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Supplemental Information

Intrinsically Active and Pacemaker Neurons in Pluripotent Stem Cell-Derived Neuronal Populations

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Inventory of all supplemental material

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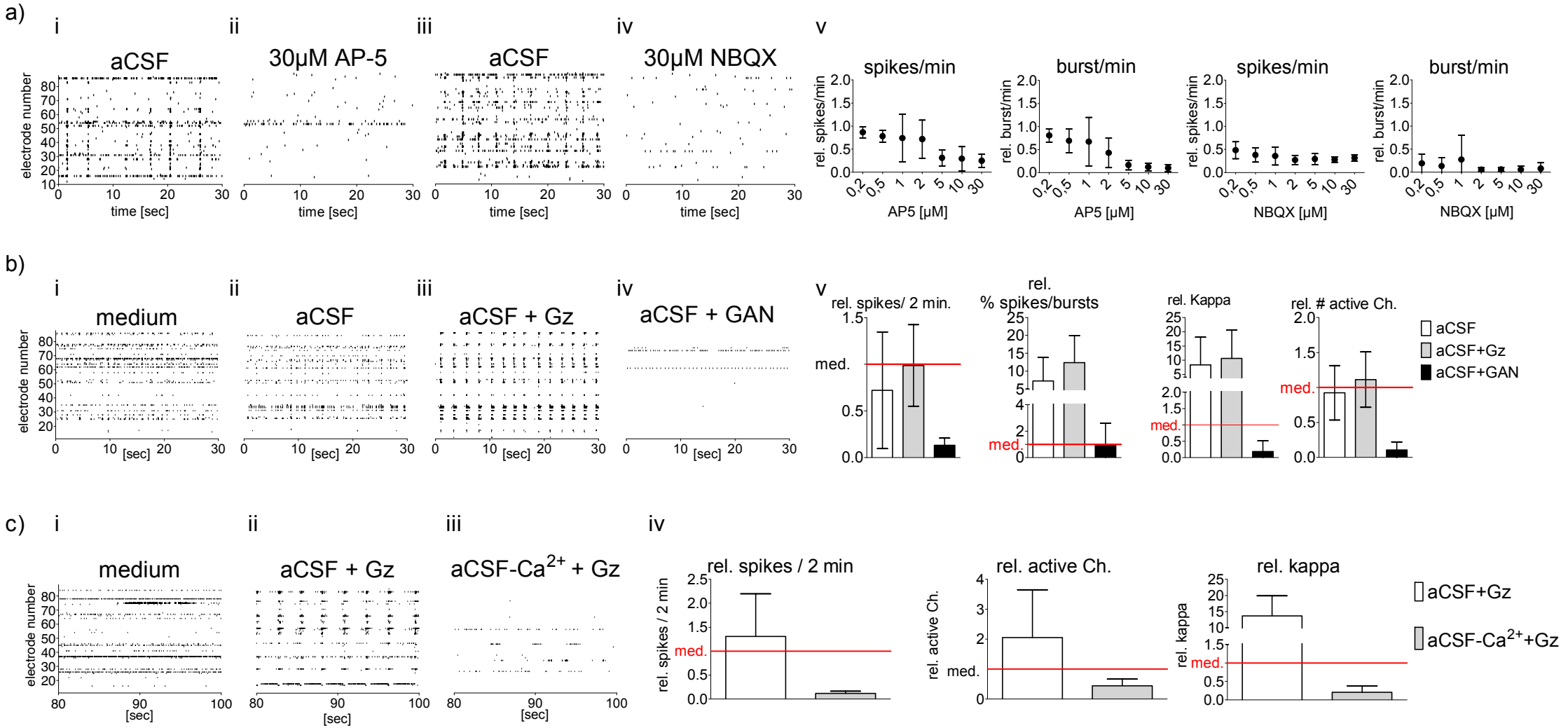
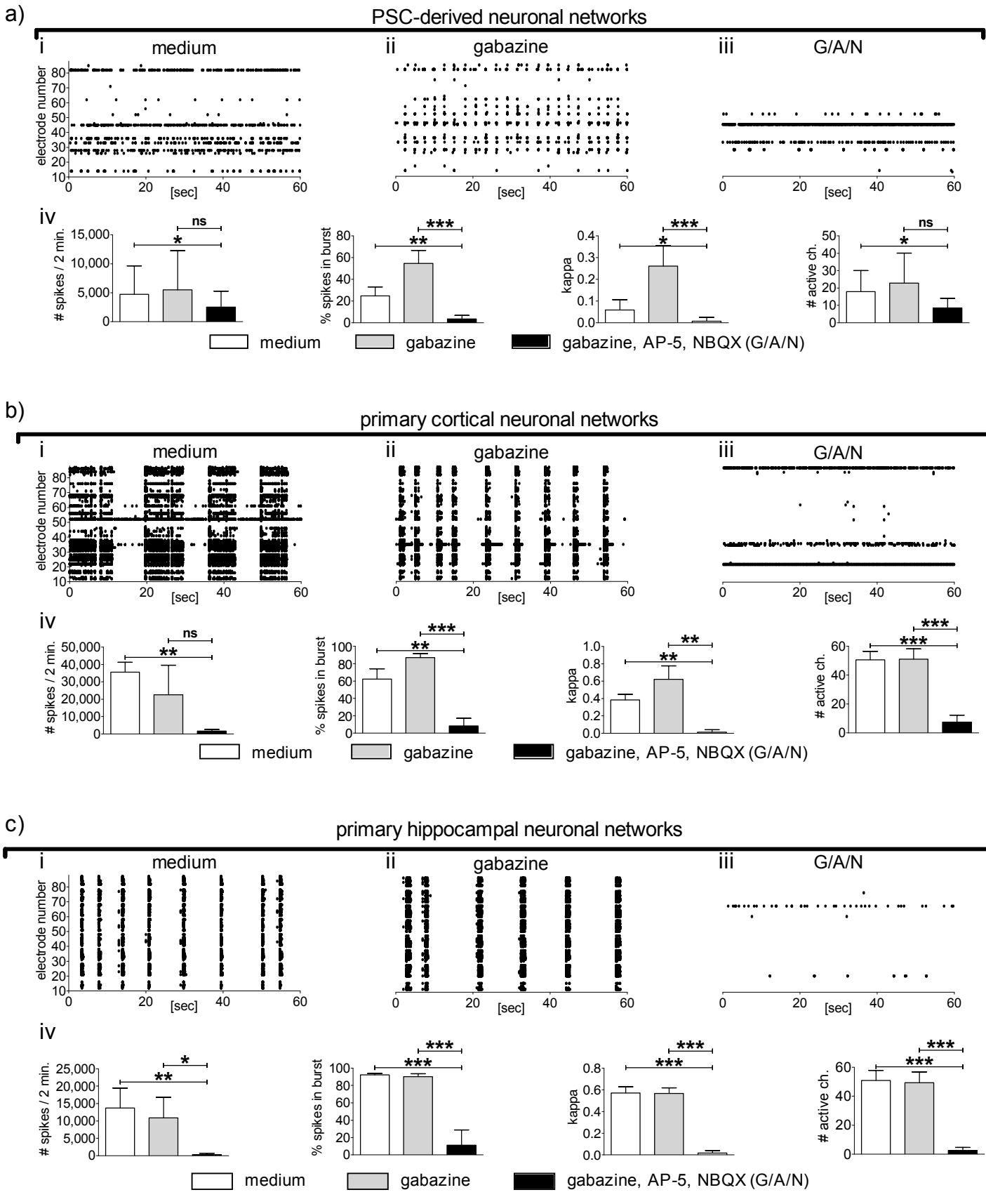
Figure S1: Activity of PSC-derived neuronal cells under aCSF in the presence or absence of the synaptic blockers AP-5, NBQX, gabazine or calciumions. Related to Figure 2.



