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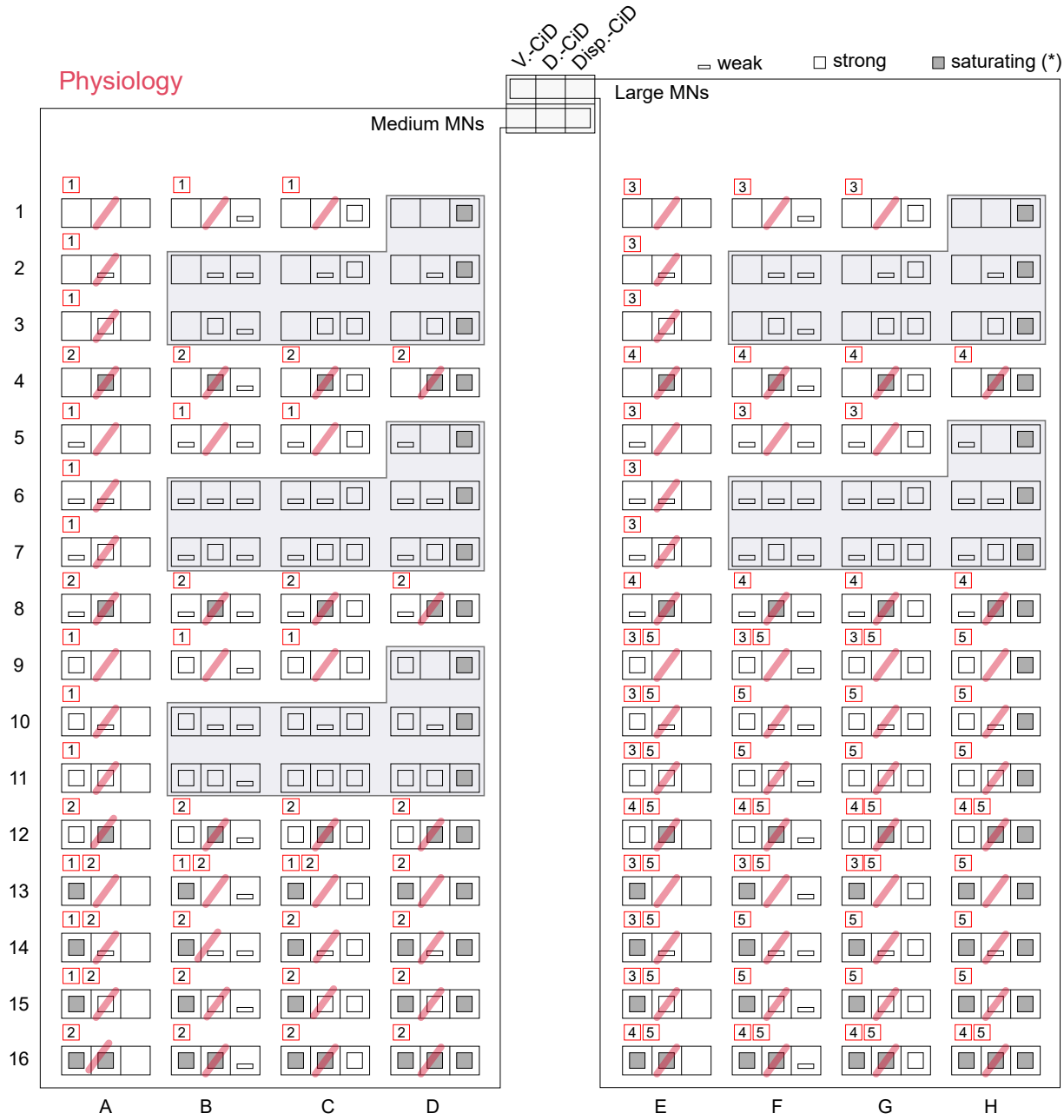
Supplemental Information

Volume EM Reconstruction of Spinal Cord Reveals Wiring Specificity in Speed-Related Motor Circuits

Fabian N. Svara, Jörgen Kornfeld, Winfried Denk, and Johann H. Bollmann



1 MMNs are maximally recruited at maximal speeds (compare Fig. 1a, b). That is, it must be possible for D-CiD → MMN + Disp.-CiD → MMN to sum to ■, therefore D-CiD/Disp.-CiD → MMN cannot be (□/ 0), (0 /□), (0 /▭) , (▭/ 0) or (0 / 0).

