Supporting Information (SI)

Targeting CD14 on blood derived cells improves intracortical microelectrode performance

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Section 1: Results

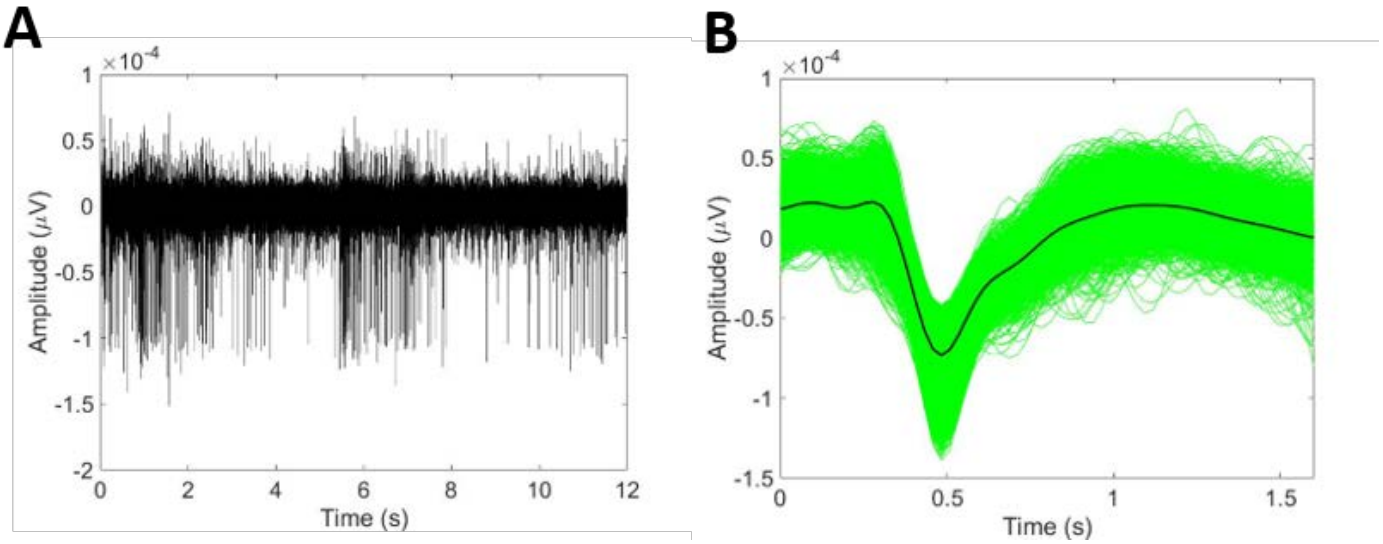

Figure S1. Representative electrophysiological recording. (A) Raw spike channel (300-3000 Hz). (B) A single unit sorted using offline spike sorting (mean waveform in black).

Signal amplitude, noise amplitude, and signal to noise ratio

Both signal and noise amplitude increased after the first week post implantation and then stabilized. Since both noise and signal increased, the signal-to-noise ratio remained relatively stable over time (**Figure S2**). There is no statistical difference in the background noise amplitude among groups (**Figure S2A**). The max amplitude of the single units detected for all groups remained constant through the sixteen-week time course. Furthermore, there is no statistical difference in the max amplitude of the single units detected among groups (**Figure S2B**). Similar to max amplitude, max SNR remained consistent over time for all groups. There was no significant difference in max SNR among groups (**Figure S2C**).

