LMN Homunculus Supplementary Material: Methods Used in Determining Homunculi Sizes

1. Overview
Heights are based on measurements of length and somatic girths are based on motor neuron density. The homunculi are drawn as close to actual measurements as reasonable, allowing the artist (JK) esthetic leeway.

2. Demographics
All nervous systems had been acquired with an Investigational Review Board and Health Insurance Portability and Accountability Act compliant informed consent process in the Benaroya Research Center ALS CNS repository as previously published [1].

3. Length Measurements Used to Estimate Homunculi Height
Methods: Fresh brains and spinal cords were measured at autopsy using pliant tape measures. Cortical determinations were measured from the Sylvian fissure to the vertex and vertex to cingulate gyrus [2]. Rostral-caudal brainstem and spinal cord determinations were estimated for midbrain, pons, medulla, upper cervical segment above the cervical enlargement, cervical enlargement, thoracic segment, lumbosacral enlargement, and sacral segment below the enlargement. Up to 11 determinations were obtained and averaged.
Results (Supplementary Materials page 2): The average lateral-medial spans along the motor gyrus from Sylvian fissure to vertex is ~10-12 cm and vertex to cingulate gyrus is ~2-3 cm. These are comparable to published data [2]. The average brainstem and spinal cord rostral-caudal spans are: pons, ~3-3.5 cm; medulla, ~1.5-2.5; cervical cord, 10-13 cm; thoracic cord, ~20-25 cm; and lumbosacral cord, ~ 5-8 cm [see Figure on page 2]. For homunculus characterizations, arm lengths (estimated at the cortex to be ~1/3 overall span and cervical enlargement) are not calculated in estimations of height.

4. Neuron "Particle" Counting Used to Estimate Girth of Somatic Regions of LMN Homunculus
Comment: Girths of the homunculi are based on motor neuron density, the technical challenges of which have been recently reviewed [3]. For the lower motor neuron homunculus, these are based on measurements of alpha motor neuron density as previously published [1]; for the upper motor neuron homunculus, these reflect what are presumed to be density of Betz cells at the cortex relatively commensurate to length, recognizing this complexity [4].
Methods: We used the control data from our previously described series [1]. There were 8 control nervous systems, 7 male and 1 female. The mean age was 63 years (range 38-80 years). None had known neurological diseases. Post-mortem intervals averaged 5.25 hours (range 2.0-12 hours). The published method is restated here for convenience.
Histological Sampling: We used 6 µm thick sections from formalin-fixed paraffin-embedded tissues stained with cresyl violet acetate. We studied 4 neuraxis levels: hypoglossal nucleus at the mid-medulla, mid-cervical spinal enlargement, mid-thoracic spinal region, and mid-lumbar spinal enlargement. We standardized rostral-caudal sampling of the hypoglossal nucleus by its relation to the medial longitudinal fasciculus and by the morphology of the 4th ventricle [5]. We standardized rostral-caudal sampling of spinal cord regions by choosing middle segments—the cervical region has uniform counts between C4-C8 [6,7], the lumbar region has uniform neuron counts between L3-S2 [8,9,10], and we assume the mid-thoracic region has uniform counts. Since motor neuron presence varies from one histological section to another [6,11], we evaluated a total of 8 sections from each neuraxis level, choosing every 10th section for a sampling interval of 60 µm from a series of 84 consecutive sections spanning 504 µm, a sampling technique modified from Method 1 of Tomlinson et al [11]. We separately imaged each side of the spinal anterior horns and hypoglossal nucleus under 50X with a Leica DM2500 microscope with a Spot Insight 4 digital camera and Spot Advanced V4.5.7 software (Diagnostic Instruments, Inc., Sterling Heights, MI) and stored each image in Tagged-Image File Format.
Counting: To meet the realities of the counting task, we devised a method of counting neuron "particles" to index relative neuron presence rather than determining absolute neuron counts [3,12]—stereology technologies do not readily apply to the motor neurons [13] and automated particle counting software was inefficient, experience reported by others [14]. Our method had 3 observers independently count motor neuron particles in each image file according to the following criteria learned in training sessions to standardize counting: neurons were located in the anterior portion of the anterior horns of the spinal cord segments or in the hypoglossal nucleus [5], had relatively deep Nissl staining, were generally multi-concave, and were larger than 25 µm in diameter in cervical and lumbar regions and larger than 15-20 µm in thoracic and hypoglossal regions. Both right and left sides were counted. Counting neurons in the perihypoglossal nuclei, the intermediolateral cell column, and Clarke's column was avoided. Because we were not concerned with pathological change or cell shrinkage, we did not correct for split cell error [15,16].
Statistics and Calculations: We compiled particle counts in a spreadsheet and collated and processed them with simple statistics.
Results (Supplementary Materials Page 3): Hypoglossal nucleus in medulla = 32 motor neuron particles/cross-sectional area/side; cervical level = 33 motor neuron particles/cross-sectional area/side; thoracic level = 10 motor neuron particles/cross-sectional area/side; lumbar level 48 motor neuron particles/cross-sectional area/side. Neuron counts, contrary to other reports [7.8,17], did not have pronounced age-related changes. The ratio of medulla:cervical:thoracic:lumbar= ~3:3:1:5.

