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High affinity, slowly desensitizing $GABA_A$ receptors mediate tonic inhibition in hippocampal dentate granule cells

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Running title page

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Abbreviations:

GABA – gamma-aminobutyric acid

DGCs - dentate granule cells

sIPSCs- spontaneous inhibitory postsynaptic currents

RMS – root mean of square

THDOC - tertrahydrodeoxycorticosterone

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Abstract

The tonic form of GABA-mediated inhibition requires the presence of slowly desensitizing $GABA_A$ receptors with high affinity, which has not yet been directly demonstrated in hippocampal neurons. Low concentration of GABA (1μM) persistently increased baseline noise, increased membrane slope conductance but did not affect sIPSCs in dentate granule cells (DGCs). Higher concentrations of GABA (10-100 μM) desensitized synaptic currents quickly and there was a large residual current. Saturating concentration of GABA (1mM) completely desensitized synaptic currents and revealed a slowly desensitizing, persistent current. Penicillin (300 μM) inhibited baseline noise without affecting mean current and inhibited decay time of sIPSCs. $GABA_A$ receptors mediating baseline noise in DGCs were sensitive to allopregnanolone, furosemide and loreclezole and insensitive to diazepam and zolpidem. These studies demonstrate persistently open $GABA_A$ receptors on DGCs with high affinity for GABA, slow desensitization rate and pharmacological properties similar to those of recombinant receptors containing α_4, β_1 and the δ subunits.

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Introduction

Fast synaptic inhibition in the forebrain is mediated by transient activation of synaptic $GABA_A$ receptors by high concentrations of γ-aminobutyric acid (GABA) released from the presynaptic terminals. GABA in the extracellular space mediates a slow inhibition of neurons by persistent activation of extrasynaptic receptors, which is commonly termed tonic inhibition. Tonic inhibition has been well characterized in cerebellar granule cells (Brickley *et al.*, 1996; Hamann *et al.*, 2002) and more recently in hippocampal dentate granule cells (DGCs) (Nusser and Mody, 2002; Stell *et al.*, 2003). In order for $GABA_A$ receptors to be activated by the low concentrations of GABA present in the extracellular space, these receptors must have a high affinity for GABA. Because persistent activation of many types of $GABA_A$ receptors leads to their desensitization, specific subsets of receptors with slow rates of desensitization would be required for maintaining tonic inhibition. Recombinant $GABA_A$ receptors containing the δ subunit in combination with the α_4 or α_6 subunit form receptors have a high affinity for GABA as well as a slow rate and limited degree of desensitization (Wohlfarth *et al.*, 2002; Brown *et al.*, 2002). The δ subunits are expressed perisomatically in hippocampal DGCs (Wei et al., 2003) and extrasynaptically in cerebellar granule cells, suggesting that these receptors are capable of mediating tonic inhibition.

In keeping with this hypothesis, previous studies on cerebellar and hippocampal granule cells demonstrated that tonic inhibition has pharmacological properties similar to those of recombinant receptors containing the δ subunit. For instance, zolpidem does not affect holding (mean) current, whereas it enhances synaptic currents in DGCs *in vivo* (Nusser and Mody, 2002) and in cultured hippocampal neurons (Yeung et al., 2003). In

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cerebellar granule cells, receptors mediating tonic inhibition are sensitive to the neurosteroid THDOC (Stell et al., 2003), furosemide (Hamann et al., 2002;Wall, 2002), and insensitive to diazepam (Hamann et al., 2002). These pharmacological properties are shared with recombinant receptors containing the α_6 and δ or α_4 and δ subunits. Post-embedding electron microscopic studies further demonstrated that δ subunit –containing $GABA_A$ receptors are present on extrasynaptic membranes in cerebellar granule cells (Nusser et al., 1998). Based on these studies it has been proposed that δ subunit-containing receptors mediate tonic inhibition. However, earlier studies do not provide direct evidence of the presence of high affinity, slowly desensitizing $GABA_A$ receptors in neurons that are necessary to mediate tonic inhibition.

We directly demonstrate that DGCs express persistently open $GABA_A$ receptors with high GABA affinity. Furthermore, these cells express $GABA_A$ receptors with a slow rate and limited extent of desensitization, which remain open when synaptic receptors have desensitized. We also characterized pharmacological properties of tonic currents, which were similar to those of recombinant receptors containing α_4, β_1 and δ subunits.