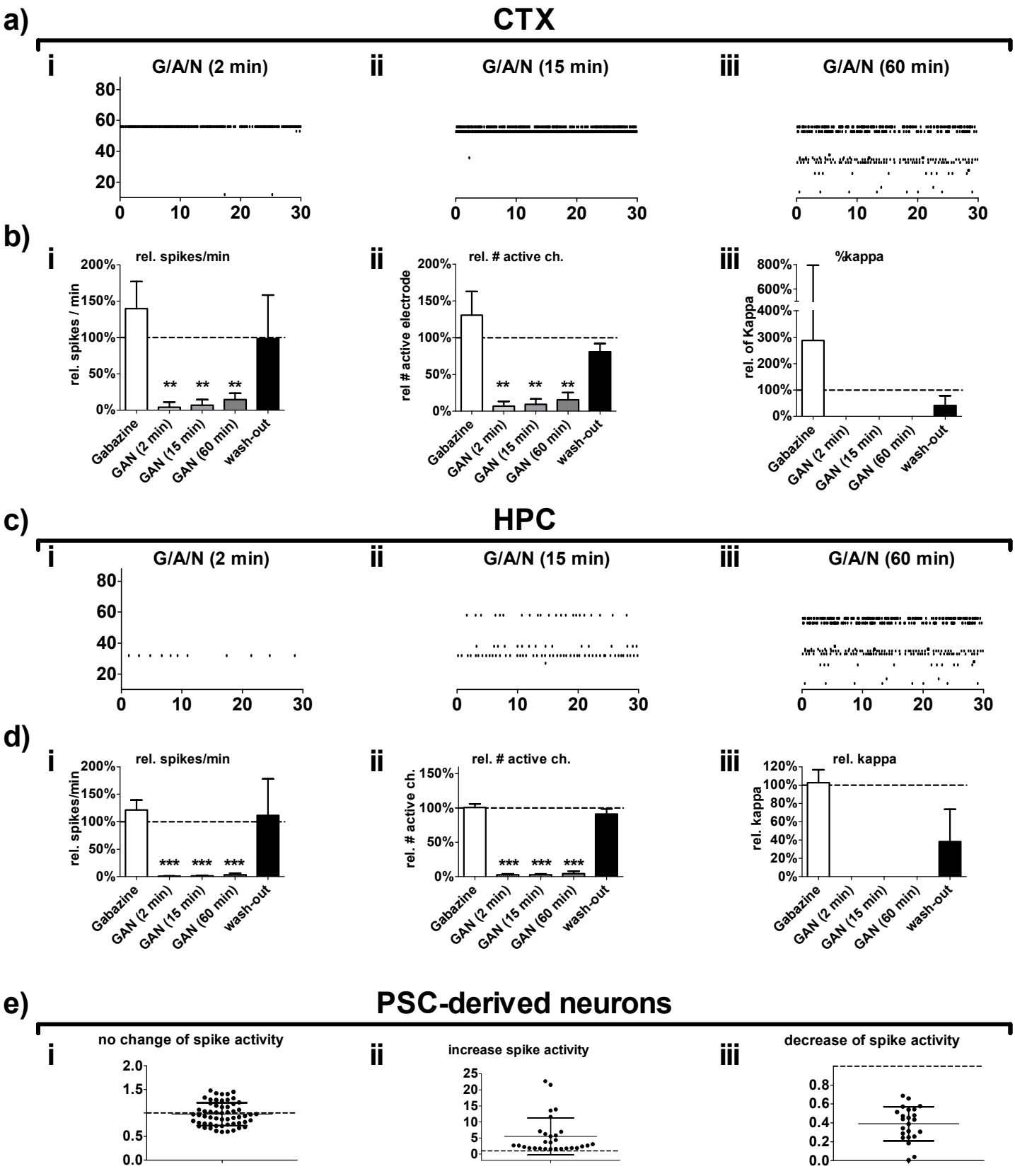
(**a**) Representative spike raster plots show activity of PSC-derived neurons under aCSF, (**i, iii**) before and after the application of high-concentration of (**ii**) AP-5 or (**iv**) NBQX. (**b**) Neuronal paramater describing the concentration dependent influence of the application of AP-5 (N=5) and NBQX(N=5) on PSC-derived neuronal spiking and bursting in relation to baseline recordings performend in aCSF. (**b**) Representative spike raster plots show activity of PSC-derived neurons under (**i**) medium, (**ii**) aCSF, (**iii**) aCSF+gabazine ([30µM], Gz) and (**iv**) aCSF+gabazine+ AP-5+NBQX ([30µM, each], G/A/N). (**v**) Neuronal paramater describing the influence of aCSF, aCSF+Gz and aCSF+G/A/N on PSC-derived neuronal network activity in relation to baseline recordings performend in medium (N=7). (**c**) Representative spike raster plots and neuronal parameters describe the impact of aCSF supplemented with gabazine ([30µM] (**ii**) in the presence or (**iii**) absence of calciumions within aCSF in relation to baseline recordings performend in (**i, line in iv**) medium) (N=7).