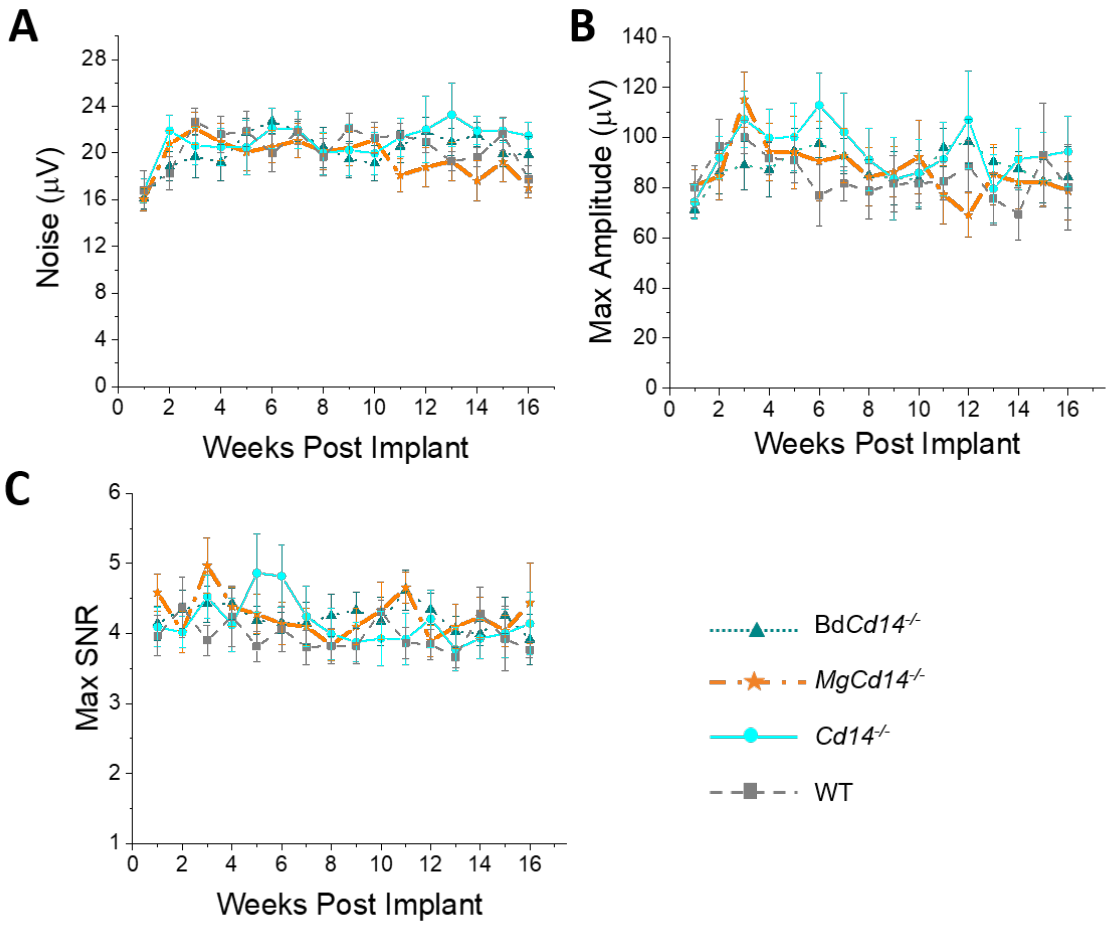

Figure S2. Recording performance for all four conditions (continued). Background noise amplitude (A), max single unit amplitude (B), max single unit signal to noise ratio (C). No significant differences were found for any of the conditions or comparisons.