5, References

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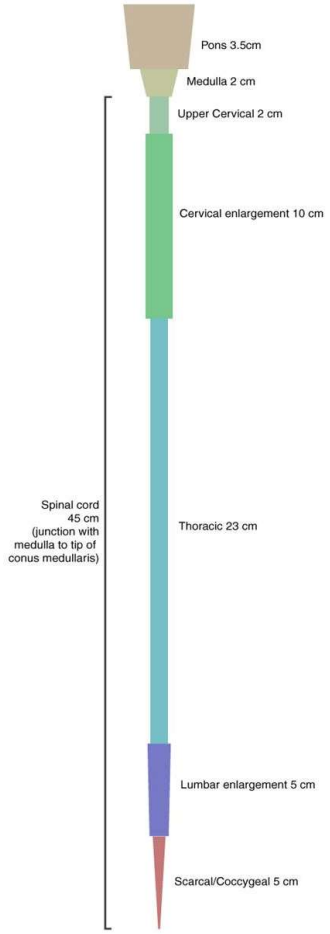
Homunculi Height Determinations

Supplemental Table 1: Motor Cortex, Cranial, and Spinal Lengths (cm)

CNS#	Age	Sex	Dx	Motor Cortex	Pons	Med-ulla	Cervical Cord (Upper)	Cervical (main)	Thor-acic Cord	Lumbo-sacral Cord
25	53	M	SALS	10	3.5	NA	NA	NA	NA	NA
28	49	F	SALS	10.5	3.5	NA	NA	NA	NA	NA
37	57	M	Control	10.7	3.5	NA	NA	NA	NA	NA
39	77	M	Control	10	NA	NA	NA	NA	21	5
40	76	F	Control	9.5	3.5	1.5	NA	10	24.5	5.5
41	81	M	Control	9.5	3.5	NA	NA	11	24	7
42	61	M	Control	10	3.2	1.5	2.5	10	21	8
43	74	M	SALS	9.5	NA	1.8	2	10	25	5
44	80	F	Control	9.5	3.1	NA	NA	10	23	6
46	51	F	SALS	10		NA	NA	10.5	20	5
47	65	F	SALS	10.7	3.5	2.5	2.5	10.5	24	6



Lower Motor Neuron Counts (reproduced from supplementary reference 1)

Supplementary Table 2: Demographics

CNS#	Dx	Age	Sex
7	Control	61	M
10	Control	78	M
19	Control	80	F
20	Control	38	M
23	Control	77	M
26	Control	49	M
31	Control	67	M
37	Control	57	M