Figure S2: Ia-neurons in prolonged culture of PSC-derived, primary cortical and hippocampal neuronal networks. Related to Figure 2.



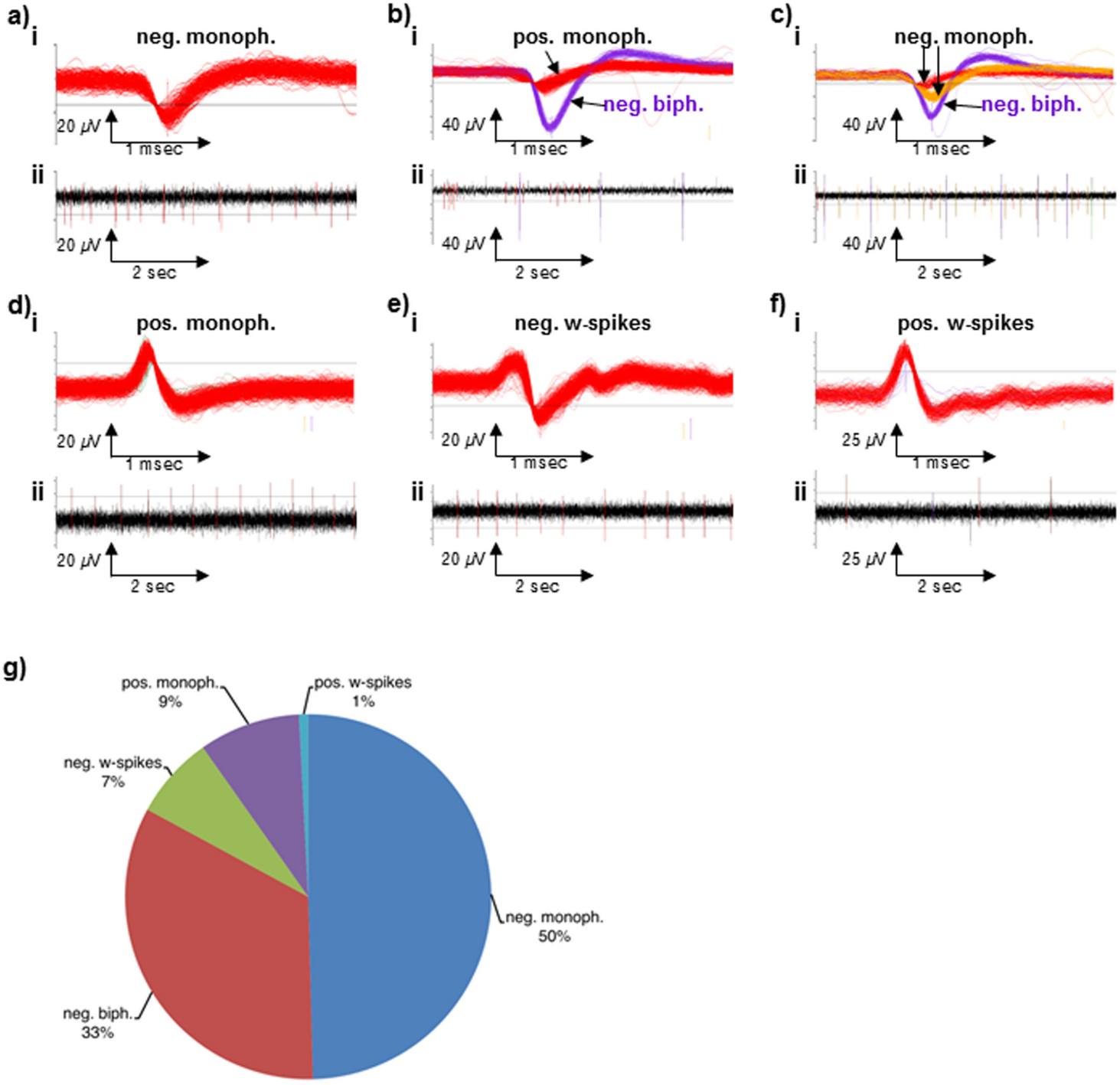
Representative spike raster plots show activity of (**a**) PSC-derived neuronal assemblies cultivated for 4 month on MEAs, (**b**) primary cortical and (**c**) primary hippocampal neuronal assemblies under (**i**) medium, (**ii**) disinhibition cond. (Gz. = gabazine-treatment) and (**iii**) in the absence of FSC (G/A/N = gabazine-, AP-5- and NBQX-treatment). Each dot represents a spike recorded by one electrode at a certain time point. (**iv in a, b, c**) Neuronal parameter describing the mean number (#) of spikes, percentages of spikes organized as bursts, the network synchrony indicated as Cohens´kappa and number of spike detecting electrodes under medium, disinhibition cond. (Gz. = gabazine-treatment) and in the absence of FSC (G/A/N = gabazine-, D-AP-5- and NBQX-treatment). (PSC-derived neuronal cultures (n=7), cortical (n=4) and hippocampal neuronal cultures (n=6); for details of statistical analysis see table S1. bars indicate mean± s. d.)

