fixation. (Photo Credit: Tomokazu Tsurugizawa, Ajinomoto Co., Inc.) (**B**) The head bar and dental cement on the mouse head and schematic figure of head fixation system. (**C**) Schedule of surgery and acclimation training for awake fMRI. (**D**) Heat rate (n=15), (**E**) Respiratory rate (n=15) and (**F**) Serum corticosterone level (n=5, 4 and 5 in control, day1 and day4, respectively) during the acclimation training. *p < 0.05 by Tukey-Kramer multiple comparison test. (**G**) Heat rate and respiratory rate at day4 in WT (n=15) and *15q dup* groups (n=10).

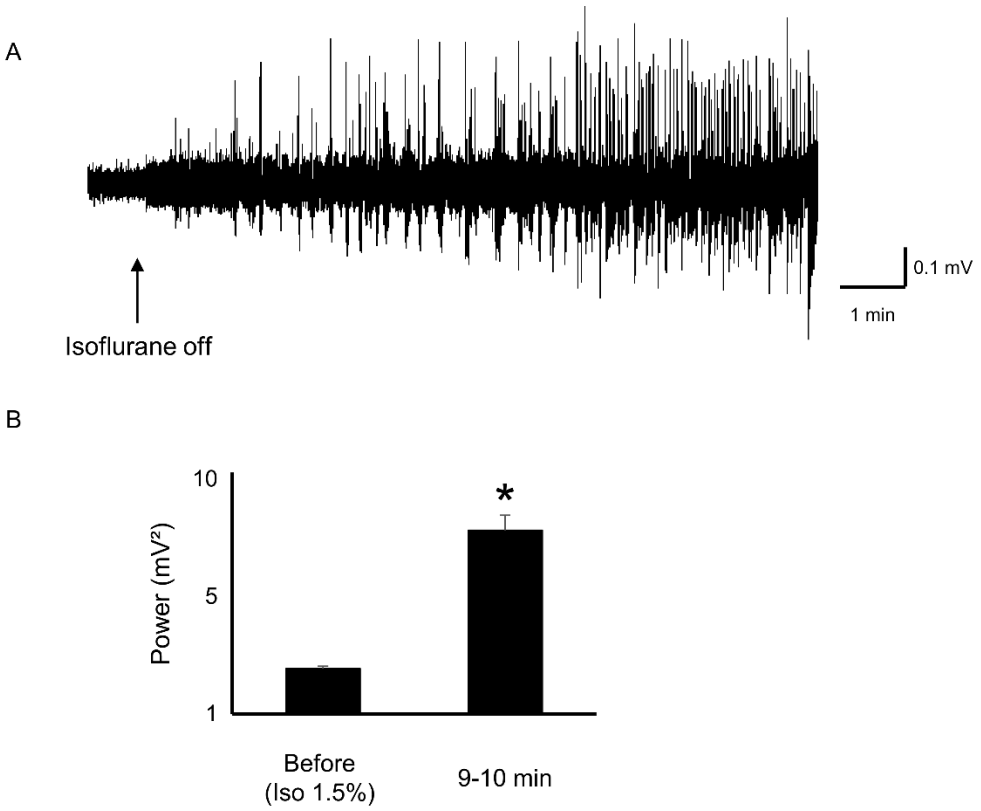

Fig. S2. EEG recording. (**A**) Time-course of EEG waves after turning off (arrow) in a representative mouse. (**B**) Averaged power of gamma frequency band during isoflurane anesthesia and at 9-10 min after turning off the isoflurane (n=4). *$p < 0.05$ by paired t-test.