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Materials and methods

Adult male Sprague-Dawley rats (150-200 g) were anesthetized with halothane prior to decapitation according to the University of Virginia Animal Care and Use Committee guidelines. Brains were dissected free and immersed in ice-cold (2-4°C) artificial cerebrospinal fluid (ACSF) saturated with 95%O_2-5%CO_2. The ACSF consisted of: (in mM) NaCl 127, KCl 2, $CaCl_2$1.5, $MgSO_4$ 1.5, $NaHCO_3$ 25.7, KH_2PO_4 1.1, glucose 10 (osmolarity, 300-305 mOsm). After cooling, the brains were blocked and mounted on a vibratome stage (Camden Instruments, UK) and 300 μM thick horizontal sections containing the ventral hippocampus were cut. Slices were maintained in continuously oxygenated ASCF, at 32°C in a holding chamber for 30 - 45 min, and then at room temperature in a recording chamber mounted on the stage of an Olympus BX51 microscope equipped with a 40x water-immersion objective, IR-DIC optics and video. DGCs were identified in dentate granule layer as small and medium size neurons with typical oval shaped soma and single process. Patch electrodes (final resistances 6 - 8 MΩ) were pulled from borosilicate glass (Sutter Instruments, Novato, CA) on a horizontal Flaming-Brown microelectrode puller (model P-97, Sutter Instruments), using a 2-stage pull protocol. Electrode tips were filled with a filtered internal recording solution consisting of (in mM): CsCl 153.3, $MgCl_2$ 1.0, N-[2-Hydroxyethyl]piperazine-N -[2-ethansulfonic acid] (HEPES) 10.0, and glycol-bis(α-aminoethyl ether) N,N,N ,N - tetraacetic acid (EGTA) 5.0, pH 7.2 (with CsOH), osmolarity was 285 - 295 mOsm. The electrode shank contained (in mM): ATP Mg^{2+} salt 3, GTP Na^+ salt 0.1. Neurons were voltage-clamped to -65 mV with an Axopatch 1D or Axopatch 200B amplifier (Axon Instruments, Union City, CA). Whole cell capacitance and series resistance were

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compensated by 70 - 75% at 10 μs lag. Recording was performed when series resistance after compensation was 20 MΩ or less. Access resistance was monitored with a 10 msec, -5 mV test pulse once every 2 min and if the series resistance increased by 25% at any time during the experiment, then the recording was terminated. Currents were filtered at 5 kHz then digitized using a Digidata 1322 digitizer and acquired using Axoscope 8.0 software (Axon Instruments) on an IBM PC compatible computer hard drive.

For recording of $GABA_A$ receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) and tonic GABAergic current, 50 μM DL-2-Amino-5-phosphonopentanoic acid (DL-AP5) and 20 μM 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris-Cookson Ellisville, MO) were included in the ACSF to block NMDA and AMPA/Kainate receptor-mediated currents, respectively. All other reagents were obtained from Sigma (St. Louis, MO).

Acquisition and analysis

The digitized current traces were analyzed with Mini Analysis software (Synaptosoft, Leonia NJ). The software was used to detect and analyze sIPSCs. To detect sIPSCs, detection threshold 5 times of root mean square of amplitude (RMS) was used. RMS noise, (I_{rms}) was defined as a square root of the average of the squares of the deviation from the I_{avg} (amplitude) over the chosen time interval using the equation (1)

$$I_{rms} = \left[\frac{(I_{avg} - I_1)_2 + (I_{avg} - I_2)_2 + \ldots + (I_{avg} - I_n)_2}{n} \right]^{1/2}$$

where I_{avg} is an average amplitude, I_n is amplitude in an individual point and n is a number of measurements in epoch.

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To study the tonic inhibition, transient events were manually removed from the current trace, so that it consisted only of holding current, the current required to voltage clamp the cell. Two features of the holding current were studied: the mean current and the noise. Mean current was measured in 100 msec epochs with 1 sec interval between epochs in 30 epochs. The measurements were taken 30 sec before and 5 min after application of a drug. The segments of a current trace that contained synaptic event were omitted. Mean current (I_{avg}) was defined as an arithmetic mean of peak-to-peak amplitudes of individual points during that epoch. In order to assess the effect of a drug on I_{avg} in an individual neuron, the distribution of I_{avg} before application of the drug during the baseline period was compared to that following drug application by means of a Kolmogorov-Smirnov (KS) test. In order to compare the data obtained from a group of neurons mean of I_{avg} from all neurons before and after drug application were compared.