Figure S3: Activity of cortical, hippocampal and PSC-derived ia-n 15 and 60 min. after the application of synaptic blockers. Related to Figure 2.



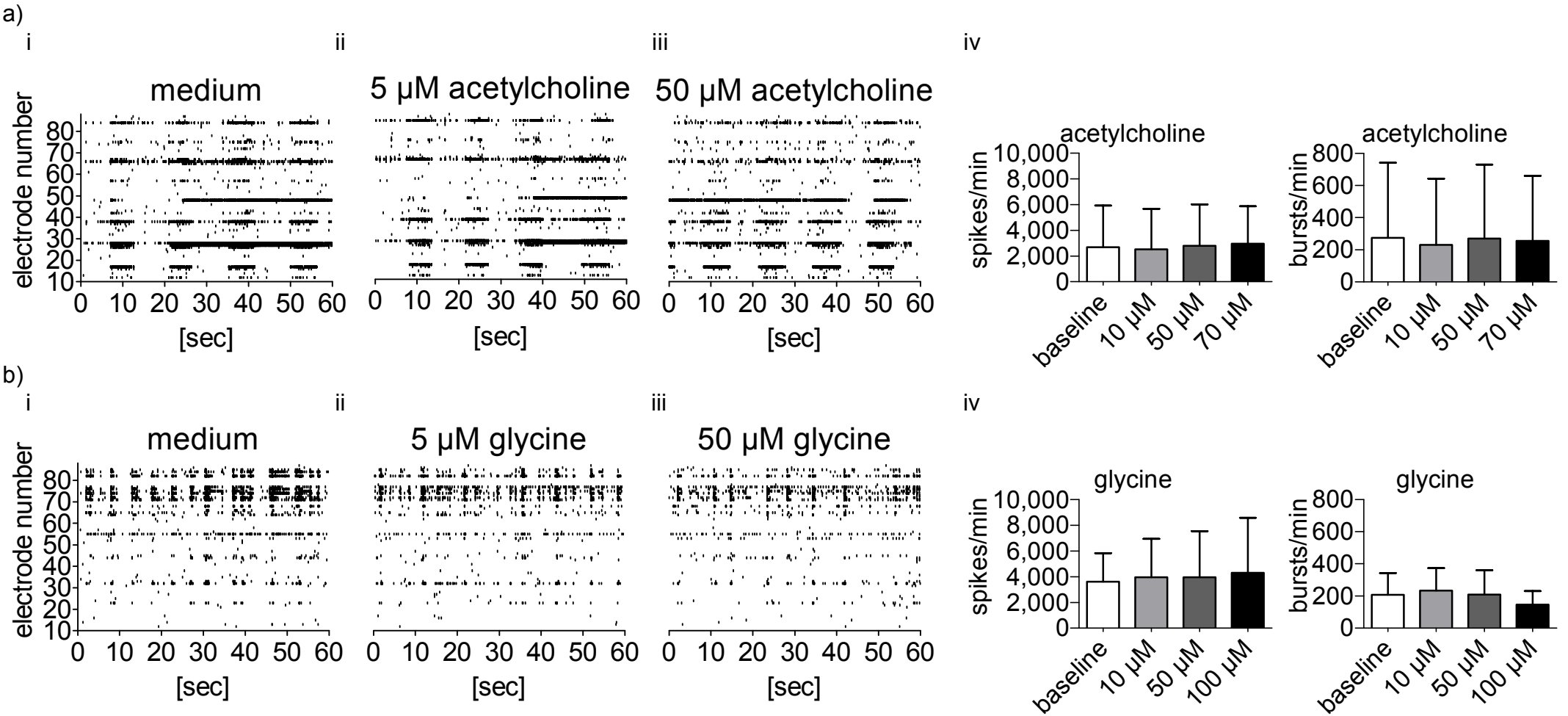
Representative spike raster plots show activity of (**a-b**) cortical and (**c-d**) hippocampal neurons (**i**) 2, (**ii**) 15 and (**iii**) 60 minutes after the application of gabazine, D-AP-5, NBQX (G/A/N). (**b, d**) Neuronal parameter describing the change of (**i**) spikes/min., (**ii**) number of active channels and (**iii**) the network synchrony indicated as Cohens´kappa relative to the medium conditions in (**b**) cortical and (**d**) hippocampal neurons. **e**) Scatter plots describing the change of the spike activity of PSC-derived ia-n recorded 15 and 60 min. after the application of G/A/N. Ia-n initially active 15 min after the application of G/A/N are still active 60 min. after the application of G/A/N and showing either (**i**) no change (±50%, N=58), (**ii**) an increase (>50%, N=31) or a (**iii**) decrease (<50%, N=31) of spike activity.

