# Supporting Information

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## SI Methods

**Dissection, in Ovo Injections.** Spinal cords from stage 36 (E10) embryos were isolated with intact muscle nerves. The dissection and recording periods took place in the absence of gabazine, CNQX, and APV unless stated otherwise. To determine the effective concentrations of receptor antagonists needed to significantly block activity in ovo, multiple doses of lidocaine or TEA plus 4-AP were injected into the egg. We selected a concentration of lidocaine or TEA plus 4-AP that when added alone only transiently altered in ovo limb movements. The $K^+$ channel antagonists did reduce SNA transiently. This may result from a depolarizing block, because higher concentrations of TEA plus 4-AP reduced SNA more significantly.

**Whole-Cell Electrophysiology.** Whole-cell current clamp recordings of rheobase currents were acquired by using an AxoClamp 2B amplifier, controlled by pClamp 10.1 software (Axon Instruments) running on a laptop computer (Apple). Voltage clamp experiments to measure individual currents and current clamp recordings of firing rates were performed by using an AxoPatch ID amplifier, controlled by pClamp 10.1. Series resistance and whole-cell capacitance were estimated by using the amplifier's built-in circuitry. Currents were analyzed by using Clampfit (Axon Instruments). Series resistance was compensated by 70–80%. Linear leak and residual capacity currents were subtracted by using a P/4 subtraction protocol. Junction potentials for the various solutions were 3–7 mV and were left uncorrected. Currents were analyzed by using Clampfit (Axon Instruments).

To obtain the $K^+$ current activation curves, values of $K^+$ chord conductance (G) were calculated from the respective peak currents. The $K^+$ equilibrium potential was determined by using the Nernst equation. Activation curves were fit with the Boltzmann equation described by the following function: $y = 1/[1 + (\exp(V - V_{1/2})/\text{slope})]$, where V is the activation voltage and $V_{1/2}$ is the half-activation voltage (1).

**Extracellular and Intracellular Solutions.** All recordings were performed in oxygenated (5% $CO_2$/95% $O_2$) saline solution at 27 °C. For all external solutions, the osmolarity was 305–315 mOsm. The osmolarity for all intracellular solutions was 289–291 mOsm. All solutions had a pH of 7.2–7.3.

The external Tyrode's solution for recordings of rheobase currents and instantaneous firing rates contained (in mM): NaCl 139, KCl 5, $CaCl_2$ 3, $MgCl_2$ 1, $NaHCO_3$ 17, and D-glucose 12. The intracellular solution was (in mM): NaCl 10, KCl 36, K-gluconate 94, $CaCl_2$ 0.1, $MgCl_2$ 1, Hepes 10, EGTA 1.1, $Na_2$-ATP 1, and Mg-GTP 0.1.

The external solution for recording $I_{Na}$ was the same as Tyrode's, with the addition of (in mM): CdCl 0.4, TEA-Cl 30, 4-AP 5, gabazine 0.02, CNQX 0.02, and APV 0.05. The intracellular solution was the same as above, with the addition of (in mM): TEA-Cl 10, verapamil 0.1, Bapta 11, and CsCl 5.

For whole-cell recordings of $I_{Ca}$, the external solution contained (in mM): NaCl 33, choline chloride 97, TEA-Cl 30, $MgCl_2$ 2, Hepes 20, $CaCl_2$ 10, 4-AP (5), D-glucose 12, and TTX 0.001. Intracellular solution was (in mM): $CsMeSO_4$ 120, TEA-Cl 10, $MgCl_2$ 5, Hepes 10, EGTA 10, $Na_2$-ATP 1, and Mg-GTP 0.5. For all extracellular calcium-free solutions, $CaCl_2$ was replaced by an equimolar concentration of $MgCl_2$.

The external solution for $I_K$ and $I_A$ experiments contained (in mM): NaCl 145, KCl 5.4, $MgCl_2$ 0.8, $CaCl_2$ 3.4, Hepes 13, D-glucose 5, and TTX 0.001. The intracellular solution contained (in mM): KCl 120, $MgCl_2$ 2, Hepes 10, EGTA 10, $Na_2$-ATP 1, and Mg-GTP 0.5.

TTX, gabazine, CNQX, and $CsMeSO_4$ were purchased from Tocris Cookson. All other chemicals and drugs were purchased from Sigma–Aldrich.

1. Brown AM (2001) A step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet. *Comput Methods Programs Biomed* 65:191–200.

