Supplemental Material

Data Tables

	Parameter	Fed	Fasted	N	p Value
DBA	SWS Count	3.2 ± 1.1	9.4 ± 1.7	10	0.0078
	SWS Duration (s)	2.6 ± 0.7	3.4 ± 0.4	10	0.375
	Seizure Burden (%)	0.08 ± 0.03	0.2 ± 0.03	10	0.0098
	Blood Glucose (mg/dL)	165.6 ± 14.3	132.1 ± 10.8	10	0.048
	β-hydroxybutyrate (mM)	2.0 ± 0.2	8.2 ± 0.8	10	0.002
	Blood Glucose (mg/dL) vs SWS Counts	r = -0.23	r^2 = 0.053		p = 0.31
	β-hydroxybutyrate (mM) vs SWS Counts	r = 0.52	r^2 = 0.27		p = 0.020
WAG	SWS Count	21.9 ± 7.10	34.9 ± 9.7	13	0.0044
	SWS Duration (s)	8.2 ± 1.7	10.1 ± 1.5	13	0.17
	Seizure Burden (%)	1.8 ± 0.7	3.1 ± 0.9	13	0.0006
	Blood Glucose (mg/dL)	114.1 ± 6.2	75.4 ± 4.8	11	0.00097
	β-hydroxybutyrate (mM)	1.1 ± 0.9	3.3 ± 0.4	11	0.002
	Blood Glucose (mg/dL) vs SWS Counts	r = -0.37	r^2 = 0.14		p = 0.094
	β-hydroxybutyrate (mM) vs SWS Counts	r = 0.15	r^2 = 0.023		p = 0.49

Table S1 SWSs during acute fasting. *N* refers to animal number. Data correspond to those in Figure 1 and Supplementary Figure 1. Data values are rounded to the nearest tenth.

	Parameter	Saline	Insulin	N	p Value
DBA	SWS Count	6.1 ± 1.8	13.4 ± 2.9	11	0.0195
	SWS Duration (s)	2.8 ± 0.4	3.3 ± 0.2	11	0.83
	Seizure Burden (%)	0.10 ± 0.03	0.3 ± 0.06	11	0.049
	Blood Glucose (mg/dL)	157.3 ± 17.3	63.0 ± 4.0	4	0.13
	β-hydroxybutyrate (mM)	1.7 ± 0.2	1.2 ± 0.07	4	0.13
	Blood Glucose (mg/dL) vs SWS Counts	r = -0.31	r^2 = 0.096		p = 0.45
	β-hydroxybutyrate (mM) vs SWS Counts	r = -0.34	r^2 = 0.12		p = 0.42
WAG	SWS Count	15.6 ± 4.1	28.6 ± 7.5	12	0.036
	SWS Duration (s)	8.5 ± 1.6	8.6 ± 1.8	12	0.92
	Seizure Burden (%)	0.98 ± 0.3	1.7 ± 0.6	12	0.012
	Blood Glucose (mg/dL)	111.6 ± 4.3	46.4 ± 5.1	9	0.0039
	β-hydroxybutyrate (mM)	1.1 ± 0.2	1.0 ± 0.1	9	0.36
	Blood Glucose (mg/dL) vs SWS Counts	r = -0.48	r^2 = 0.23		p = 0.046
	β-hydroxybutyrate (mM) vs SWS Counts	r = -0.24	r^2 = 0.058		p = 0.33
	Parameter	**Saline**	**2-DG**	**N**	**p Value**
	SWS Count	9.2 ± 2.4	19.7 ± 4.6	9	0.01
	SWS Duration (s)	6.1 ± 1.9	6.8 ± 0.36	9	0.56

Table S2 SWSs during acute insulin and 2-DG. *N* refers to animal number. Data correspond to those found in Figure 2 and Supplementary Figure 2. Data values are rounded to the nearest tenth.

	Manipulation		Control	Metformin	n (N)		p Value
Rebound Action Potentials (#)	10 mM Metformin	-140 pA	5.7 ± 0.8	6.5 ± 1.0	13 (7)		0.70
Depolarization-Evoked Action Potentials (#)	10 mM Metformin	+20 pA	0 ± 0	0 ± 0	13 (7)		-
		+40 pA	0.8 ± 0.7	2.6 ± 1.2			0.11
		+60 pA	2.6 ± 2.4	9.7 ± 4.3			0.054
		+80 pA	6.7 ± 3.5	21.4 ± 8.5			0.039
		+100 pA	29.1 ± 15.4	36.8 ± 12.4			0.36
		+120 pA	29.3 ± 12.3	49.6 ± 15.7			0.023
		+140 pA	39.2 ± 15.3	64.8 ± 20.3			0.023
		+160 pA	48.8 ± 16.9	80.8 ± 25.2			0.009
		+180 pA	61.6 ± 19.7	101.2 ± 7.0			0.016
		+200 pA	74.6 ± 23.1	113.2 ± 28.5			.018
AMPKAR-FRET	10 mM Metformin		1.00 ± 0.0021	1.004 ± 0.0042	13(3)		0.0002
	100 nM A-769662		1.001 ± 0.0028	1.013 ± 0.014	10(5)		0.0129
	Manipulation		**Control**	**n (N)**	**Drug**	**n (N)**	**p Value**
Baclofen Puff	1 mM AMP		96% ± 4%	5 (6)	110% ± 6%	9 (8)	0.06
	1 mM Metformin		88% ± 9%	7 (6)	102% ± 3%	9 (6)	0.02
	100 nM A-769662		85% ± 10%	5 (6)	104% ± 4%	10 (6)	0.02

Table S3 Neuron excitability, AMPKAR-FRET and baclofen application. *n* refers to cell number. *N* refers to animal number. Data correspond to those found in Figure 3 and Supplementary Figure 4. Data values are rounded to the nearest tenth.