Figure S4: Classification of extracellular recorded spikes generated from ia-neurons. Related to Figure 3.



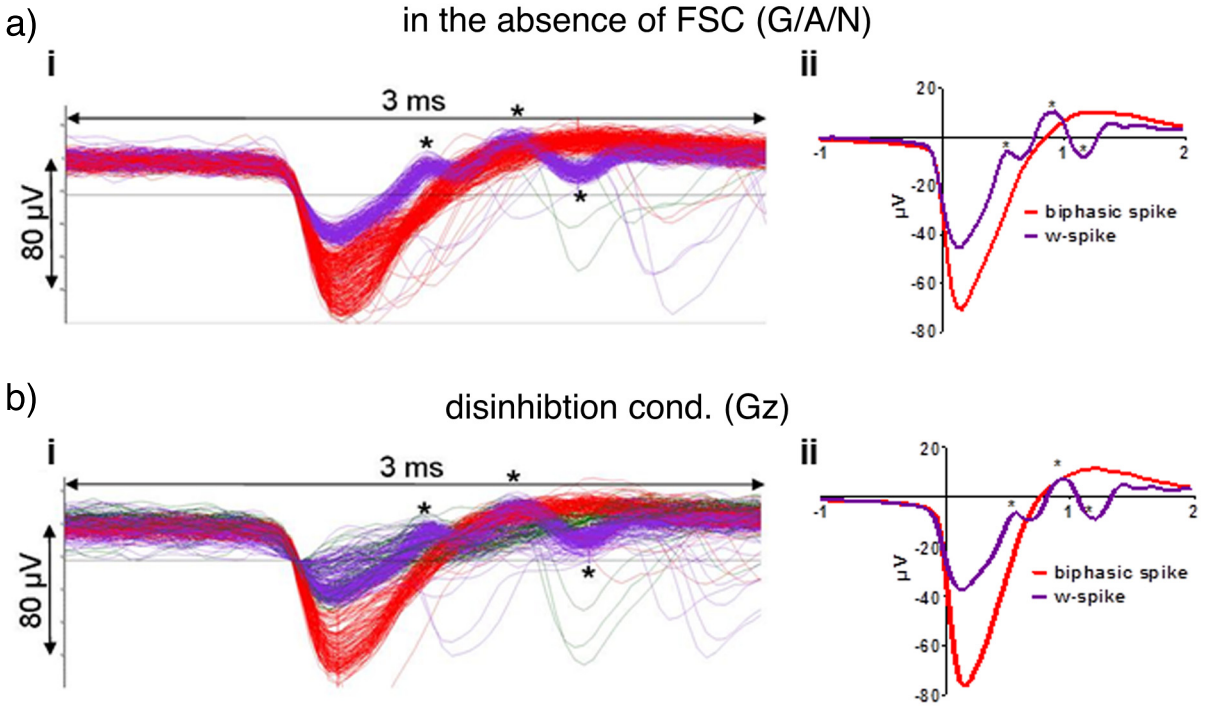
Examples of different spike morphologies recorded in PSC-derived neuronal assemblies treated with gabazine/D-AP-5/NBQX. Sorted spikes recorded by one electrode are visualized in (**i in a-f**) electrode data and (**ii in a-f**) as superimposed spikes (temporal resolution of 25kHz). Examples of electrodes detecting (**a**) one, (**b**) two or (**c**) three different spikes. Five different spike morphologies are shown: (**a, b, c**) monophasic spikes with negative amplitudes, (**b, c**) biphasic spikes with negative amplitudes followed by a positive slope, (**d**) monophasic spikes with positive amplitudes and (**e, f**) w-spikes(Chorev and Brecht, 2012) with either a (**e**) negative or (**f**) a positive amplitude followed by additional slopes. (**g**) Quantification of spike morphologies (n=124 spikes).

Figure S5: Acetylcholine and glycine has no impact on neuronal activity of PSC-derived neuronal networks. Related to Figure 5.



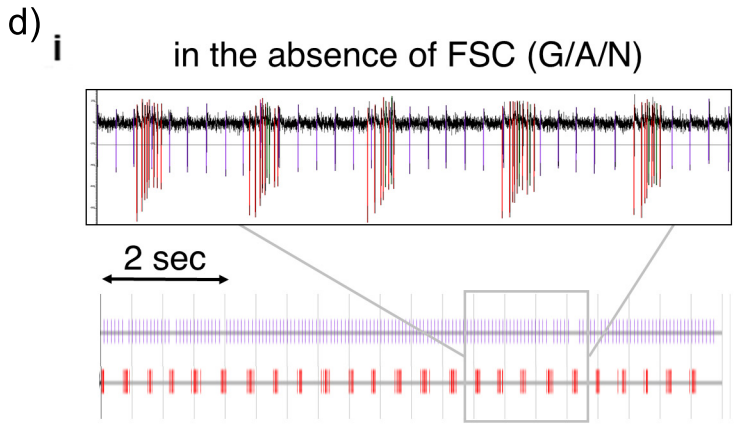
Representative spike raster plots show activity of PSC-derived neurons under medium,**i**)(before and after the application of (**ii**) low and (**iii**) high-concentration of (**a**) acetylcholine or (**b**) glycine. Neuronal parameter describing the concentration dependent impact of the application of(**iv in a**) acetylcholine (N=5) or (**iv in b**) glycine (N=5) on PSC-derived neuronal networks.