2 The population firing rate of MMNs does not yet reach maximal levels when only V-CiDs or D-CiDs are maximally recruited (compare Fig. 1a, b), i.e. V-CiD → MMN and D-CiD → MMN are not ■.

3 LMNs are maximally recruited at maximal speeds (compare Fig. 1a, b). That is, it must be possible for D-CiD → LMN + Disp.-CiD → LMN to sum to ■, therefore D-CiD/Disp.-CiD → LMN cannot be (□/ 0), (0 /□), (0 /▭) , (▭/ 0) or (0 / 0).

4 LMNs are not recruited maximally at 40 Hz (compare Fig. 1a, b), i.e. D-CiD → LMN ≠ ■.

5 LMNs are not recruited below 40 Hz (compare Fig. 1a, b) i.e. V-CiD → LMN cannot be □ or ■.

Figure S1. Exclusion of potential wiring models by physiological arguments. Related to Figure 6
(Legend on next page)

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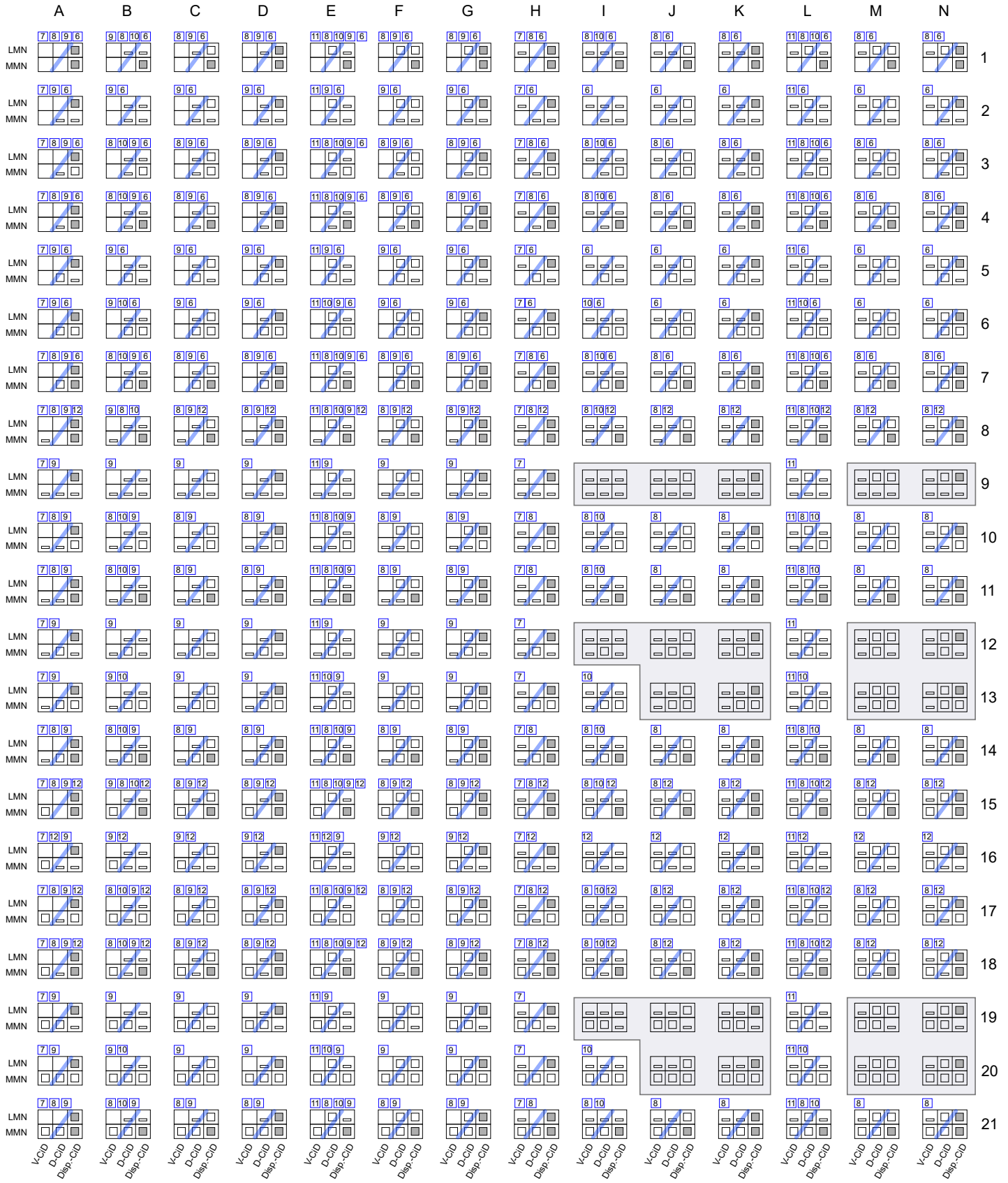
Enumeration of all potential wiring models between CiDs (V-CiDs, D-CiDs and displaced CiDs) and MNs (medium and large). Entries in each box indicate for a given CiD population, to what degree its recruitment alone would evoke spiking activity in a typical MN in the MMN or LMN class.
It is assumed that: MN synaptic input from recruitment of the V-CiD population is maximal in the 25-35 Hz band with negligible input from dorsal CiDs; MN synaptic input from recruitment of D-CiDs predominates in the 40-60 Hz band with increasing contribution from displaced CiDs; and MN synaptic input from D-CiDs and displaced CiDs is maximal in the 60-80 Hz band, when both of these CiD types are maximally active. An empty box indicates there is no synaptic connection. A white horizontal bar (▭) indicates the MN receives, from a given CiD population, synaptic input that is nonzero, but too weak to trigger action potentials when active alone ("weak"). A white square (□) indicates that the MN receives input from a CiD population that is sufficiently strong to trigger action potentials alone, but not at the maximal firing reliability of 100%, i.e. at least one spike in phase with each (fictive) tail bend (McLean et al., 2008) ("strong"). A gray square (■) indicates that synaptic input from a given CiD population is, alone, strong enough to drive the MN at maximal firing reliability of 100% ("saturating"). A saturating connection means that the maximal firing rate of the motoneuron is reached, which may or may not coincide with a saturating excitatory current.
Red crossed out models are incompatible with physiological prior knowledge based on the arguments #1 - #5.
Note: The V-CiDs are the only CiD-type active at slow frequencies (<30 Hz), which suggests that their connectivity to MMNs cannot be zero or subthreshold. However, a distinct population of excitatory commissural interneurons active at very slow speeds (MCoDs) could provide synaptic drive in the range 15-40 Hz (McLean et al., 2008), which is why we cannot rule out zero or weak connections between V-CiDs and MMNs based on physiology.