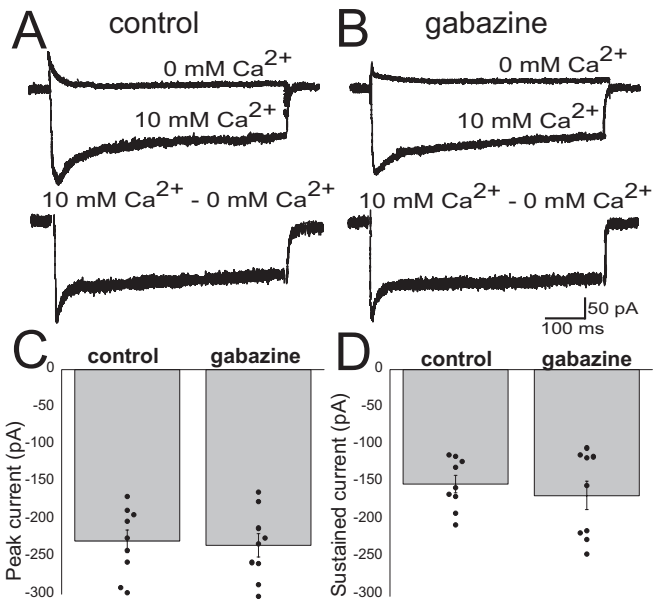


**Fig. S1.** HVA calcium currents were not affected by 12 h of gabazine treatment. Sample traces of control (*A*) and gabazine-treated (*B*) $Ca^{2+}$ currents elicited by depolarizing voltage steps to −10 mV from a holding potential of −60 mV in the presence and absence of external $Ca^{2+}$ (*Upper*). Net currents were assessed by subtracting the current elicited without $Ca^{2+}$ from current elicited with external $Ca^{2+}$ (*Lower*). The average currents produced reveal that $GABA_A$ block ($n$ = 9) did not alter either peak (*C*) or sustained (*D*) calcium currents compared with control ($n$ = 9).

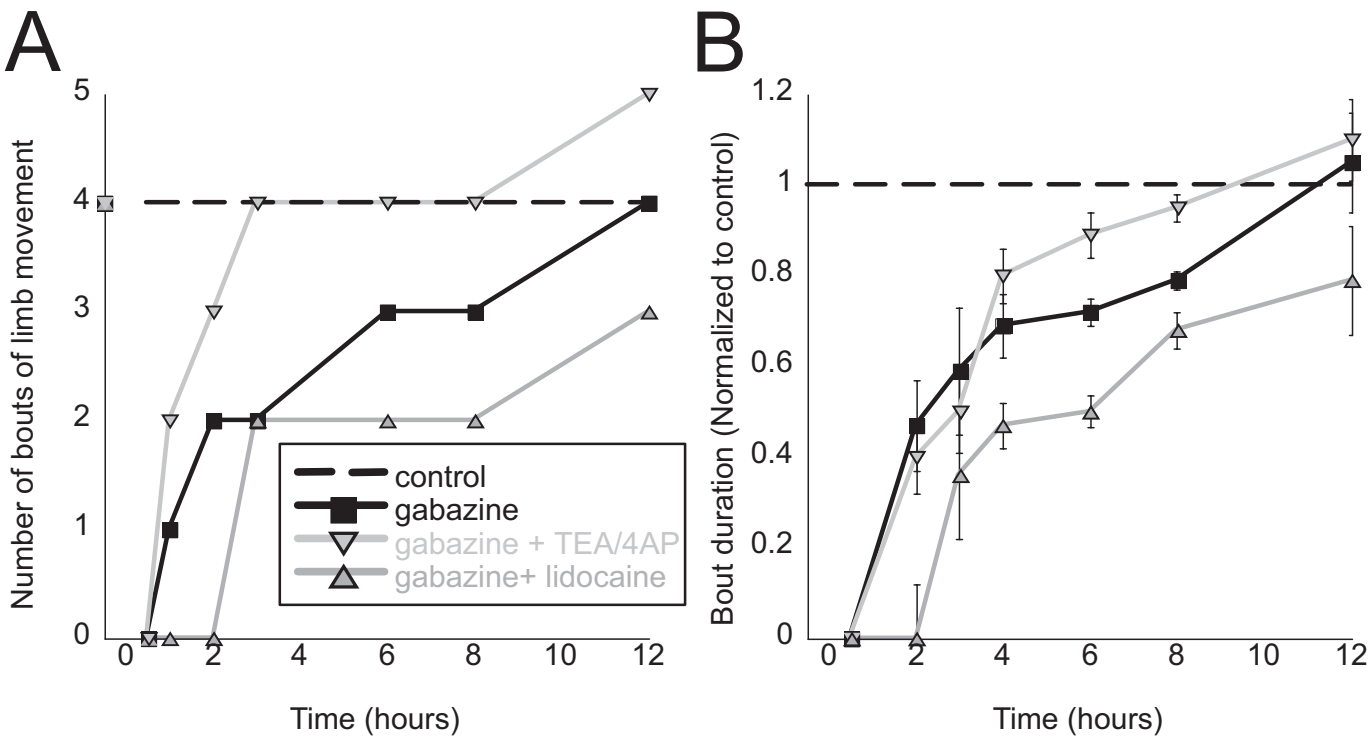


**Fig. S2.** Reducing $Na^+$ or $K^+$ currents in addition to blocking $GABA_A$ receptor activation altered the recovery of limb movements. Graph of the median number of bouts or episodes of SNA (*A*) or average bout duration (*B*) during a 5-min period of observation. Error bars indicate SEM.