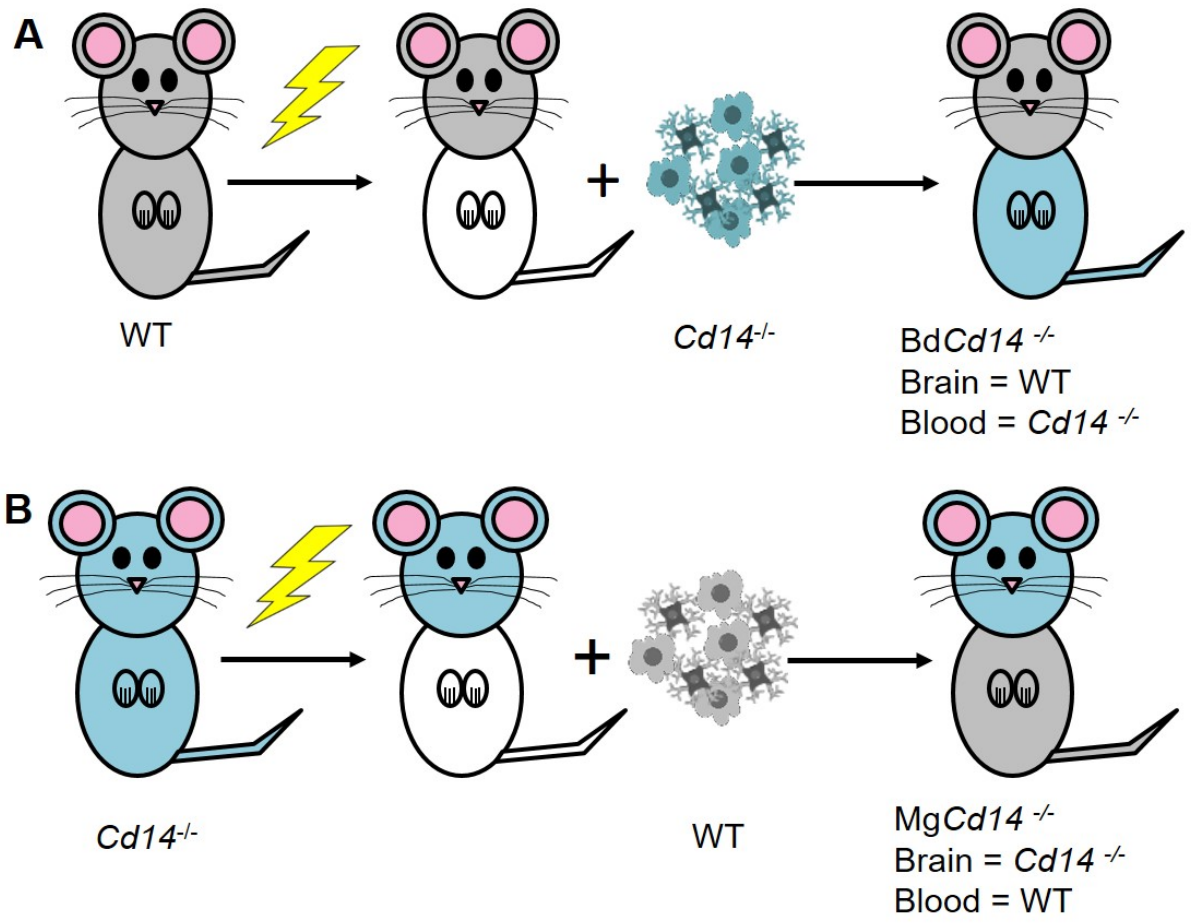

Figure S3. Schematic of creation of bone marrow chimeras. (A) Wildtype (WT) mice were irradiated; bone marrow (BM) cells isolated from non-irradiated *Cd14* $^{-/-}$ mice were transplanted into the irradiated wildtype mice creating Bd*Cd14* $^{-/-}$ chimeras where the CD14 gene was selectively knocked out from only the blood derived cells. (B). *Cd14* $^{-/-}$ mice were irradiated; BM cells isolated from non-irradiated wildtype mice were transplanted into the irradiated *Cd14* $^{-/-}$ mice creating Mg*Cd14* $^{-/-}$ chimeras where the CD14 gene was selectively knocked out from only the resident brain microglia.

Section 2: Methods

Table S1: Complete blood count (CBC) analysis on whole blood samples from WT, *Cd14*$^{-/-}$, Bd*Cd14*$^{-/-}$ chimera, Mg*Cd14*$^{-/-}$ at two weeks post implantation.

Parameter and Normal Range			Wildtype	*Cd14*$^{-/-}$	Bd*Cd14*$^{-/-}$	Mg*Cd14*$^{-/-}$
Leukocytes						
White Blood Cell	1.8 - 10.7	(K/µL)	9.73 ± 2.46	9.96 ± 0.83	9.82 ± 0.65	9.88 ± 1.04
Neutrophil	0.1 - 2.4	(K/µL)	1.70 ± 0.85	1.61 ± 0.25	1.48 ± 0.37	1.44 ± 0.25
Lymphocyte	0.9 - 9.3	(K/µL)	7.37 ± 1.31	7.90 ± 0.66	7.88 ± 0.35	7.79 ± 0.81
Monocyte	0.0 - 0.4	(K/µL)	0.35 ± 0.14	0.30 ± 0.09	0.32 ± 0.09	0.38 ± 0.04
Esonophil	0.0 - 0.2	(K/µL)	0.21 ± 0.11	0.10 ± 0.05	0.10 ± 0.03	0.19 ± 0.03
Basophil	0.0 - 0.2	(K/µL)	0.09 ± 0.07	0.05 ± 0.03	0.04 ± 0.01	0.07 ± 0.01
Erythrocytes						
Red Blood Cell	6.36 - 9.42	(m/µL)	6.53 ± 0.88	6.31 ± 0.93	6.43 ± 0.28	6.80 ± 0.32
Hematocrit	35.1 - 45.4	(%)	38.9 ± 0.91	37.8 ± 2.04	36.5 ± 1.67	36.6 ± 1.19
Thrombocytes						
Platelet	592 - 2972	(K/µL)	715 ± 136	603 ± 65.3	610 ± 56.1	607 ± 85.0