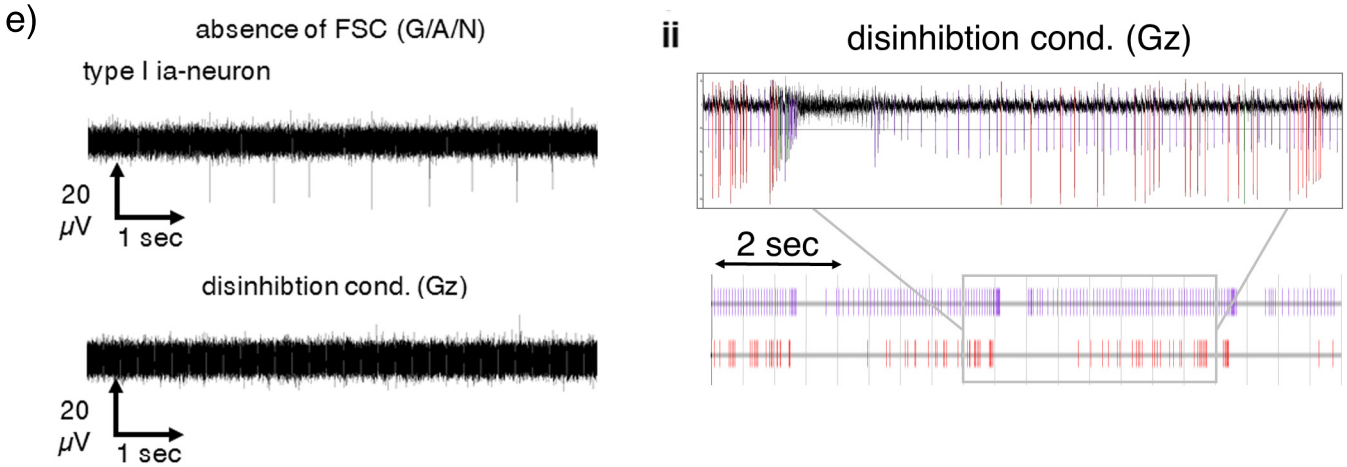
Figure S6: Spike morphologies of type IV ia-neurons in the absence and presence of FSC and example of the firing behaviour of type I ia-neurons. Related to Figure 7.



c)

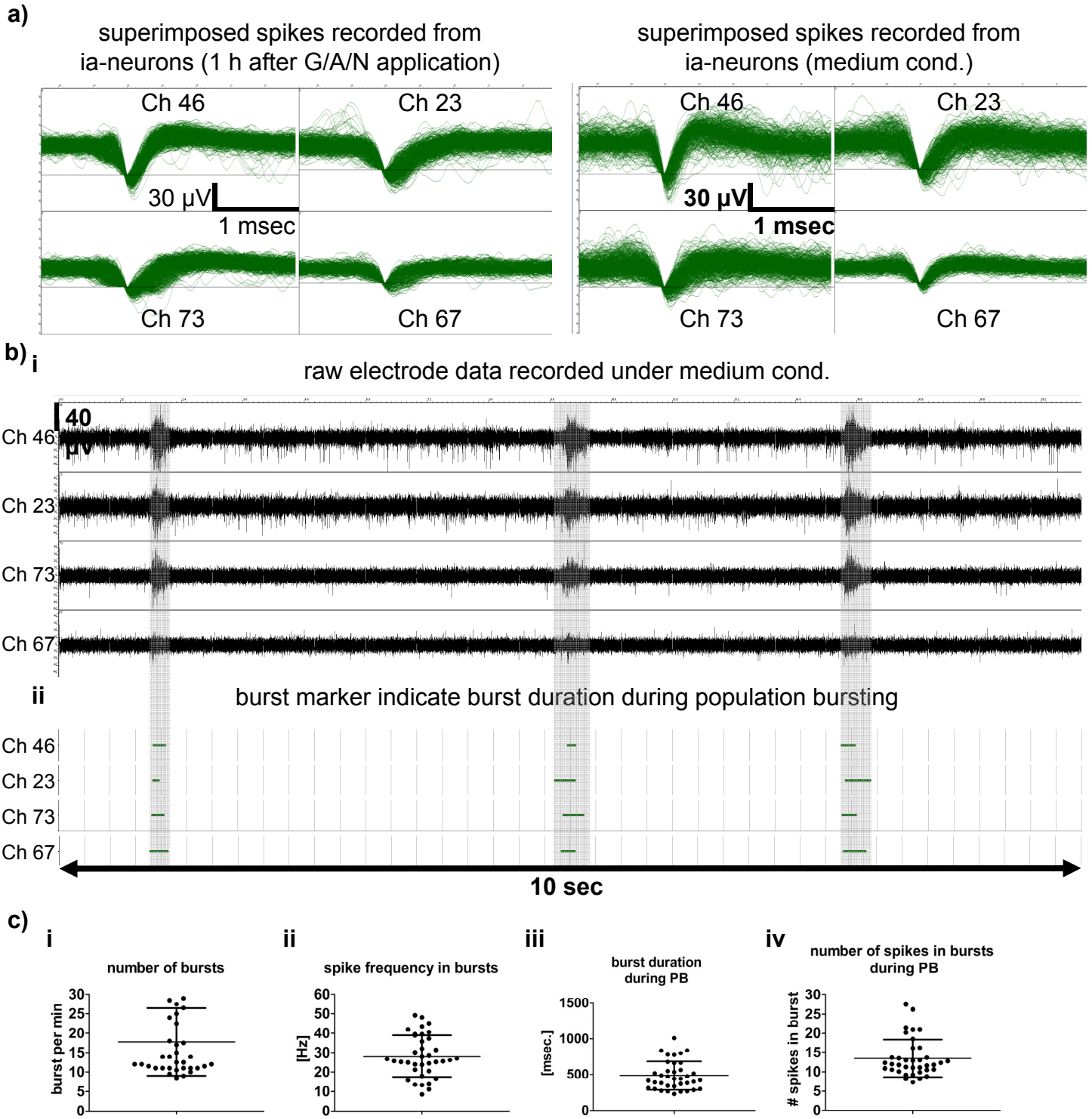
values in 2 min. extrac. recording	bursting ia-neuron		regular spiking ia-neuron	
	Gz	Gz+A+N	Gz	Gz+A+N
# of spikes	1154	2564	2237	1984
spike frequency [Hz]	9.6	21.3	18.6	16.53
# of bursts	62	335	39	0
bursts/sec.	0.51	2.79	0.32	-
bursts/min.	31	167.5	19.5	-
% of spikes in bursts	43.7	91.2	16.9	0
mean burst duration [ms]	114.40	62.74	92.1	-
mean spikes in burst	8.11	6.97	9.69	-
mean ISI in burst [ms]	16	10	10.6	-
mean spike frequency in bursts [Hz]	62	95	94	-
mean interburst interval [ms]	1.9	357.8	3.1	-