A second measure of holding current, RMS of noise (I_{rms}) was studied. The time interval (epoch) for each measurement was 50 msec and contained 250 amplitude measurements (5 kHz digitization rate). The time interval between two epochs was 500 msec. Sixty epochs were analyzed for each experimental condition (60 control and 60 after a drug application in each cell). In order to asses the effect of a drug on I_{rms} in an individual neuron, the distribution of I_{rms} in epochs before the application of a drug (during the baseline period) was compared to that following drug application by means of KS test. In order to compare data obtained from a group of neurons, I_{rms} values in individual epochs before and after drug application were averaged. Mean I_{rms}, mean I_{avg}, mean sIPSC frequency and amplitude values were compared using *t*-tests unless specified otherwise.

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Results

Tonic inhibition revealed by competitive antagonists

Several recent studies have used competitive antagonists to measure tonic inhibition. In order to measure tonic inhibition, spontaneous inhibitory postsynaptic currents (sIPSCs) interspersed with the mean current were recorded from DGCs in the hippocampal slices by blocking excitatory neurotransmission with DL-AP5 (50 μM) and CNQX (20 μM). The neurons were voltage clamped to -65 mV, under near-symmetrical Cl^- conditions, at a room temperature (20 – 22ºC, see Methods for details). After the input resistance became stable, sIPSCs and mean current were recorded for 5 minutes and then 100 μM bicuculline was bath-applied. A typical recording with 100 μM bicuculline application is shown on figure 1A. In response to bicuculline, a slow outward current occurred, accompanied by loss of spontaneous inhibitory synaptic currents (sIPSCs) and reduction in baseline noise.

For analysis of tonic currents, I_{avg} and I_{rms} were measured. I_{avg} was analyzed in 30 successive 100 msec epochs (2000 points), and at every second interval were analyzed after manually excluding rapid transient, synaptic currents from each epoch. A typical experimental change in I_{avg} after application of bicuculline was 67 pA. The experiment was replicated on 4 DGCs and the mean reduction of I_{avg} was 72 ± 19 pA. I_{rms}, the deviation from the mean of 250 individual digitized current measurements in each epoch lasting 50 msec (RMS noise, figure 1C), was measured in 60 epochs during the baseline period and 60 epochs during application of bicuculline after manually removing rapid transient synaptic currents. Each epoch was separated from the subsequent epoch by 500 msec intervals. The I_{rms} during application of bicuculline was less than that during the

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baseline period in each of 4 cells (KS test, $p < 0.001$). Pooled values from all 4 cells were compared using a paired *t*-test, and mean I_{rms} during the baseline period was 7.082 ± 0.56 pA and during 100 μM bicuculline application it was 5.997 ± 0.20 pA ($p = 0.001$).

Application of another competitive $GABA_A$ receptor antagonist, SR 95531 (100 μM) to 5 DGCs produced similar results on synaptic currents in each cell (figure 1B). Unlike bicuculline, SR 95531 did not have an effect on I_{avg} in 3 DGCs (82.8 ± 6.9 pA vs. 80.1 ± 3.6 pA, $p = 0.57$, paired *t*-test). The I_{rms} was significantly decreased (figure 1C). Data were pooled from all 5 cells and during baseline period the mean I_{rms} was 6.671 ± 0.11 pA and during SR 95531 application it was 5.496 ± 0.07 pA (KS test, $D = 0.24$, $p < 0.001$).

High affinity $GABA_A$ receptors

Studies with bicuculline and SR 95531 confirmed that tonic inhibition was present in DGCs, however these drugs blocked synaptic inhibition along with tonic inhibition. It has been suggested that the $GABA_A$ receptors specifically mediating tonic inhibition possess the high affinity for GABA that makes them less sensitive to competitive $GABA_A$ receptor antagonists than those receptors mediating synaptic inhibition. However, this high affinity should render these receptors mediating tonic inhibition more sensitive to agonists than synaptic receptors. We determined whether a low concentration of GABA (1μM), similar to that found in the extracellular space, could selectively activate $GABA_A$ receptors mediating tonic inhibition without altering synaptic currents.