Experiment	Baseline Duration (sec)	Drug Duration (sec)	n (N)	p-Value
Oscillations Metformin (5 mM)	5.5 ± 0.8	6.9 ± 0.8	11 (7)	0.0027
Oscillations A-769662 (10 µM)	7.8 ± 1.2	8.9 ± 1.2	15 (11)	0.0082
Oscillations CGP-54626 (20 nM)	2.2 ± 0.51	2.3 ± 0.54	11 (6)	0.43
Oscillations A-769662 (Fig. S5)	4.8 ± 1.3	6.1 ± 1.7	5 (3)	0.06
Oscillations Bicuculline (Fig. S5)	7.7 ± 0.8	7.1 ± 0.8	6 (3)	0.23

A-769662 (10 µM) EEG and Infusion	**# Saline SWSs**	**#A-769662 SWSs**	**p – Value**	**Saline SWS Duration**	**A-769662 SWS Duration**	**p - Value**	**N**
	11.2 ± 4.5	22.5 ± 5.2	0.017	7.9 ± 0.5	7.75 ± 0.5	0.72	10

Table S4 AMPK activation and thalamic oscillations. *n* refers to slice number. *N* refers to animal number. Data correspond to those found in Figure 4 and Supplementary Figure 5. Data values are rounded to the nearest tenth.

	Rat Strain	# Saline SWSs	# Metformin SWSs	N	p Value	Lactate Saline (mmol/L)	Lactate Metformin (mmol/L)	N	p Value
150 mg/kg metformin	WAG/Rij	1.5 ± 4.6	32.7 ± 5.8	6	0.22	0.46 ± 0.1	0.88 ± 0.2	7	0.16
	Wistar	0 ± 0	0.2 ± 0	5	1	0.51 ± 0.1	2.7 ± 1.4	5	0.19
200 mg/kg metformin	WAG/Rij	14.2 ± 2.8	49.8 ± 11.5	6	0.036	0.42 ± 0.11	5.1 ± 1.4	7	0.016
	Wistar	0 ± 0	0.2 ± 0	5	1	0.49 ± 0.04	6.8 ± 1.9	5	0.03

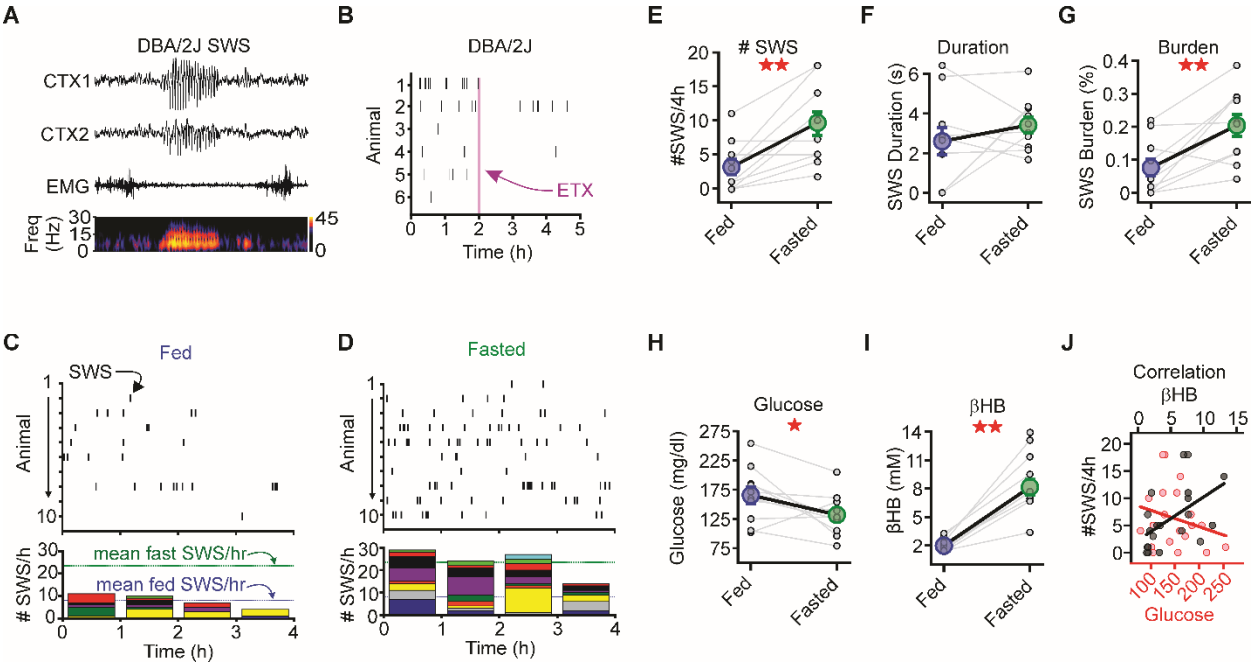
	Baseline Duration (sec)	3Cl-OH-BA Duration (sec)	n (N)	p Value
Evoked Oscillations	4.8 ± 1.2	6.7 ± 1.5	4 (4)	0.025

Table S5 SWSs and metformin. *n* refers to cell number. *N* refers to animal number. Data correspond to Figure 5 and Supplemental Figure 6. Most data values are rounded to the nearest tenth.