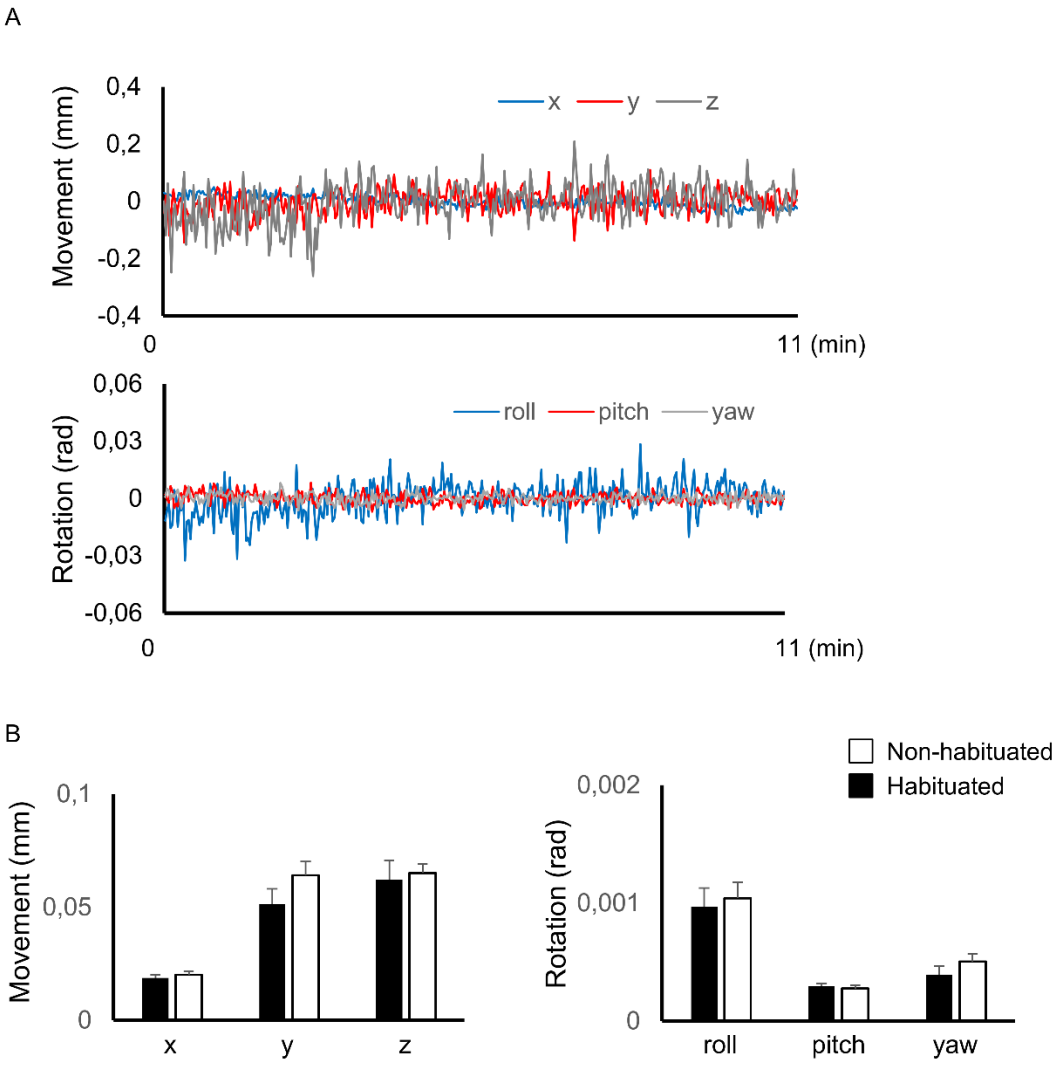

Fig. S3. Head motion parameters. (**A**) Six motion parameters (three translation and three rotation) during the MRI scanning in a representative WT-saline mouse. The x and y-direction corresponds to the in-plane direction and slices are acquired in the z- with 2D-gradient echo EPI parameters (**B**) The averaged motion parameters in habituated (after the training) and non-habituated (1st of the training in MRI bore) WT mice.