After making a baseline recording for 5 minutes, a low concentration of GABA (1 μM) was bath applied and 5 minutes were allowed to elapse to allow the drug equilibrate

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within the slice. The holding current and sIPSCs in subsequent 5 minutes were compared to those during 5-minute baseline period prior to GABA application. A typical experiment is shown in figure 1. Note that there was no apparent change in I_{avg} on visual examination of the trace (figure 2A). A detailed quantitative examination of the I_{avg} confirmed the visual impression. The distribution of I_{avg} in 30 epochs before GABA application was compared to that in 30 epochs after application KS test (figure 2 B), and the two distributions were not different (71.3 ± 11.2 pA vs. 69.3 ± 6.7 pA, D = 0.04, p = 0.94). The experiment was repeated in 4 DGCs and 1 μM GABA did not change $I_{avg.}$in any of the cells.

Visual inspection of the trace also suggested that the baseline trace was thicker after application of 1 μM GABA, and when expanded further it appeared to possess greater noise (figure 2 C). The I_{rms} during baseline period was 5.30 pA (figure 2 D, upper trace), and 5 min after application of 1 μM GABA it was 6.0 pA. The distribution of I_{rms} measurements from each of 60 epochs before and after GABA application was compared using the KS test, indicating that they were significantly different, (D = 0.68 and p = 0.001, figure 2E). This finding was confirmed in 4 DGCs and in each one the KS test revealed that I_{rms} was significantly increased. To further understand the impact of 1 μM GABA on I_{rms}, an I_{rms} amplitude frequency distribution histogram was constructed (figure 2F), which demonstrated that 1 μM GABA increased the frequency of large I_{rms} epochs. In 4 DGCs tested, the mean I_{rms} increased from 6.29 ± 0.02 pA to 6.66 ± 0.03 pA ($p < 0.0001$) in response to 1 μM GABA.

Using a different approach, we studied the slope of the *I-V* relationship (slope conductance) to confirm that 1 μM GABA indeed increased $GABA_A$ receptor

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conductance. Recordings were obtained in DGCs at baseline and after application of 1 μM GABA voltage-clamped to -65, -35 and -15 mV. I_{rms} in 60 epochs was obtained for each holding potential, and these values were plotted against the membrane holding potential (figure 2G). During the baseline period, the I_{rms} decreased proportionally to the driving force (r^2 = 1,) and ordinate intercept was 2.9 pA. The regression line crossed the abscissa at close to 0 mV, the chloride reversal potential, suggesting that passage of chloride ions through the membrane contributed to I_{rms}. The slope of the RMS noise (current) voltage relationship in the control condition was 18 pS. GABA (1 μM) increased I_{rms} at each holding potential and the ordinate intercept was also close to 0 (2.9 mV). The slope of the RMS noise (current) voltage relationship in the presence of 1 μM GABA in this neuron was 26.2 pS, therefore 1 μM GABA increased the slope conductance. These findings were confirmed in 4 DGCs, and in each cell 1 μM GABA increased the slope conductance of I_{rms}.

We tested whether increased I_{rms} by 1 μM GABA was accompanied by inhibition of sIPSCs, because low concentrations of GABA are known to desensitize synaptic $GABA_A$ receptors and diminish synaptic currents in DGCs (Overstreet and Westbrook, 2001). The mean amplitude of sIPCSs remained unchanged after application of 1 μM GABA, 31.2 ± 5.3 pA in baseline and 33.7 ± 8.6 pA (approx. 500 events, paired *t*-test, p = 0.82, n = 4). The mean decay time constant was also unchanged (9.9 ± 0.9 msec vs. 10.12± 1.1, p = 0.88). The frequency of sIPSCs was 1.38 ± 0.7 Hz at baseline and 1.49 ± 0.7 Hz after application of 1 μM GABA (p = 0.92). Therefore, 1 μM GABA selectively enhanced baseline noise but did not have an effect on synaptic currents.