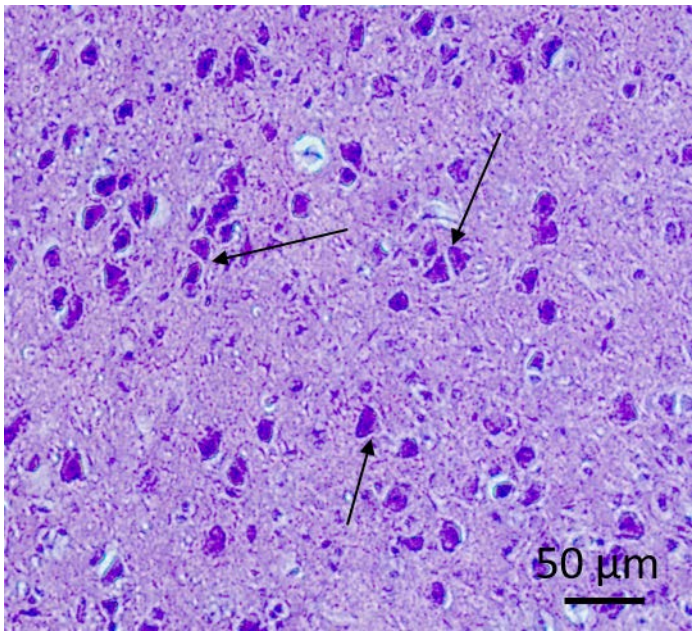

Figure S4. Representative H&E stain of motor cortex about ~640 µm deep from surface of brain. Black arrows show representative large pyramidal neurons. Scale bar: 50 µm.

Immunohistochemistry

Brain slices were incubated in phosphate buffered saline (1X) containing 0.1% Triton X 100 (Sigma) for 15 minutes to permeabilize the cells. Brain tissue sections were then blocked in 4% v/v chicken serum (Invitrogen) for one hour prior to addition of primary antibodies targeting specific antigens and, incubated overnight at 4 °C (**Table 3**). Unbound primary antibody was washed away, and AlexaFluor™ conjugated secondary antibodies corresponding to each of the primary antibodies were then added to their respective tissue sections for two hours at room temperature. DAPI (Molecular Probes D3571) was also added to the secondary antibody solution to stain cell nuclei. After subsequent washes to deplete brain tissue of unbound secondary antibody, tissue autofluorescence was minimized by treating tissue sections with a ten minute incubation of 0.5mM copper sulfate buffer solution, according to protocols previously described (1). Following $CuSO_4$ treatment, all slides were washed thoroughly with MilliQ H_2O, coverslipped using Fluoromount-G, and stored in the dark at 4 °C until imaged.

Mixed effects linear model

A mixed effects linear model is a model that incorporates both fixed (researcher defined) and random effects (a random sample of the population) into the model. Because our experimental design consisted of both fixed (Epoch and group) effects and random effects (subject) a mixed effects linear model was used in this study. Mixed effects linear model is hugely broad in both theoretical content and applicability and any additional information seems unnecessary for the scope of the paper.

Table S2. Primary antibodies used in immunohistochemistry to assess inflammation

Primary antibody	Inflammatory marker	Dilution Factor	Catalogue Number	Company
rat mAb to CD68 [FA-11]	Activated microglia and macrophages	1:500	ab53444	Abcam
rabbit pAB to GFAP	Astrocytes	1:500	RA22101	Neuromics
Rabbit anti-IBA1	Total microglia and macrophage population	1:1000	SAG4318	Wako
Rabbit anti-Ms IgG	BBB permeability	1:1000	STAR26B	AbD Serotec
Goat anti-NeuN IgG1	Neuronal cell bodies	1:250	MAB377	EMD Millipore

3. References:

1. Potter KA, Simon JS, Velagapudi B, & Capadona JR (2012) Reduction of autofluorescence at the microelectrode-cortical tissue interface improves antibody detection. *J Neurosci Methods* 203(1):96-105.