Supplementary Table 3: Summary

Region	Observer: JR	Observer: YF	Observer: PL	AVERAGE
Medulla AVE	33	36	25	32
Medulla SD	12	11	9	
Cervical AVE	30	37	32	33
Cervical SD	18	19	18	
Thoracic AVE	8	13	9	10
Thoracic SD	3	4	4	
Lumbar AVE	43	53	48	48
Lumbar SD	10	10	9	

Supplementary Table 4: Particle Counts

Region	Observer: JR	Observer: YF	Observer: PL
Medullas	10	19	8
Medullas	12	19	10
Medullas	14	20	11
Medullas	15	20	12
Medullas	16	21	12
Medullas	17	22	13
Medullas	18	22	13
Medullas	18	23	13
Medullas	19	24	14
Medullas	19	24	14
Medullas	19	24	14
Medullas	20	24	15
Medullas	20	25	15
Medullas	20	25	15
Medullas	21	26	15
Medullas	21	26	16
Medullas	21	26	16
Medullas	22	26	16
Medullas	22	26	16
Medullas	22	27	16
Medullas	22	27	16
Medullas	22	27	17
Medullas	23	27	17
Medullas	23	28	18
Medullas	23	28	18
Medullas	24	28	18
Medullas	24	28	18
Medullas	24	28	19
Medullas	25	28	19
Medullas	25	28	19
Medullas	25	29	19
Medullas	26	29	19
Medullas	26	29	19
Medullas	26	29	19
Medullas	26	29	20
Medullas	26	29	20
Medullas	26	29	20
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Medullas	28	31	21
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Medullas	29	31	21
Medullas	29	32	21
Medullas	30	32	22
Medullas	30	32	22
Medullas	30	32	22
Medullas	30	32	22
Medullas	31	32	22
Medullas	31	33	23
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Medullas	31	33	23
Medullas	31	33	23
Medullas	32	33	23
Medullas	32	33	24
Medullas	32	33	24
Medullas	32	34	24
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Medullas	35	36	26
Medullas	35	36	26
Medullas	35	36	26
Medullas	36	36	26
Medullas	36	36	26
Medullas	36	36	26
Medullas	37	37	27
Medullas	37	37	27
Medullas	37	37	27
Medullas	37	37	28
Medullas	37	38	28
Medullas	37	38	28
Medullas	37	39	28
Medullas	38	39	28
Medullas	38	39	29
Medullas	38	39	29
Medullas	38	40	29
Medullas	38	40	29
Medullas	39	40	29
Medullas	39	41	30
Medullas	39	41	31
Medullas	39	41	31
Medullas	40	41	32
Medullas	40	41	32
Medullas	40	42	33
Medullas	41	42	33
Medullas	41	43	33
Medullas	41	43	34
Medullas	41	44	34
Medullas	42	44	34
Medullas	42	44	35
Medullas	42	45	36
Medullas	43	46	36
Medullas	43	46	36
Medullas	43	46	37
Medullas	43	47	37
Medullas	44	47	37
Medullas	44	47	37
Medullas	45	48	38
Medullas	47	49	39
Medullas	47	49	39
Medullas	47	50	39
Medullas	48	50	41
Medullas	48	50	41
Medullas	50	51	42
Medullas	54	55	42
Medullas	56	55	43
Medullas	57	57	43
Medullas	57	60	44
Medullas	60	62	45
Medullas	63	64	46
Medullas	65	66	54
Medullas	67	69	*20
Medullas	67	77	*24
AVE	33.48	36.08	25.40650407
SD	11.5182884	10.7497937	9.052521973