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GABA concentration- $\triangle I_{rms}$ relationship

Theoretical studies on receptor-gated channel noise and subsequent recordings predict that the relationship between agonist concentration and I_{rms} is parabolic, with minimum noise occurring at low and high concentrations of the agonist and maximum noise at intermediate concentrations (Traynelis and Jaramillo, 1998). As noted above, 1 μM GABA increased mean I_{rms} by 0.37 pA. In 3 DGCs, 10 μM GABA increased mean I_{rms} by 1.69 pA at the maximum change of I_{avg}. However, when the concentration of GABA was increased further to 30 μM the increase in mean I_{rms} was smaller, 0.41 pA. These results indicated that the GABA concentration versus I_{rms} noise change relationship was parabolic, thus further confirming that $GABA_A$ receptor conductance contributes to I_{rms}.

Penicillin modulates tonic and synaptic inhibition

Bicuculline and SR 95531 blocked synaptic inhibition along with tonic inhibition. When the $GABA_A$ receptor is going through open and desensitized states, the agonist remains bound to it (agonist trapping) and competitive antagonists can not bind to the receptor or close it (Bianchi and Macdonald, 2001). Therefore, bicuculline can not selectively inhibit tonic currents, which are mediated by bound open receptors. We reasoned that a drug that preferentially binds to $GABA_A$ receptors in the open state is likely to discriminate between receptors mediating tonic and synaptic inhibition. Penicillin G is a non-competitive antagonist of the $GABA_A$ receptor (Macdonald and Barker, 1977) that inhibits $GABA_A$ receptors by blocking the chloride channel in all open states (Twyman et al., 1992). Penicillin at a concentration as low as 250 μM reduces total average current in a single channel by 38% (Twyman et al., 1992).

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The effect 300 μM penicillin on tonic and synaptic currents was studied in 7 DGCs. A typical experiment is shown on figure 3, where penicillin did not change the I_{avg} but it did diminish I_{rms} (figure 3A, B). The decrease was significant for each cell, as confirmed by KS test with $p < 0.001$. The mean I_{rms} decreased from 4.364 ± 0.30 pA to 3.609 ± 0.21 pA ($p = 0.002$, $n = 7$). At the same time, penicillin effects on synaptic currents were subtle. It did not change peak amplitude and frequency of sIPSCs, but significantly decreased decay time constant (figure 3C). The mean frequency of sIPSCs during the baseline period in 7 neurons was 1.190 ± 0.19 Hz and in the presence of 300 μM penicillin it was 1.25 ± 0.25 Hz ($p = 0.84$). The mean amplitude of sIPSCs was unchanged, 45.1 ± 13 pA during the baseline period and 43.7 ± 14 pA ($p = 0.95$) in the presence of penicillin. However, penicillin is an open channel blocker and it is believed to inhibit channels following activation. We tested whether the decay of sIPSCs was accelerated by penicillin. The average decay time constant of sIPSCs decreased from 9.310 ± 0.9 msec to 5.240 ± 0.9 msec ($p = 0.002$, $n = 4$,). The decrease of decay resulted in decreased charge transfer. It decreased from 512.1 ± 56.9 pAmsec to 256.0 ± 33.4 pAmsec ($p = 0.006$, $n = 4$).

Desensitizing and non-desensitizing $GABA_A$ receptor currents elicited by 10 and 100 μM GABA

Several studies have shown that δ subunit-containing receptors desensitize less extensively and more slowly than γ2 subunit-containing receptors (Saxena and Macdonald, 1994; Haas and Macdonald, 1999; Bianchi et al., 2002). We tested whether higher concentrations of GABA (10 and 100 μM) could differentially desensitize lower affinity synaptic $GABA_A$ receptors while persistently activating high affinity-slowly-desensitizing receptors that are believed to mediate tonic inhibition.

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After 5 minutes of baseline recording, 10 μM GABA was applied for 10 minutes. This resulted in an inward shift in I_{avg}, which reached a maximum (trough) and then decayed to persistent current that did not return to baseline I_{avg} for the duration of application of GABA (figure 4A, B). In 5 DGCs tested, the peak I_{avg} evoked by 10 μM GABA was 134.7 ± 0.81 pA. The I_{avg} decayed to a persistent current (90.25 ± 0.57 pA), which was measured 5 minutes after application of 10 μM GABA was started, and the residual non-desensitizing component I_{avg} was 44.5 ± 1 pA (n = 5). Therefore, the extent of desensitization of I_{avg} was 33 %. The channel noise can decrease due to opening or closure of channels and therefore I_{rms} was not used to study desensitization of receptors.