6 V.-CiD → MMN ≠ 0

7 D.-CiD → LMN ≠ 0

8 D.-CiD → MMN ≥ Disp. → MMN, therefore D-CiD/Disp. CiD → MMN cannot be (0 / ■), (▭ / □), (▭ / ■) or (□ / ■).

9 V.-CiD → LMN ≠ 0

10 The contact area from Disp. CiDs to LMN is much larger than that onto MMN (by a factor of 42, compare Fig. 6D). If, despite of this, Disp. CiD → LMN has to be subthreshold with Disp. CiD → MMN being suprathreshold, the ratio between the LMN firing threshold and the MMN firing threshold would have to be unrealistically large (compare Menelaou et al., 2012). Therefore, Disp. CiD → [LMN / MMN] cannot be [▭ / □] or [▭ / ■].

11 D.-CiD → LMN ≤ Disp.-CiD → LMN, therefore D-CiD/Disp. CiD → LMN cannot be (□ / ▭).

12 V.-CiD → MMN ≈ D.-CiD → MMN (≠ 0), therefore V-CiD/D-CiD → MMN cannot be (▭ / 0), (□ / 0) or (□ / ▭).

Figure S2. Exclusion of potential wiring models by connectivity arguments. Related to Figure 6
(Legend on next page)

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All models that remained after exclusion by physiological arguments (Supplemental Fig. S1). Blue crossed out models are incompatible with our connectivity results (due to arguments #6 - #12). The remaining models are compatible both with physiology and with our wiring data, and cannot be distinguished further based on wiring alone.