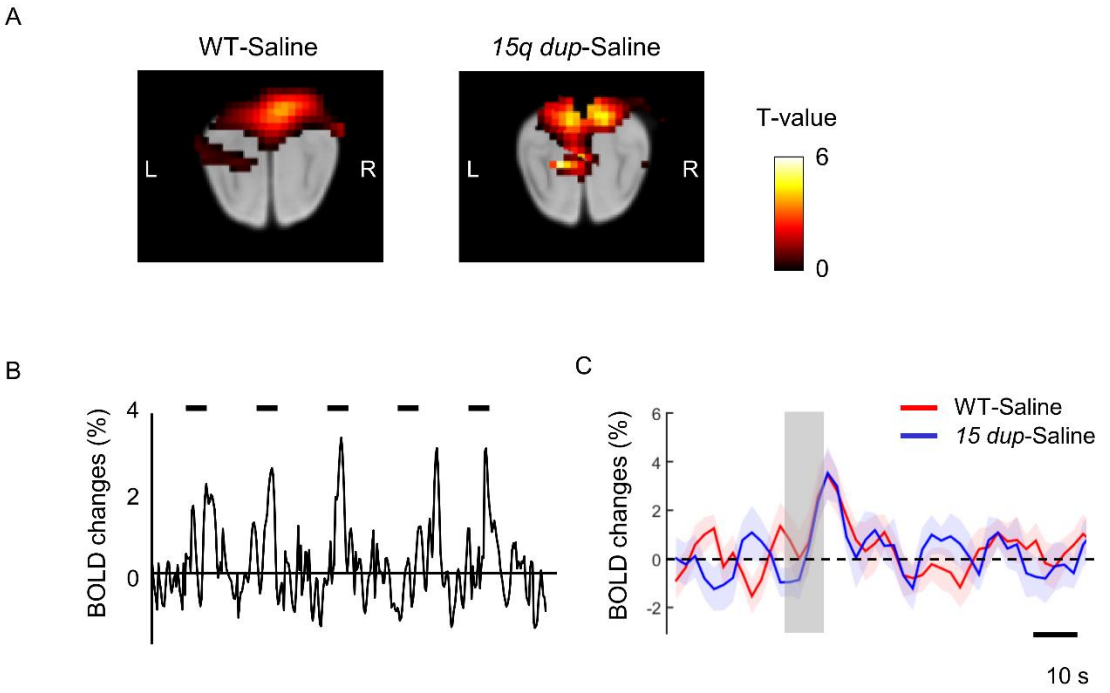

Fig. S4. Similar BOLD response to odor stimulation in the olfactory bulb between WT and *15q dup*-saline mice. (**A**) Significant BOLD response to other-odor stimulation in the olfactory bulb in WT-saline compared to 15q dup-saline ($p<0.05$, FDR-corrected at cluster level). Color bar represents t-statistic value. L/R represents the left/right hemisphere. (**B**) Time-course of BOLD signal changes within the activated voxels in the olfactory bulb in a representative WT mouse. (**C**) Time-course of averaged BOLD signals in WT-saline (n=15) and 15q dup-saline (n=10) mice. Data are represented as mean ± SEM.