We determined the extent of desensitization of synaptic currents by 10 μM GABA. GABA (10 μM) was applied after recording of sIPSCs at baseline from a DGC and it reduced the amplitude and number of sIPSCs. The reduction of sIPSC amplitude was likely due to desensitization of synaptic receptors, however reduction of number of sIPSCs could be due to diminished presynaptic release of GABA (decrease of frequency), or could be due to disappearance of smaller amplitude events into increased baseline noise (Stell and Mody, 2002). In order to determine whether or not reduction of number of sIPSC was merely increase of failures to detect events, the Cl^- driving force was diminished. sIPCSs were recorded from 3 DGCs voltage-clamped to -65 mV for 5 minutes and then to -20 mV, thus reducing the driving force by 60%. The frequency of sIPSCs decreased from 2.05 Hz to 1.23 Hz (by 40%) and the mean amplitudes decreased from 4.6 ± 18.6 pA to 40.6 ± 12.9 pA. Therefore, decrease of number of sIPSCs was proportional to decrease of driving force, which suggests that decrease frequency with

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decrease of driving force reflects increase of failures to detect, rather than changes in presynaptic release of GABA.

In each of 8 DGCs studied, 10 μM GABA inhibited sIPSC amplitude and frequency (figure 4C, D). The mean amplitude of sIPSC decreased from 64.3 ± 6.6 pA to 47.1 ± 6.1 pA ($p < 0.05$) (figure 4E) and the frequency decreased from 1.26 ± 0.3 Hz to 0.36 ± 0.04 Hz ($p = 0.01$). The total charge transfer mediated by sIPSCs prior to the application of GABA was 120.24 pA per second, and in the presence of GABA it was 42.39 pA per second. Therefore prolonged application of 10 μM GABA desensitized 65% of synaptic GABA release and contributed to the elimination of sIPSCs as well. To test this possibility, 100 μM GABA was currents, substantially more than desensitization of mean current, which was 33%, suggesting synaptic currents were more susceptible to desensitization than mean current.

We confirmed these findings using a higher concentration of GABA. In 5 DGCs, higher concentration of GABA (100 μM) produced a 1642 ± 164 pA inward shift of I_{avg} at the maximum (trough), which decayed to a persistent 676.5 ± 148.5 pA current. The effect of 100 μM GABA on sIPSCs was more dramatic, as they disappeared in all 5 cells. The mean frequency of sIPSCs before GABA application was 0.92 ± 0.12 Hz and no transient event could be detected after the mean current reached the peak.

Activation of presynaptic $GABA_B$ receptors by GABA could have reduced applied to DGCs in the presence of CGP 55548 (10 μM) to block $GABA_B$ receptors. There was no change in frequency of sIPSCs when recordings without CGP 55548 were compared to recordings in the presence of CGP 55548 (0.92 ± 0.17 Hz, n=8 and 1.22 ± 0.05 Hz, $n = 3$, $p = 0.36$). The sIPSCs were completely inhibited by 100 μM GABA in

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the presence of 10 μM CGP 55584. Therefore, desensitization of postsynaptic $GABA_A$ receptors alone without activation of $GABA_B$ receptors could explain the loss of synaptic currents.

Saturating concentration of GABA to measure total residual conductance

Studies with 10 and 100 μM GABA suggested that GABA could be used to differentially desensitize two kinds of receptors that mediate tonic and phasic inhibition in DGCs. GABA activated both kinds of receptors but a fraction of receptors desensitized rapidly while others were persistently active in the presence of GABA. We determined the residual $GABA_A$ receptor conductance in the presence of a saturating concentration of GABA. GABA (1 mM) produced a large, whole-cell inward I_{avg} with maximum amplitude of 1628 ± 194 pA (n = 8, see an example in figure 5A). Continuous application of 1 mM GABA (~10 min) produced desensitization of $GABA_A$ receptors. However, residual current remained regardless of duration of GABA application. This residual current had mean amplitude of 534 ± 126 pA. This residual current was likely mediated by a combination of slowly and rapidly desensitizing rceptors.