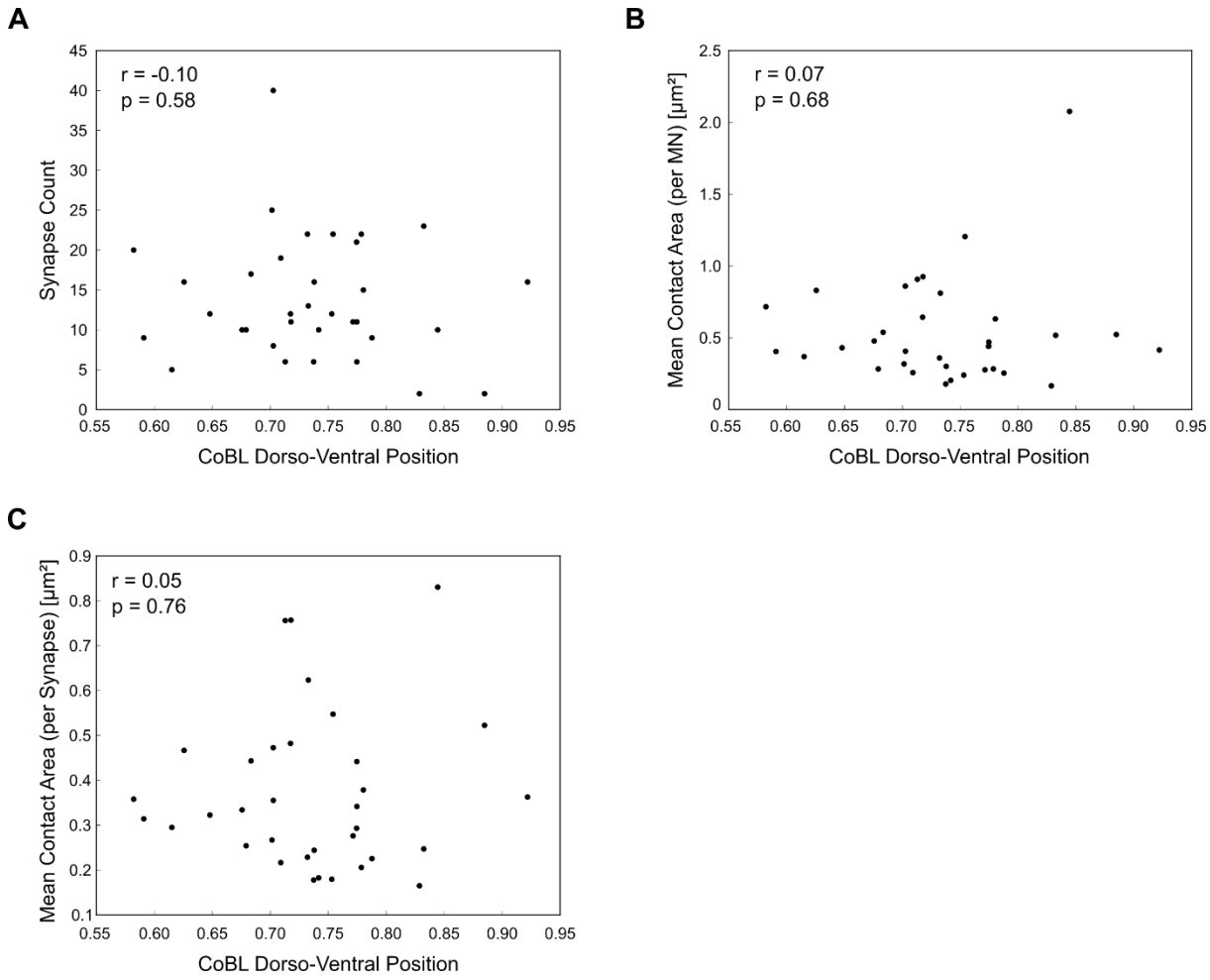

Figure S3. Properties of CoBL synapses by dorso-ventral soma position. Related to Figure 7
(A) Synapse count *vs.* CoBL soma dorso-ventral position.
(B) Mean synaptic contact area per MN *vs.* CoBL soma dorso-ventral position.
(C) Mean synaptic contact area per synapse *vs.* CoBL dorso-ventral position. Correlation and significance refers to Pearson's correlation coefficient.