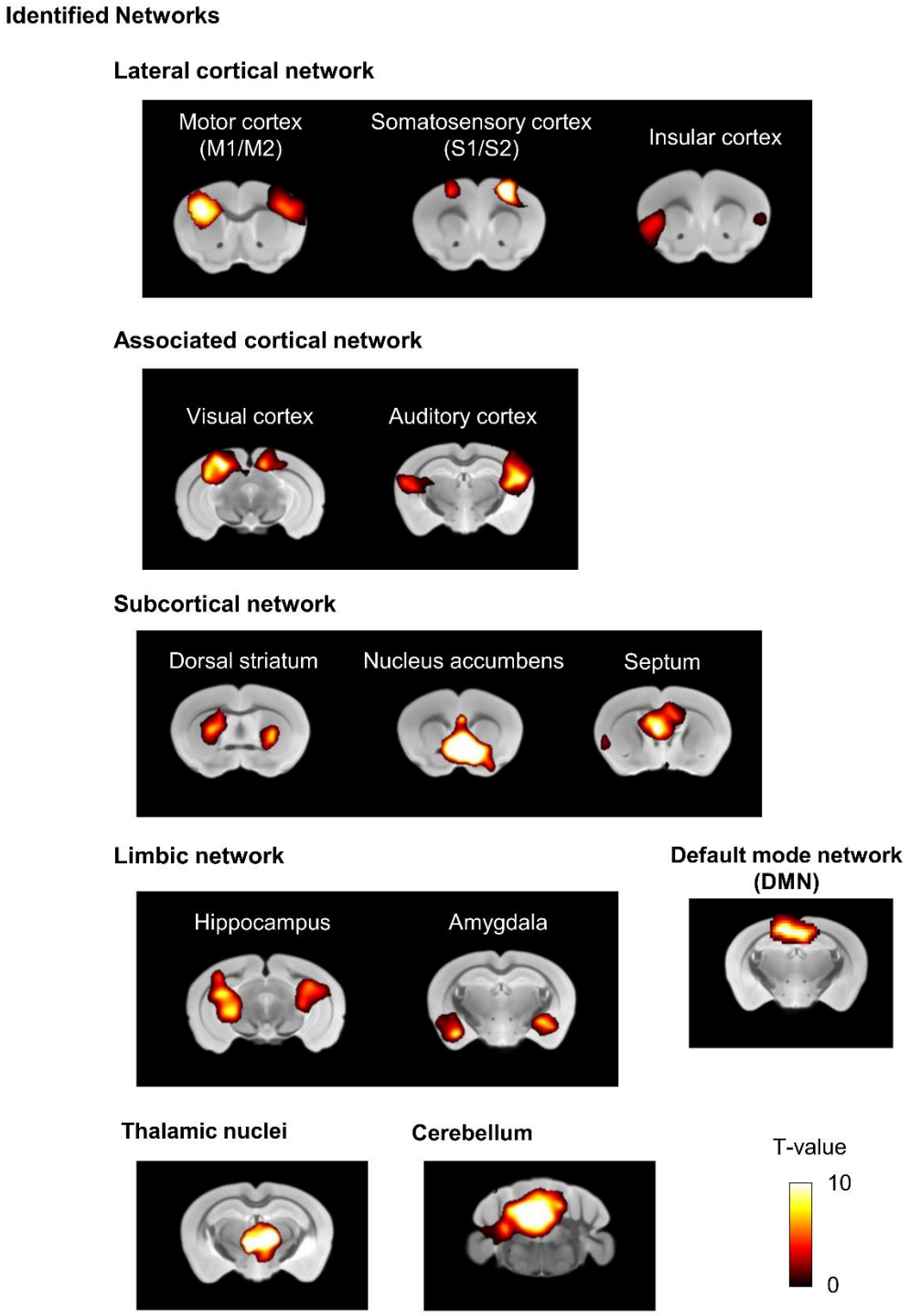

Fig. S5. Common ICA components in WT and *15q dup* mice. The common ICA components, lateral cortical network, associated cortical network, subcortical network, limbic network, default mode network, thalamic nuclei and cerebellar network were observed in WT and 15q dup mice with saline or DCS injection.