Prolonged application of a saturating concentration of GABA could substantially affect the distribution of Cl^- ions, raising the possibility that a large persistent current in the presence of 1 mM GABA could result in the re-distribution of Cl^- ions as opposed to increased conductance resulting from open $GABA_A$ receptor channels. In order to address this question, the DGCs were voltage-clamped to progressively more depolarizing potentials such that no synaptic activity could be detected, typically these potentials were +2 to +4 mV and conductance pulses (2 mV, 10 msec) at 1 Hz frequency were applied. After measuring membrane resistance by applying conductance pulses

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during 5 minutes of the baseline recording, 1 mM GABA was applied for 5 minutes. In response to 1 mM GABA, the I_{avg} changed very little (figure 5A) but the amplitude of the conductance pulse first increased to a peak and then decayed to a persistent value (figures 5B, C). In all 6 DGCs, GABA caused a small inward current (42 ± 6 pA) and reduced the membrane resistance from 211 ± 9.3 MΩ to a minimum of 57.5 ± 0.7 MΩ. The resistance then increased to a persistent level, 95.6 ± 0.8 MΩ. Therefore the persistent change in resistance was 115.4 MΩ or a 8.66 nS change in conductance, similar to that when the DGCs were voltage clamped to –65 mV, 8.21 nS.

Pharmacological properties $GABA_A$ receptors mediating tonic currents

A metabolite of progesterone, 3a-OH-5a-pregnan-20-one or allopregnanolone, is a potent allosteric modulator of $GABA_A$ receptors. Allopregnanolone, in concentrations of the physiological range (10-30 nM), enhances whole-cell $GABA_A$ receptor currents elicited from DGCs (Mtchedlishvili et al., 2001). Nanomolar concentrations of neurosteroid THDOC enhance $GABA_A$ receptor currents elicited from recombinant receptors containing the δ subunit (Wohlfarth et al., 2002), thus we tested whether low concentrations of allopregnanolone could modulate tonic inhibition in DGCs.

A physiological concentration of allopregnanolone (10 nM) was applied to a DGC after a 5-minute recording of sIPSCs, which did not alter I_{avg} but increased mean I_{rms} (figure 6A, B). The effect of allopregnanolone was studied in 5 DGCs, and it increased I_{rms} in each of the 5 DGCs tested (KS test). When the data from individual cells were pooled, 10 nM allopregnanolone increased I_{rms} from 9.165 ± 0.5 pA to 9.612 ± 0.7 pA ($p < 0.001$, $n = 5$). When data from 5 cells were combined, allopregnanolone (10 nm) increased mean I_{avg} from 46.8 ± 3.1 pA, to 48.3 ± 0.6 pA, ($p = 0.4$). A higher

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concentration of allopregnanolone, 30 nM, also increased mean I_{rms} from 7.887 ± 0.47 pA to 8.121 ± 0.4335 pA (n = 5, *t*-test, p = 0.003) without any effect on mean I_{avg} (69.1 ± 8 pA and 71.6 ± 3.2 pA, *t*-test, p = 0.38).

Previous studies suggested that neurosteroids modulate mean current, so we tested whether higher concentration of allopregnanolone (300 nM) would modulate mean current. The mean current was enhanced by 300 nM allopregnanolone as shown in figure 6D. In 3 DGCs, 300 nM allopregnanolone increased mean I_{avg} by 98.2 ± 17 pA, from 62.6 ± 4.9 to 160.8 pA ($p < 0.05$). It also increased I_{rms}, from 7.258 ± 0.06 pA to 8.151 ± 0.1 pA, (p = 0.01). These results suggest that the low nanomolar concentrations of allopregnanolone, likely to be found in the hippocampus *in vivo*, enhanced $GABA_A$ receptor-mediated tonic inhibition by increasing I_{rms}. The effect of 300 nM allopregnanolone on mean current could have been in part due to a direct effect of allopregnanolone on the chloride channel (Twyman and Macdonald, 1992).

Furosemide and loreclezole

Furosemide is a non-competitive antagonist, which inhibits $GABA_A$ receptor currents elicited from α4 subunit-containing recombinant receptors (Wafford et al., 1996) and does not discriminate between γ and δ subunit containing receptors (Korpi and Luddens, 1997). In 6 DGCs, furosemide (300 μM) decreased I_{rms} from 7.611 ± 0.54 pA during baseline to 6.498 ± 0.35 pA, ($p < 0.05$, figure 7 A), but did not change I_{avg}, which was 143.7 ± 23 pA during baseline and 145.6 ± 35 pA (p = 0.38) during drug application. The presence of tonic $GABA_A$ receptor-mediated current was confirmed by bath application of bicuculline (100 μM) in the presence of 300 μM furosemide.