Spike morphologies of bursting and regular firing type IV ia-neuron described in figure 6. Spike shapes detected (**a**) in the absence of FSC (G/A/N = gabazine-, D-AP-5- and NBQX-treatment) and (**b**) under disinhibition cond. (Gz = gabazine-treatment). (**i in a, b**) Superimposed spikes and (**ii in a, b**) mean waveform of biphasic spikes and w-spikes shapes. Stars indicate the additional slopes in w-spikes. (**c**) Neuronal parameters describing the firing behavior of bursting and regular spiking type IV ia-neuron in the absence of FSC and under disinhibition cond. (**d**) Activity of bursting and regular spiking type IV ia-n detected by Channel 34 (**i in d**) in the absence of FSC and (**ii in d**) under disinhibition cond. (**Upper traces in d**) Firing behaviors are visualized as electrode data and as (**lower traces in d**) spike raster plots. (**e**) MEA-recordings (30 sec) showing examples of the firing behavior of type I (**above**) in the absence of FSC (G/A/N = gabazine-, D-AP-5- and NBQX-treatment)and (**below**) under disinhibition (Gz = gabazine-treatment) conditions.

Figure S7: Firing characteristics of mESC-derived ia-neurons during population bursting under medium conditions. Related to Figure 7.



a-c) Images showing examples of spike morphologies (**a**), firing behaviour (**b**) and burst marker (**c**) of four ia-neurons recorded in one neuronal culture. **a)** Example of superimposed spikes recorded in the presence (**i**) and absence (**ii**) of synaptic blockers. Note, that the spike morphologies persist under both condtions indicating that the recorded activity correspond to one ia-neuron. **b**) 10 second of extracellular recordings (**i**) of four channels showing spiking and bursting activity as well as population bursting (**grey bars**) of ia-neurons (same neurons as in a). Burst marker (**green line in ii**) indicating start and end of bursts recorded from ia-neurons during synchronous population bursting. **c**) Scatter plots illustarting the number of bursts (**i**), spike frequency in bursts (**ii**), burst duration (**iii**) and number of spikes in bursts (**iv**) during PB of 37 ia-neurons recorded in three individual neuronal cultures (dots illustrate individual values from type II and III ia-neurons, lines indicate the mean±s.d.)

Table S1: Values of statistical analysis (part 1)