		Cortex	Dentate Gyrus	Reticular Thalamus		Ventrobasal Thalamus
Immunohisto-Chemistry (A.U.)		23 ± 4.6	40.5 ± 0.6	32.0 ± 3.2		23.0 ± 2.3
		Cortex	**Thalamus**			
qPCR (Copy #)		4.1 ± 0.2	4.1 ± 0.2			
ΔCt		10.9 ± 0.7	10.2 ± 0.2			
		Manipulation	**Control**		**n (N)**	**p Value**
DBA/2J Mouse	Holding Current (pA)	10 µM Diazoxide	-0.67 ± 2.3	2.3 ± 7.9	8 (2)	0.57
		100 µM Diazoxide	-2.11 ± 2.0	56.9 ± 22.7	7 (4)	0.038
		250 µM Diazoxide	-0.35 ± 2.3	123.7 ± 19.7	8 (4)	0.0016
		500 µM Diazoxide	-1.4 ± 2.7	172.7 ± 34.6	6 (5)	0.022
		100 nM Glibenclamide + 500 µM Diazoxide	-0.57 ± 0.49	-1.8 ± 1.0	4 (2)	0.69
		Low Glucose	1.1 ± 0.55	15.2 ± 10.6	14 (12)	0.18
	Membrae Resistane (MΩ)	Low Glucose	218.5 ± 20.4	210.1 ± 23.5	14 (12)	0.77
WAG/Rij Rat	Holding Current (pA)	500 µM Diazoxide	2.8 ± 1.3	69.2 ± 9.4	8 (5)	0.00016
		1 µM Glibenclamide + 500 µM Diazoxide	-0.57 ± 0.49	-1.8 ± 1.0	8 (4)	0.69
		Low Glucose	1.2 ± 1.1	-1.4 ± 13.7	11 (5)	0.29
	Membrane Resistance (MΩ)	Low Glucose	151.3 ± 23.6	147.0 ± 20.0	11 (5)	0.95

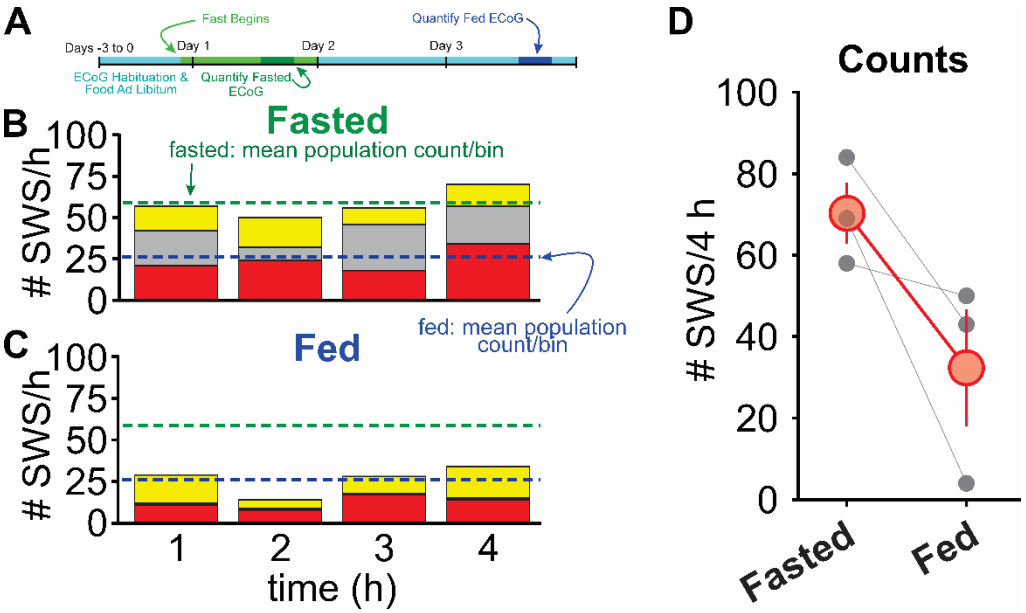
Table S6 KATP channel expression and glucose responsivity. *n* refers to cell number. *N* refers to animal number. Data correspond to those found in Supplementary Figure 3.