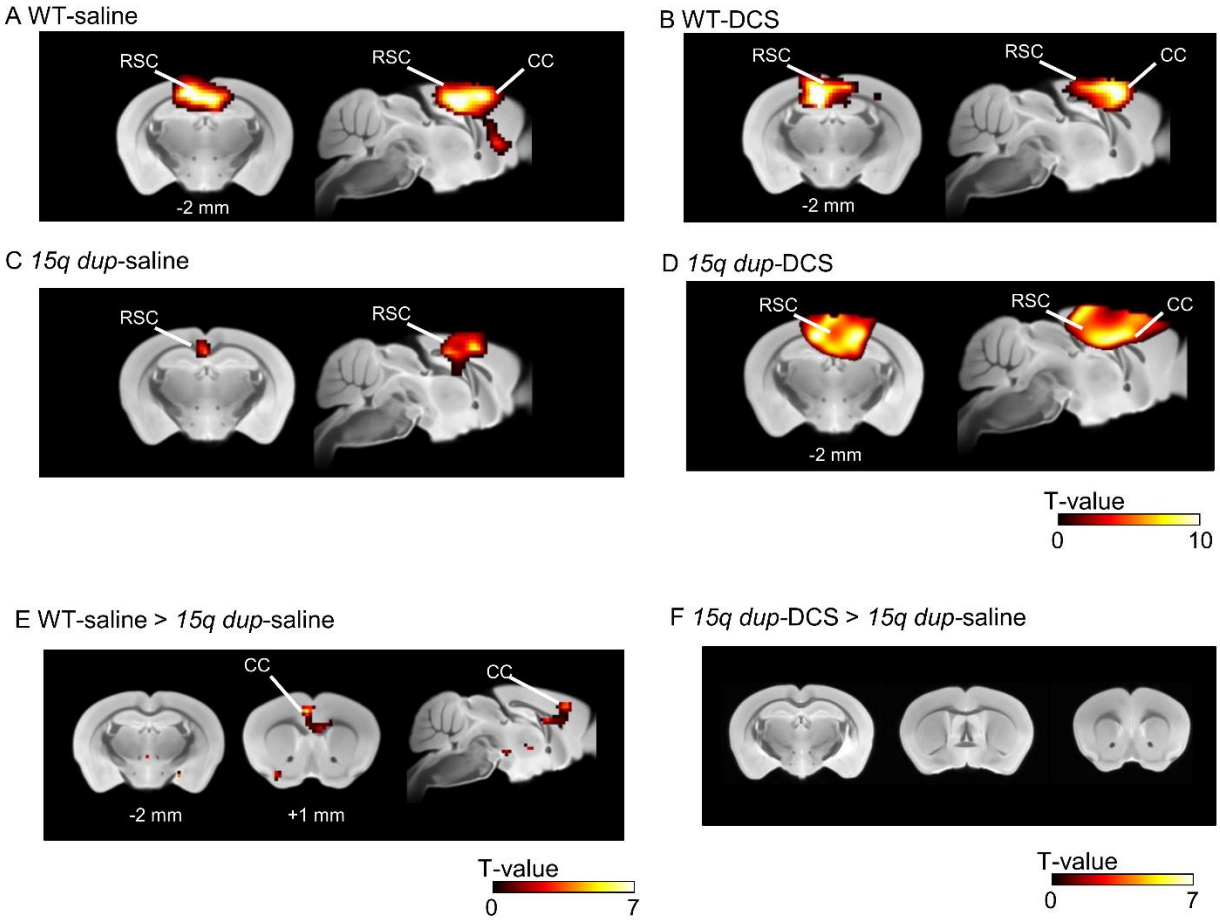

Fig. S6. DMN in WT and *15q dup* mice. DMN in (**A**) WT-saline mice (n=15), (**B**) WT-DCS (n=10), (**C**) 15q dup-saline (n=10) and (**D**) 15q dup-DCS mice (n=15). Color bar, t-values. Comparisons of DMN between (**E**) WT-saline and 15q dup-saline groups and between (**F**) 15q dup-saline and 15q dup-DCS groups. CC, anterior cingulate cortex; RSC, retrosplenial cortex.