Region	Observer: JR	Observer: YF	Observer: PL
Cervical	2	4	3
Cervical	2	6	3
Cervical	2	6	3
Cervical	3	7	3
Cervical	3	7	3
Cervical	4	7	4
Cervical	4	7	5
Cervical	5	7	5
Cervical	5	8	6
Cervical	6	8	6
Cervical	6	8	6
Cervical	6	8	6
Cervical	7	9	6
Cervical	7	9	6
Cervical	8	9	7
Cervical	8	9	7
Cervical	8	9	7
Cervical	8	10	8
Cervical	9	11	9
Cervical	9	11	9
Cervical	10	12	10
Cervical	11	12	11
Cervical	11	13	11
Cervical	11	14	12
Cervical	13	17	13
Cervical	15	17	17
Cervical	16	18	18
Cervical	16	22	18
Cervical	18	27	19
Cervical	20	28	21
Cervical	20	29	22
Cervical	20	31	24
Cervical	22	32	24
Cervical	22	32	26
Cervical	23	33	26
Cervical	23	34	26
Cervical	24	35	27
Cervical	24	36	27
Cervical	25	37	28
Cervical	25	37	28
Cervical	25	37	29
Cervical	26	37	29
Cervical	26	38	29
Cervical	27	38	30
Cervical	27	38	30
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Cervical	29	39	32
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Cervical	31	41	35
Cervical	32	41	35
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Cervical	33	42	36
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Cervical	41	48	42
Cervical	41	48	42
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Cervical	45	52	49
Cervical	46	52	49
Cervical	47	53	50
Cervical	47	54	51
Cervical	47	56	51
Cervical	48	56	52
Cervical	49	57	53
Cervical	50	58	53
Cervical	51	58	54
Cervical	52	59	54
Cervical	52	59	56
Cervical	54	60	56
Cervical	56	60	57
Cervical	56	60	59
Cervical	57	61	60
Cervical	58	62	60
Cervical	58	63	61
Cervical	61	64	61
Cervical	62	68	62
Cervical	64	70	62
Cervical	68	73	66
Cervical	74	84	70
AVE	30.06930693	36.65346535	31.91089109
SD	17.90489175	19.21532495	18.35979249

Region	Observer: JR	Observer : YF	Observer: PL
Thoracic	2	4	2
Thoracic	2	5	3
Thoracic	3	6	3
Thoracic	3	6	4
Thoracic	3	7	4
Thoracic	3	7	4
Thoracic	4	7	4
Thoracic	4	7	4
Thoracic	4	7	5
Thoracic	4	7	5
Thoracic	4	7	5
Thoracic	4	8	5
Thoracic	4	8	6
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Thoracic	12	19	15
Thoracic	12	20	15
Thoracic	12	20	15
Thoracic	12	21	16
Thoracic	12	21	16
Thoracic	12	21	16
Thoracic	13	21	16
Thoracic	13	21	16
Thoracic	14	22	17
Thoracic	15	22	17
Thoracic	16	23	19
Thoracic	17	24	22
AVE	7.57377049	12.9918	9.155738
SD	2.85226216	4.32513	3.656807

Region	Observer: JR	Observer : YF	Observer: PL
Lumbar	16	31	29
Lumbar	21	33	30
Lumbar	22	35	32
Lumbar	22	35	33
Lumbar	23	36	34
Lumbar	23	36	34
Lumbar	23	37	34
Lumbar	24	37	35
Lumbar	25	37	35
Lumbar	26	39	35
Lumbar	26	39	35
Lumbar	27	40	36
Lumbar	28	40	36
Lumbar	29	41	36
Lumbar	32	41	36
Lumbar	32	41	36
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Lumbar	46	56	49
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Lumbar	47	56	50
Lumbar	48	56	50
Lumbar	48	57	50
Lumbar	48	57	50
Lumbar	48	57	51
Lumbar	48	57	51
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Lumbar	54	67	62
Lumbar	54	68	62
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Lumbar	55	68	62
Lumbar	56	69	62
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Lumbar	57	69	63
Lumbar	57	69	63
Lumbar	58	69	64
Lumbar	59	71	64
Lumbar	60	71	66
Lumbar	62	72	66
Lumbar	62	73	67
Lumbar	65	74	68
Lumbar	66	76	69
AVE	43.4140625	53.3203	48.078125
SD	10.086654	9.90569	9.17296201