Supplemental Figures

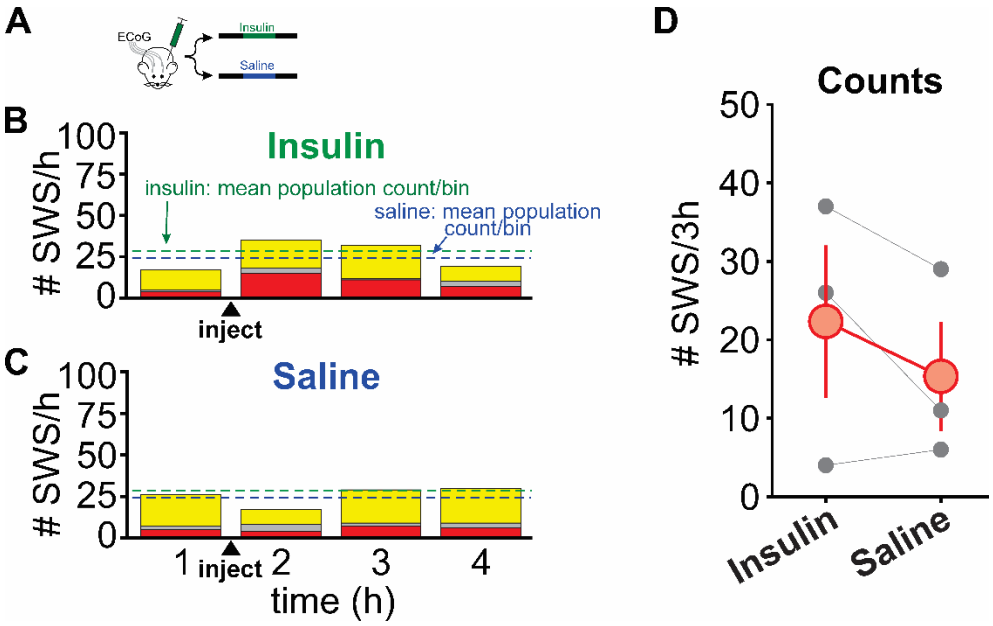


Supplemental Figure 1. Overnight fasting increases spike-and-wave discharges in DBA/2J mice.

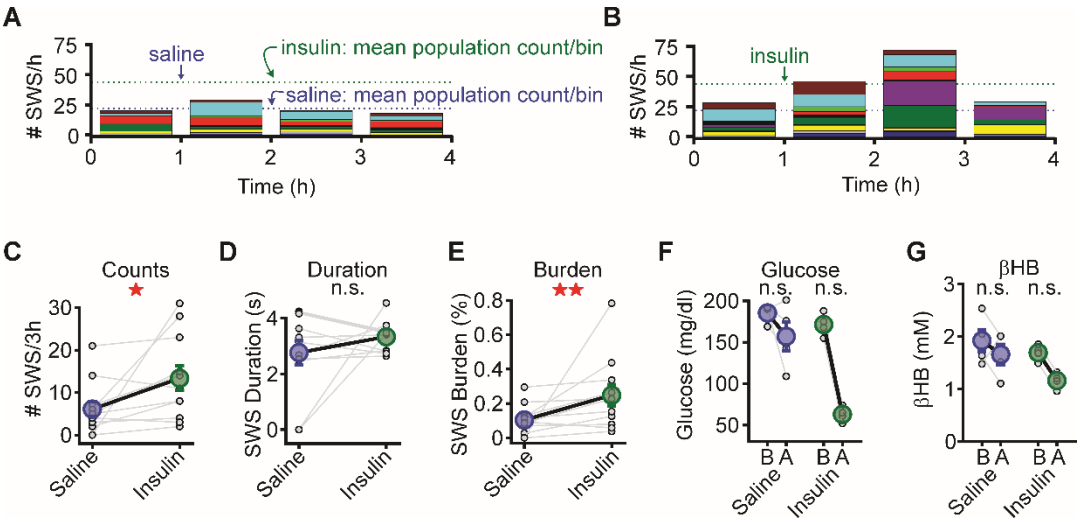
(A) *Top:* Representative SWS from DBA/2J mouse. CTX1 and CTX2 are cortical ECoG recordings while EMG recording is from the neck. EMG activity was suppressed during the SWS, corresponding to behavioral arrest. *Bottom:* Spectrograms from CTX1 showing increased power in the 5-8 Hz frequency band during the SWS. **(B)** Ethosuximide (ETX; 200 mg/kg) suppressed SWSs in the DBA/2J mouse. Purple line indicates i.p. injection of ETX. **(C, D)** *Top:* SWS rasters during the fed and fasted conditions. *Bottom:* Stacked histograms showing hourly SWS count for each mouse during fed **(C)** and fasted **(D)** conditions. Blue and green dashed lines represent mean SWSs count during fed and fasted conditions, respectively. **(E)** The number of SWSs was 3-fold higher during the fasted period than during the fed period ($p = 0.0078$, $n = 10$; Table S1). **(F)** Fasting did not affect SWS duration ($p = 0.375$, $n=10$; Table S1). Mice that had no SWSs were assigned a duration value = 0 sec. **(G)** SWS burden was 2.5-fold higher post-fast relative to the fed state ($p = 0.0098$, $n=10$; Table S1). **(H)** Fasting decreased blood glucose ($p = 0.048$, $n = 10$; Table S1). **(I)** Fasting increased β-hydroxybutyrate ($p = 0.002$, $n = 10$; Table S1) relative to the fed state. **(J)** Blood glucose (red) or serum β-hydroxybutyrate (black) versus SWS count in mice. For each panel, small circles represent data from one animal, while large circles represent the sample mean (± SE). * $p < 0.05$, ** $p < 0.01$, not significant (n.s.) from the Wilcoxon sign rank test. See Table S1 for details.