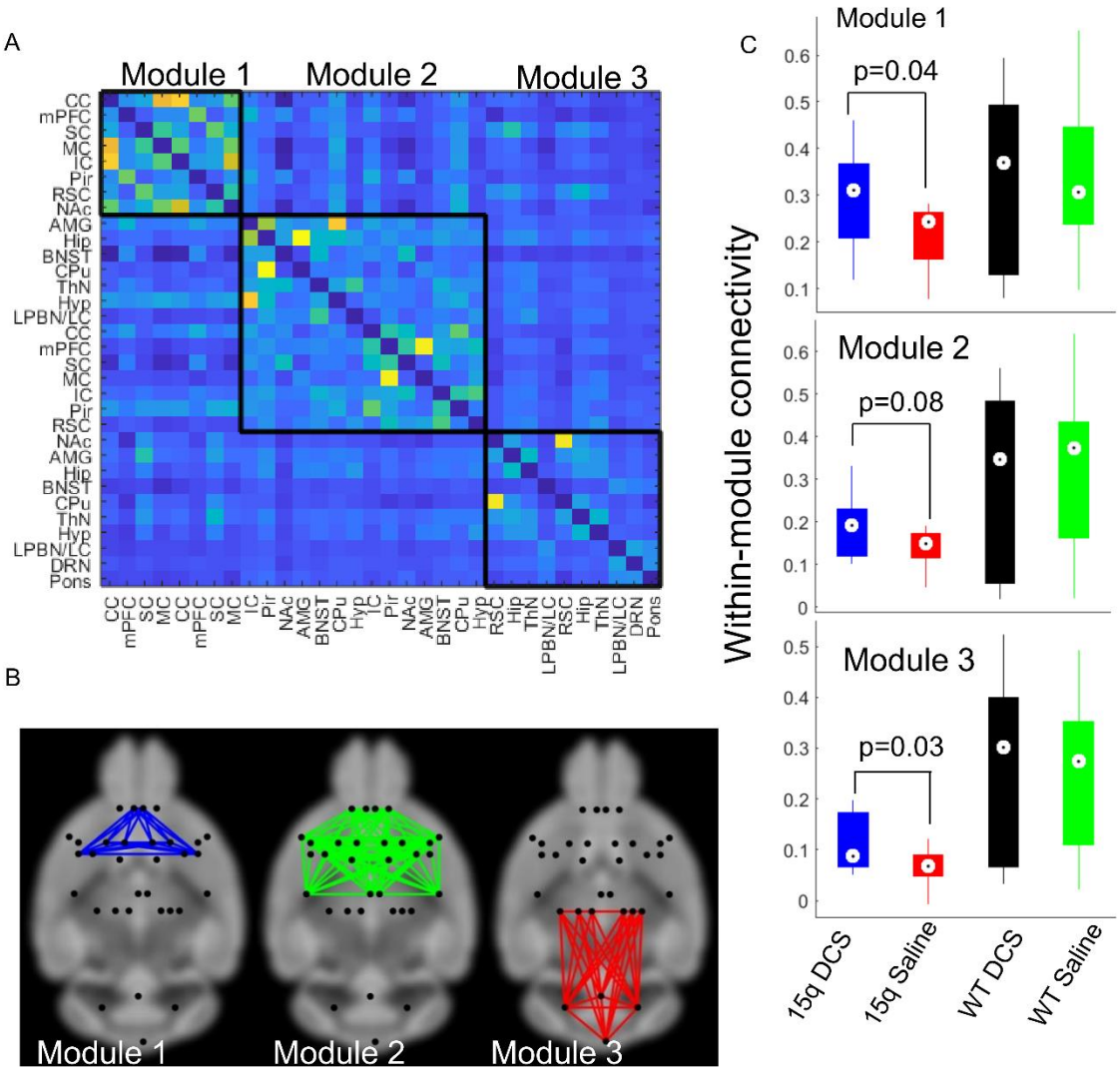

Fig. S7. Modular decomposition of functional connectivity networks and comparison of within-module–averaged connectivity. (**A**) Consensus modular decomposition yielded three distinct modules (Louvain algorithm). Modules represent a consensus across all mice. Solid black lines indicate boundaries between modules. Matrix rows/columns are reordered such that regions comprising common modules are shown consecutively. (**B**) Modules shown in planar anatomical space (axial representation). (**C**) Functional connectivity was averaged over all pair of connections comprising each module, yielding an estimate of within-module-averaged connectivity for each module. DCS did not impact within-module connectivity in the WT group. In the 15q dup group, administration of DCS significantly increased within-module connectivity for Modules 1 & 3 (two-sample, two-tailed t-test), compared to administration of saline. Between-module connectivity did not differ between DCS and saline (not shown). Circles indicate medians and boxes indicate 25th and 75th percentiles.

Table S1. Amino acid concentration in hindbrain in WT and *15q dup* mice. AABA, α-aminobutyric acid; AAAA, α-aminoadipic acid; GABA, γ-Aminobutyric acid. N.D., not detected. *p<0.05 by t-test.

	Cerebellum		Midbrain		Pons/Medulla	
Amino acids	WT	15q-dup	WT	15q-dup	WT	15q-dup
Taurine	5802.3	6456.1	3017.7	3212.8	2586.2	2461.0
Aspartic acid	1957.9	1986.7	2319.8	2395.6	2455.0	2407.4
Threonine	244.8	215.3	147.6	137.1	144.8	119.8
Serine	484.9	273.7	249.7	235.3	238.9	208.0
Asparagine	60.8	48.6	76.1	67.0	64.2	62.5
Glutamic acid	6612.9	7071.7	4685.6	4927.8	4381.2	4306.2
Glutamine	6203.5	5404.2	5469.7	3726.5	4185.8	3063.0
AAAA	25.9	29.2	12.4	13.5	8.3	10.6
Glycine	802.4	700.9	1326.5	1187.0	2930.5	2831.0
Alanine	379.2	391.0	271.0	254.4	357.4	317.9
Citrulline	24.2	24.4	6.4	5.6	11.4	10.0
AABA	5.8	5.3	9.2	5.6	6.3	5.1
Valine	71.2	45.0	58.2	52.3	45.7	43.8
Methionine	33.8	31.9	26.7	20.6	21.3	17.0
Cystathionine	31.9	27.3	N.D.	N.D.	24.0	30.1
Leucine	60.7	41.8	48.5	40.0	40.5	34.7
Isoleucine	32.4	19.5	21.0	20.2	19.7	17.5
Tyrosine	81.4	57.6	52.2	42.1	44.9	36.7
Phenylalanine	64.2	43.4	44.5	35.2	43.6	32.9
GABA	1425.6	1408.0	3310.9	3095.1	1551.1	1392.6
Tryptophan	18.1	16.0	13.4	12.4	13.6	12.5
Ethanolamine	143.7	130.5	235.6	227.9	146.4	132.3
Hydroxylysine	2.0	2.8	2.8	2.8	N.D.	N.D.
Lysine	150.2	139.4	162.4	152.7	145.7	144.2
Histidine	73.5	56.6	49.1	41.9	41.1	33.4
Anserine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Carnosine	56.4	53.8	70.9	63.6	79.3	69.5
Arginine	147.1	167.0	232.3	226.9	250.6	243.4
Hydroxyproline	13.1	12.5	10.4	10.3	11.3	9.5
Proline	75.9	60.1	52.1	47.1	41.1	39.9