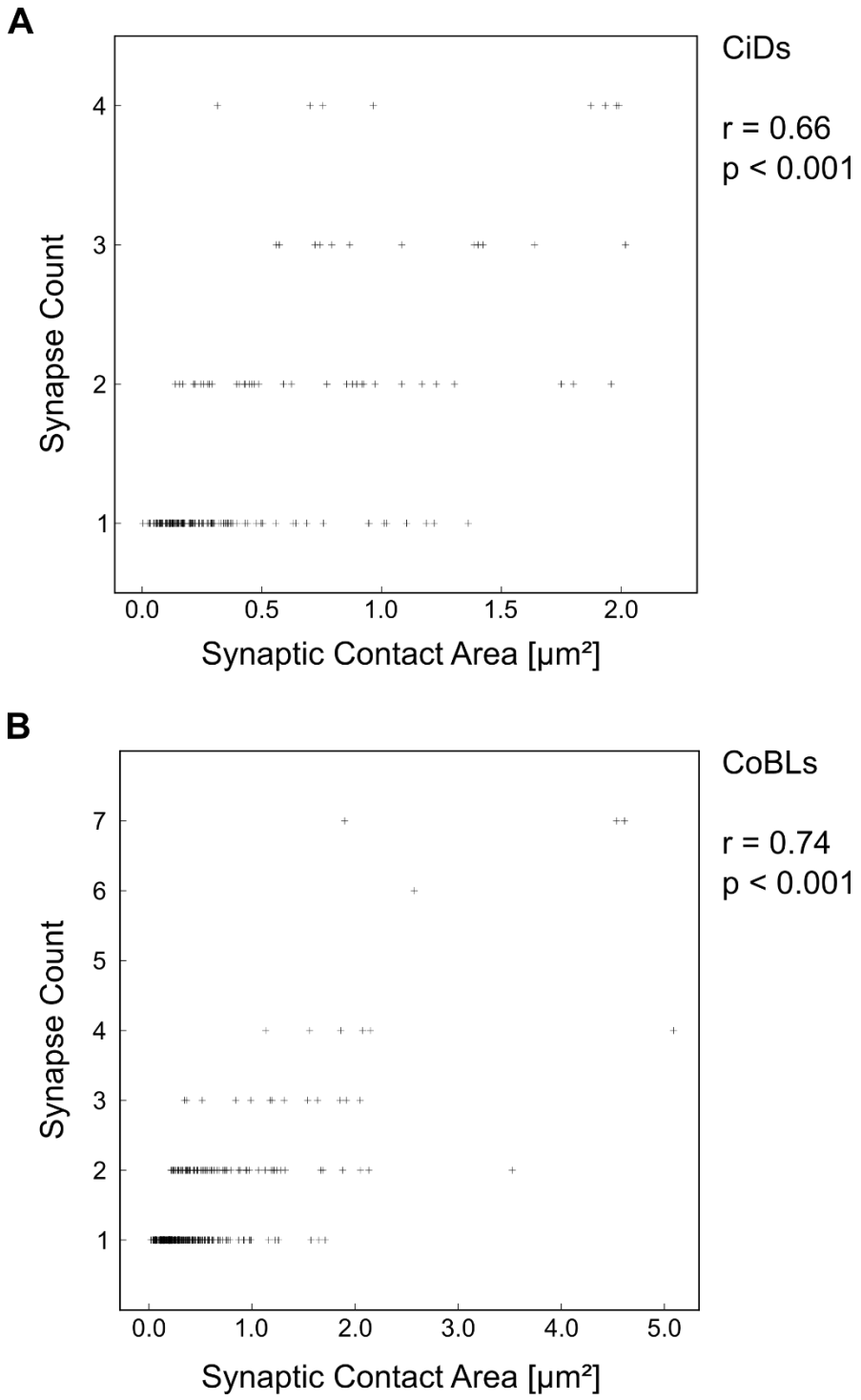

Figure S4. Correlation of number and total area of synapses between different interneuron types (CiDs, CoBLs) and MNs. Related to Figure 5 and Figure 7

Count of synaptic contacts *vs.* summed synaptic contact area between an interneuron and a MN for (A) all pairs of CiDs and MNs, and (B) all pairs of CoBLs and MNs. r- and p-values refer to Pearson's correlation coefficient.

SUPPLEMENTAL REFERENCES

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