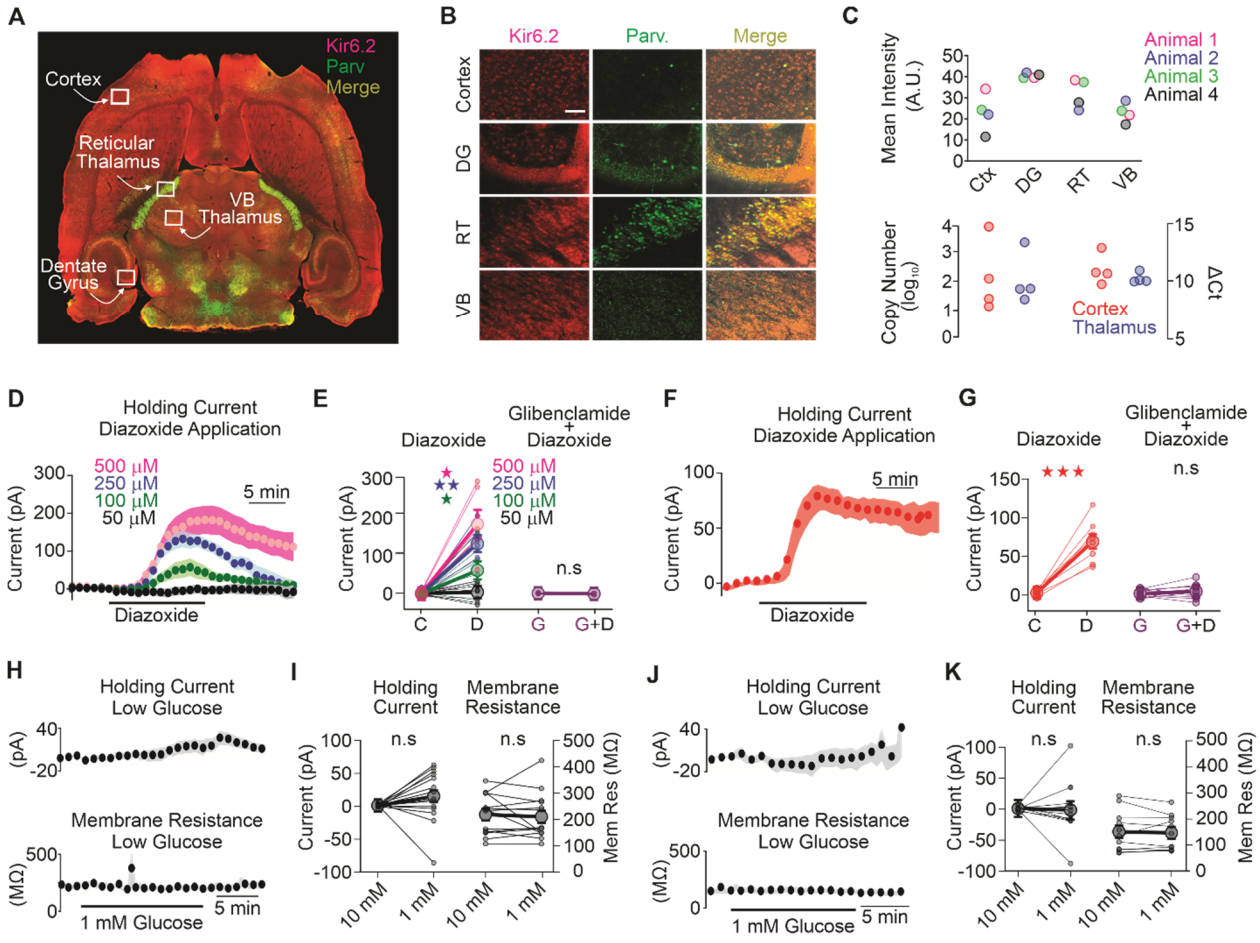
Supplemental Figure 2. SWS count is higher for fasted rats when performing fasted experiment before fed experiment. A. Experimental approach. Animals were fed ad libitum prior to experimentation. Prior to the first experimental day, food was removed and rats were fasted for 18 hours prior to evaluating SWS count. After counting for four hours, rats were supplied with food. After one day of rest, SWS count was evaluated in fed rats. Thus, the experiment was performed in the reversed order, relative to those shown in Figure 1. **B, C**. Stacked histogram of SWS count for three rats during the fasted (B) and fed (C) experiment (n=3 rats). **D.** Quantification of total SWS count.