Figure	***compared groups***	***P value***	***n***
1, e, i	med vs Gz	0,5102	17
1, e, i	med vs G/A/N (15 min)	0,0437	17
1, e, i	Gz vs G/A/N (15 min)	0,0752	17
1, e, i	G/A/N (15 min) vs G/A/N (1h)	0,1585	17
1, e, ii	med vs Gz	< 0.0001	17
1, e, ii	med vs G/A/N (15 min)	0,0016	17
1, e, ii	Gz vs G/A/N (15 min)	< 0.0001	17
1, e, ii	G/A/N (15 min) vs G/A/N (1h)	0,1728	17
1, e, iii	med vs Gz	< 0.0001	17
1, e, iii	med vs G/A/N (15 min)	0,0021	17
1, e, iii	Gz vs G/A/N (15 min)	< 0.0001	17
1, e, iii	G/A/N (15 min) vs G/A/N (1h)	0,934	17
1, e, iv	med vs Gz	0,2102	17
1, e, iv	med vs G/A/N (15 min)	0,0001	17
1, e, iv	Gz vs G/A/N (15 min)	0,0006	17
1, e, iv	G/A/N (15 min) vs G/A/N (1h)	0,228	17
4, a	baseline vs 1µM	0,0018	9
4, a	baseline vs 2µM	0,0006	9
4, a	baseline vs 5µM	< 0.0001	9
suppl. fig. 2, b, i	med vs G/A/N	0,0481	7
suppl. fig. 2, b, i	Gz vs G/A/N	0,1004	7
suppl. fig. 2, b, ii	med vs G/A/N	0,0015	7
suppl. fig. 2, b, ii	Gz vs G/A/N	< 0.0001	7
suppl. fig. 2, b, ii	med vs G/A/N	0,025	7
suppl. fig. 2, b, ii	Gz vs G/A/N	0,0003	7
suppl. fig. 2, b, iv	med vs G/A/N	0,0167	7
suppl. fig. 2, b, iv	Gz vs G/A/N	0,0654	7
suppl. fig. 2, d, i	med vs G/A/N	0,0014	4
suppl. fig. 2, d, i	Gz vs G/A/N	0,0898	4
suppl. fig. 2, d, ii	med vs G/A/N	0,0092	4
suppl. fig. 2, d, ii	Gz vs G/A/N	0,001	4
suppl. fig. 2, d, ii	med vs G/A/N	0,0029	4
suppl. fig. 2, d, ii	Gz vs G/A/N	0,0042	4
suppl. fig. 2, d, iv	med vs G/A/N	0,0001	4
suppl. fig. 2, d, iv	Gz vs G/A/N	0,0004	4
suppl. fig. 2, f, i	med vs G/A/N	0,0024	6
suppl. fig. 2, f, i	Gz vs G/A/N	0,0074	6
suppl. fig. 2, f, ii	med vs G/A/N	< 0.0001	6
suppl. fig. 2, f, ii	Gz vs G/A/N	< 0.0001	6
suppl. fig. 2, f, ii	med vs G/A/N	< 0.0001	6
suppl. fig. 2, f, ii	Gz vs G/A/N	< 0.0001	6
suppl. fig. 2, f, iv	med vs G/A/N	< 0.0001	6
suppl. fig. 2, f, iv	Gz vs G/A/N	< 0.0001	6

Table indicates individual p values and number of individual experiments (n). Data values were analyzed by paired *t*-test.

Table S1: Values of statistical analysis (part 2)

Figure	***compared groups***	***P value***	***n***
suppl. fig. 3, b, i	med vs G/A/N (2 min)	0,0001	5
suppl. fig. 3, b, i	med vs G/A/N (15 min)	0,0001	5
suppl. fig. 3, b, i	med vs G/A/N (60 min)	0,0001	5
suppl. fig. 3, b, ii	med vs G/A/N (2 min)	0,0001	5
suppl. fig. 3, b, ii	med vs G/A/N (15 min)	0,0001	5
suppl. fig. 3, b, ii	med vs G/A/N (60 min)	0,0001	5
suppl. fig. 3, b, iii	med vs G/A/N (2 min)	0,0001	5
suppl. fig. 3, b, iii	med vs G/A/N (15 min)	0,0001	5
suppl. fig. 3, b, iii	med vs G/A/N (60 min)	0,0001	5
suppl. fig. 3, d, i	med vs G/A/N (2 min)	0,0004	5
suppl. fig. 3, d, i	med vs G/A/N (15 min)	0,0004	5
suppl. fig. 3, d, i	med vs G/A/N (60 min)	0,0003	5
suppl. fig. 3, d, ii	med vs G/A/N (2 min)	0,0018	5
suppl. fig. 3, d, ii	med vs G/A/N (15 min)	0,0015	5
suppl. fig. 3, d, ii	med vs G/A/N (60 min)	0,00017	5
suppl. fig. 3, d, iii	med vs G/A/N (2 min)	0,0001	5
suppl. fig. 3, d, iii	med vs G/A/N (15 min)	0,0001	5

Table indicates individual p values and number of individual experiments (n). Data values were analyzed by paired *t*-test.

Table S2: Used mESC-cell lines for the individual experiments

Figure	used mESC cell lines
2	C57Bl6
3	C57Bl6
4	C57Bl6
5	SV-129
6	C57Bl6 and SV-129
7	C57Bl6
S1	C57Bl6
S2	SV-129
S4	C57Bl6 and SV-129
S5	C57Bl6
S6	C57Bl6 and SV-129
S7	C57Bl6
S8	C57Bl6

Suppl. method: generation of murine PSC-derived neural cultures

Shortly, undifferentiated ES cells were grown on murine embryonic fibroblasts in the presence of leukemia inhibitory factor (Chemicon) and 20% fetal bovine serum in a ES cell medium described elsewhere(18). For neural differentiation of ES cells, ES cell colonies were harvested without dissociation and replated on bacterial culture dishes (Greiner, Germany) in ES cell medium containing 10% FBS without LIF. After 24h, free-floating embryoid bodies had developed and were re-plated in neural induction medium (NIM) consisting of DMEM/F12 and neurobasal medium (1:1) (both from Gibco-BRL), 2 mM Glutamax (Gibco-BRL) and N2 supplement as well as B27 supplement (Gibco-BRL). After 7 days, serum-free, floating cultures of embryoid body-like aggregates (SFEB) had developed consisting predominantly of neural precursor cells (18, 21, 40). Floating SFEBs were cultivated for 7 to 14 days in the presence of neural proliferation medium (NPM) consisting of DMEM/F12, 2 mM Glutamax, fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF) (PeproTech, each 20 ng/ml) in order to enrich NSC within the neural aggregates(18). After reaching a critical size (> 500 µm) neural precursor cell-enriched SFEBs (nSFEBs) have been disintegrated using cell-strainer (pore size: 100µm, BD Falcon). After 7 to 14 days nSFEB has been dissociated into cell suspensions and stored at -160° Celsius until use.

References S1

Chorev, E., and Brecht, M. (2012). In vivo dual intra and extracellular recordings suggest bi-directional coupling between CA1 pyramidal neurons. Journal of neurophysiology.