Table S2. Amino acid concentration in forebrain in WT and *15q dup* mice. AABA, α-aminobutyric acid; AAAA, α-aminoadipic acid; GABA, γ-Aminobutyric acid. N.D., not detected. *p<0.05 by t-test.

	Hippocampus		Hypothalamus		Olfactory bulb		Frontal cortex	
Amino acids	WT	15q-dup	WT	15q-dup	WT	15q-dup	WT	15q-dup
Taurine	6377.6	**6164.6 ***	3609.6	3496.7	10890	12144	8171.7	8204.4
Aspartic acid	1392.7	1269.2	2232.6	2399.7	2108.4	2021.6	2504.2	2300.1
Threonine	148.9	131.0	182.0	154.3	202.1	198.1	158.5	144.4
Serine	522.4	438.4	378.0	325.7	283.2	284.8	583.1	**434.9 ***
Asparagine	60.7	57.1	91.5	91.3	61.3	66.9	85.0	83.4
Glutamic acid	6882.2	6145.1	5450.6	5683.3	5129.0	5288.5	8703.8	9501.7
Glutamine	5206.7	3872.5	6187.2	4956.0	4644.6	4742.0	5246.3	4609.0
AAAA	11.0	12.3	21.6	26.3	13.8	17.0	18.4	**29.1 ***
Glycine	722.9	588.7	913.1	864.0	523.7	502.5	698.0	593.4
Alanine	651.9	589.9	312.0	255.8	546.5	508.3	680.3	656.6
Citrulline	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
AABA	9.1	6.5	9.8	7.4	7.9	5.7	8.6	6.8
Valine	61.4	52.2	77.1	68.8	74.9	72.8	69.4	59.4
Methionine	29.3	23.2	31.5	26.3	28.2	30.6	28.3	26.8
Cystathionine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Leucine	48.4	36.5	60.9	53.3	66.6	55.8	51.9	42.9
Isoleucine	21.8	18.1	26.6	23.2	29.2	28.9	24.6	23.0
Tyrosine	56.9	45.3	64.0	54.1	53.0	56.9	55.7	47.8
Phenylalanine	46.3	36.8	50.9	39.9	46.5	45.3	44.0	40.3
GABA	1783.5	1511.4	4525.1	3953.8	4797.5	4468.3	1458.0	1438.7
Tryptophan	12.3	13.2	16.5	15.1	11.4	15.8	11.6	11.8
Ethanolamine	264.6	237.7	412.0	362.5	215.9	214.5	347.6	328.1
Hydroxylysine	2.7	3.8	2.7	2.7	N.D.	N.D.	3.8	3.1
Lysine	137.8	117.8	160.0	141.8	166.8	148.5	114.5	101.6
Histidine	68.2	52.2	72.8	61.6	50.3	51.8	62.4	55.2
Anserine	N.D.	N.D.	N.D.	N.D.	9.9	8.8	N.D.	N.D.
Carnosine	24.9	22.6	46.3	33.9	1210.3	1155.0	90.9	91.0
Arginine	102.9	95.2	137.4	129.9	95.7	100.8	80.6	88.6
Hydroxyproline	11.1	11.2	13.4	11.6	10.8	10.1	13.7	13.3
Proline	64.1	56.8	62.7	55.5	112.3	116.7	82.6	78.0