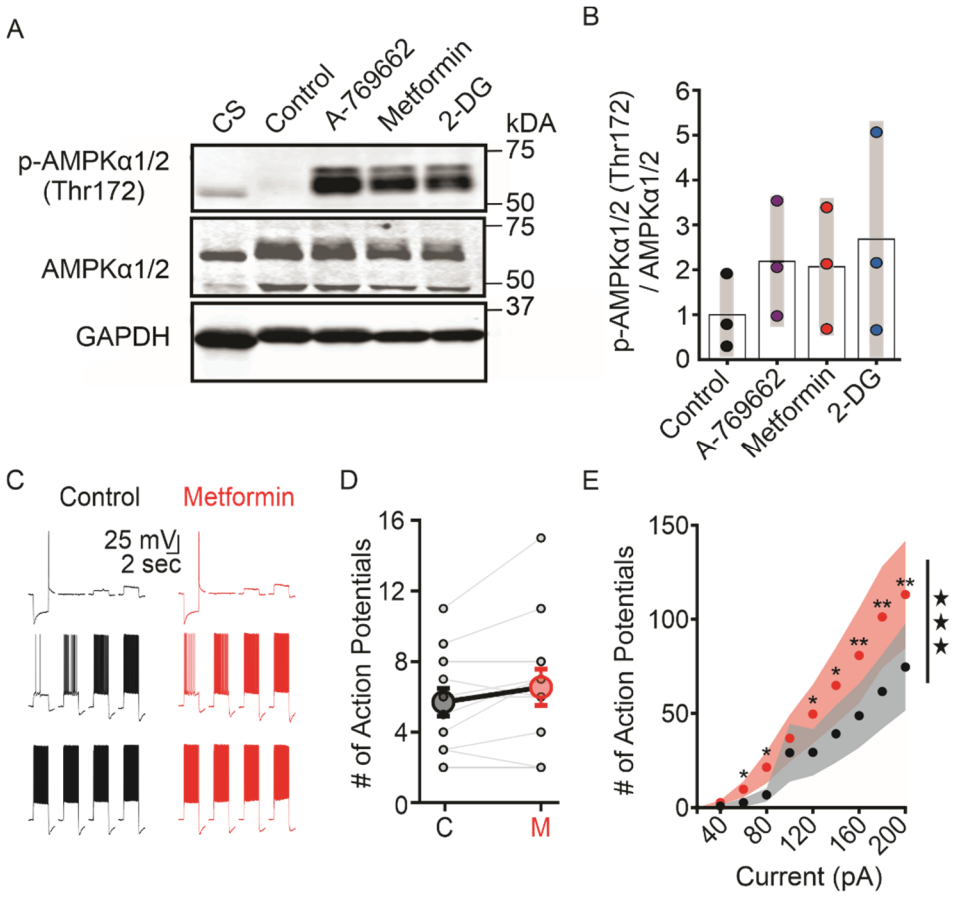
Supplemental Figure 3. SWS count is higher for fasted rats when performing insulin experiment before saline experiments. A. Experimental approach. Animals were fed ad libitum throughout experimentation. On the first experimental day, insulin was injected and SWS count was evaluated for three hours. After one day of rest, SWS count was evaluated in rats injected with saline. Thus, the experiment was performed in the reversed order, relative to those shown in Figure 2B. **B, C**. Stacked histogram of SWS count for three rats following insulin injection (B) and following saline injection (C) experiment (n=3 rats). **D.** Quantification of total SWS count.



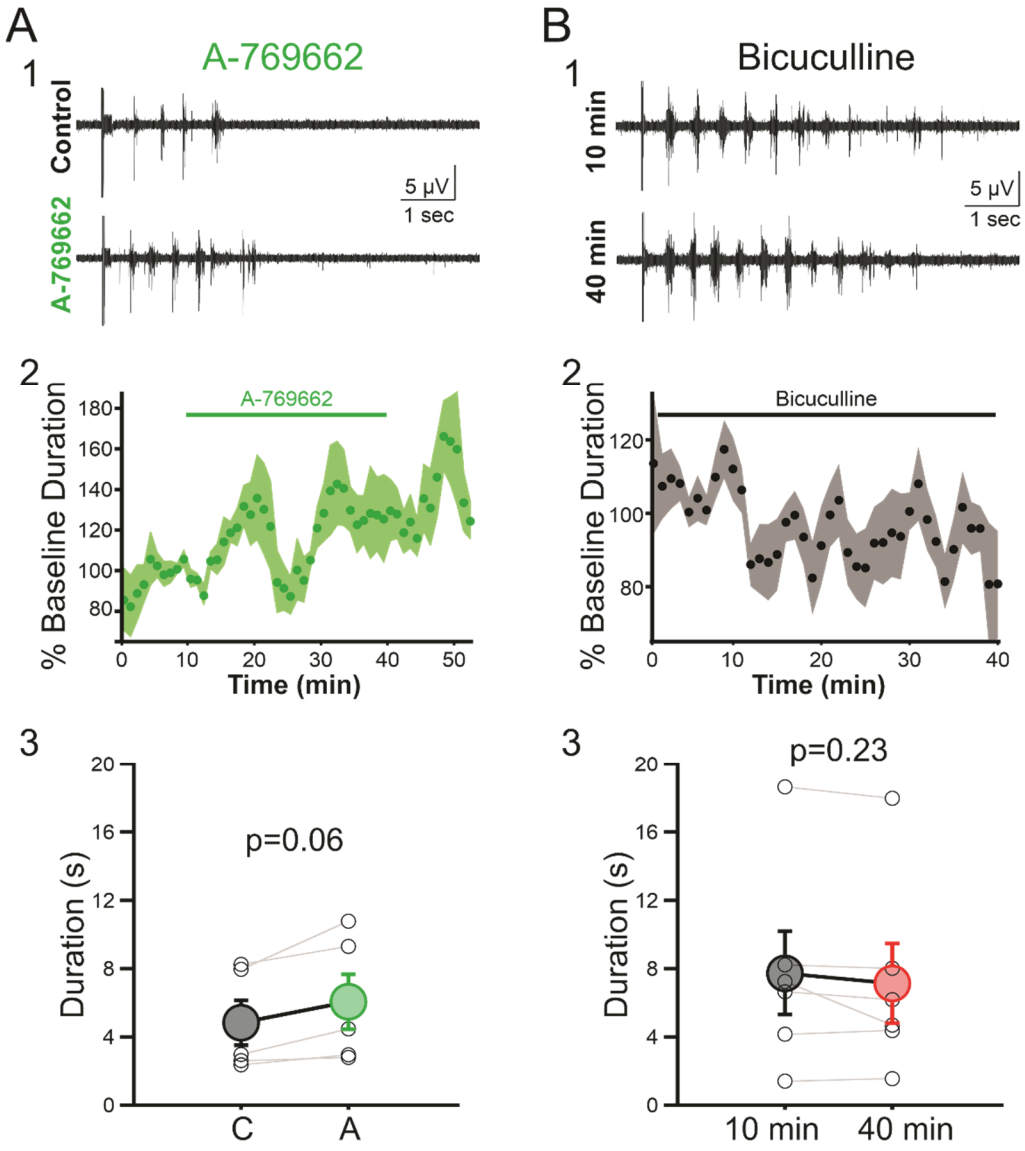
Supplemental Figure 4. Elevated SWS count tracks with low blood glucose in DBA/2J mice. **(A, B)** Stacked histograms showing SWS counts for DBA/2J mice after saline **(A)** or insulin **(B)** injection. Blue dashed lines represent the mean SWS count after saline. Green dashed lines represent the mean SWS count after insulin. **(C)** Insulin increased mean SWS count ($p = 0.0195$, $n = 11$; Table S2). **(D)** SWS duration did not change after insulin injection ($p = 0.83$, $n = 11$; Table S2). Mice that had no SWSs were assigned a duration value = 0 sec. **(E)** Insulin increased SWS burden ($p = 0.049$, $n = 11$; Table S2). **(F)** Insulin produced a trend towards decreased blood glucose ($p = 0.13$, $n = 4$; Table S2). **(G)** Insulin had no effect on β-hydroxybutyrate ($p = 0.13$, $n = 4$; Table S2). In each panel, small circles represent data from one animal, while large circles represent the sample mean (± SE). * $p < 0.05$, ** $p < 0.01$, not significant (n.s.) from paired t-test. See Table S2 for details.



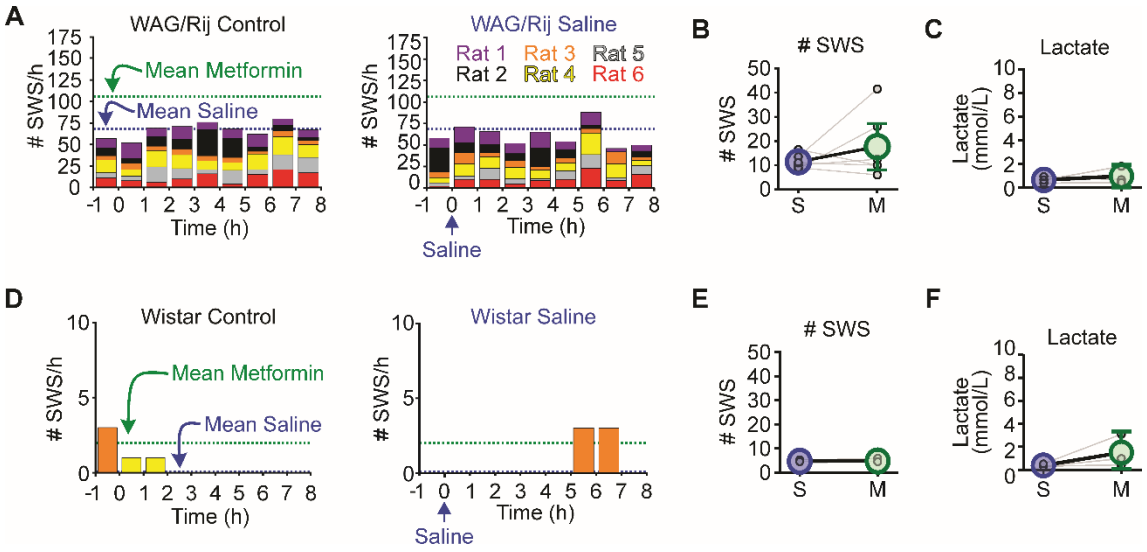
Supplemental Figure 5. K_{ATP} channel expression in thalamocortical neurons.
(A) Kir6.2 (magenta) and parvalbumin (green) expression in the mouse brain. White boxes indicate structures shown in Fig. 3B. Scale bar = 500 µM. **(B)** Kir6.2 expression in cortex, dentate gyrus (DG), reticular (RT) and ventrobasal (VB) thalamus was qualitatively similar. Scale bar = 50 µM. **(C)** *Top:* Mean Kir6.2 intensity in each structure across four mice. Each circle represents one animal. *Bottom*: Absolute and relative Kir6.2 mRNA expression in mouse thalamus and cortex. *Bottom, left axis*: total Kir6.2 copy number. *Bottom, right axis*: Kir6.2 mRNA expression relative to cyclophilan A. Each circle represents one sample. **(D)** Diazoxide, a K_{ATP} channel opener, induced a dose-dependent outward current in mouse thalamocortical neurons, indicating functional channel expression. **(E)** *Left*: Mean holding current change at each diazoxide dose. *Right*: Mean holding current in 500 µM diazoxide + 100 nM glibenclamide, a selective K_{ATP} channel blocker. **(F, G)** As in mice, 500 µM diazoxide application induced a glibenclamide-sensitive outward current in WAG/Rij thalamocortical neurons. **(H)** Low glucose did not alter thalamocortical neuron activity in DBA/2J mice. *Top*: holding current; *Bottom:* membrane resistance. **(I)** Mean holding current (*left*) and mean membrane resistance (*right*) did not change in 1 mM glucose relative to 10 mM glucose for DBA/2J mouse. **(J, K)** Likewise, low glucose did not affect these parameters in WAG/Rij rat thalamocortical neurons. In each panel, small circles represent data from one animal, whereas large circles represent the sample mean (± SE). * $p < 0.05$, **$p< 0.01$, ***$p<0.001$, not significant (n.s.) from paired t-test. See Table S6 for details.



Supplemental Figure 6. P-AMPK protein expression and firing properties in thalamocortical neurons with pharmacological activation of AMPK.
(A) Relative p-AMPK/total AMPK protein expression in acute thalamic slices incubated with AMPK agonists. Representative western blot illustrating p-AMPK expression after 30-minute exposure to control (standard ACSF) or solutions containing AMPK activators (*left* to *right*): ACSF + 100 nM A-769662, 10 mM metformin or 27 µM 2-DG. CS lane represents a standard sample of a whole tissue lysate mixture of liver, heart, and skeletal muscle. **(B)** Fold change in p-AMPK expression relative to total AMPK across conditions normalized to mean control p-AMPK values. Results of one-way ANOVA: $F(3,8) = 0.63$, $p = 0.62$. Each circle represents an experiment. **(C)** Representative recording of WAG/Rij thalamocortical neuron during hyperpolarizing and depolarizing current injections (black: ACSF; red: ACSF + 10 mM metformin). **(D)** Metformin did not significantly alter the number of rebound burst action potentials relative to saline following hyperpolarizing current injections. **(E)** Metformin altered the number of action potentials evoked by depolarizing current injection. The F-I plot shows the effect metformin had on thalamocortical neuron firing activity during depolarizing current steps (+20 pA increments). Each circle represents mean ± SE current responses at each step; *p <0.05, **p< 0.01, ***p<0.001, not significant (n.s.); Friedman's test, Wilcoxon sign rank test for pairwise comparison. See Table S4 for details.



Supplemental Figure 7. Oscillations evoked in the naïve thalamic slice trend towards longer durations during AMPK activation. (A) 400 µm slices from WAG/Rij rats were recorded in the absence of any GABA receptor (A or B) blockade. (1) Oscillatory activity was evoked with a 20Hz burst of 10 electrical stimuli and AMPK was activated with A-769662. (2) AMPK activation produced a trend towards longer duration oscillations (n=5). This trend was not significant (p=0.06). **(B)** The duration of thalamic oscillations evoked only in the presence of $GABA_A$ receptor blockade (i.e., 10 µM bicuculline, (1) are generally stable (2) insofar the duration evoked at 10 minutes is similar to the duration evoked at 40 minutes (3). See Table S4 for further details.



Supplemental Figure 8. SWSs and lactate measurements at 150 mg/kg metformin
(A) WAG/Rij rat SWS counts for control and saline injections for the 150 mg/kg metformin experiment. Saline injection did not affect SWS occurrence relative spontaneous (i.e. not injected) epochs. Stacked histograms showing SWS counts per animal for all conditions. Dotted lines indicate mean SWS count following saline (blue) and 150 mg/kg metformin (green) injection, respectively. **(B, C)** SWS counts and lactate (mmoL/L) trended higher two hours after 150 mg/kg metformin injection. **(D)** Non-epileptic Wistar rat SWS counts for control and saline injections for the 150 mg/kg metformin experiment. **(E, F)** 150 mg/kg metformin did not change SWS counts in Wistar rats despite similar changes in lactate (mmol/L) between the two strains. In each panel, small circles represent data from one animal, whereas large circles represent the sample mean (± SE); not significant (n.s.) from Wilcoxon test (WAGs) and paired student's t-test (Wistars). See Table S5 for details.