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Bicuculline abolished all synaptic currents and decreased mean I_{avg} by 47 ± 8.31 pA, (n = 3).

Loreclezole decreased tonic current

Anticonvulsant loreclezole enhances β_2 or β_3, but inhibits β_1 subunit-containing $GABA_A$ receptors (Fisher et. al., 2000). Loreclezole (30 μM, figure 8) decreased I_{rms} from 7.573 ± 0.75 pA to 7.355 ± 0.63 pA ($p < 0.05$,) in 4 DGCs. 30 μM loreclezole had the tendency to reduce mean I_{avg} from 174.0 ± 1.1 pA during baseline to 168 ± 0.57 pA during drug application ($p > 0.05$, n = 4).

Diazepam and zolpidem did not modulate tonic current

Diazepam (100 nM) did not alter I_{avg} and I_{rms} (figure 9A, B, C). In order to confirm the presence of tonic currents, bicuculline was bath applied to the cell, which caused a reduction in mean current. This result was confirmed and in 4 cells, diazepam did not change mean I_{avg}, which was 46.8 ± 3.1 pA during baseline and 48.3 ± 0.6 pA ($p = 0.4$) during drug application. The mean I_{rms} was slightly but not significantly diminished from 3.62 ± 0.05 pA during baseline to 3.39 ± 0.12 pA ($p = 0.15$, figure 9). Thus, tonic inhibition was not modulated by diazepam.

The imidazopiridine, zolpidem, acts on the benzodiazepine binding site and exerts maximal potentiation of $GABA_A$ receptor currents in the presence of α_1 subunits (Pritchett at al., 1989) and least enhancement in the presence of α_5 subunits. Zolpidem (100 nM) was bath applied to DGCs. Mean current and RMS noise was measured 30 seconds prior and 5 minutes after the flow of diazepam was started. There was no change in mean current (56.1 ± 2.1 pA and 59.3 ± 0.6 pA, n = 4, $p = 0.48$, *t*-test). The RMS noise also remained unchanged (3.62 ± 0.05 pA versus 3.39 ± 0.12 pA, n = 4, $p = 0.15$, *t*-

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test, figure 10). In order to confirm that tonic currents were present in cells insensitive to zolpidem, bicuculline was applied and it caused reduction of mean current (figure 10A).

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Discussion

This study for the first time demonstrated high affinity, slowly desensitizing $GABA_A$ receptors in hippocampal neurons. Furthermore, this study suggested that persistently open $GABA_A$ receptors contribute to membrane current fluctuations (I_{rms}) recorded from DGCs in baseline conditions. $GABA_A$ receptors contributing to tonic I_{rms} in DGCs were sensitive to allopregnanolone, furosemide, and loreclezole, and insensitive to diazepam and zolpidem. These pharmacological properties were similar to those of recombinant receptors containing α_4, β_1 and the δ subunits.

Tonic $GABA_A$ receptor conductance contributes to membrane noise

Membrane current fluctuations have long been used to study the properties of ion channels and receptors, but there are few studies demonstrating $GABA_A$ receptor contribution to membrane noise. Several experiments in the present study suggested that persistently open $GABA_A$ receptor channels contribute to the membrane noise (I_{rms}) in DGCs in hippocampal slices. The current fluctuations recorded from DGCs diminished in proportion to the chloride driving force suggesting that passage of chloride ions through the membrane contributed to I_{rms}. Penicillin, which blocks open $GABA_A$ receptors reduced I_{rms}. GABA enhanced I_{rms} suggesting that activation $GABA_A$ receptors could contribute to I_{rms}. Pharmacological studies suggested that only a subset of $GABA_A$ receptors contribute to baseline membrane noise. I_{rms} was enhanced by neurosteroid, allopregnanolone whereas benzodiazepine site agonists, diazepam and zolpidem, which enhance synaptic $GABA_A$ receptor currents in DGCs, did not enhance membrane noise.

We found that the membrane noise was more sensitive to GABA, penicillin and allosteric modulators of $GABA_A$ receptors than mean current, I_{avg}. Previous studies using

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competitive antagonists such as bicuculline found that these drugs reduce mean current (I_{avg}) as well as membrane noise I_{rms} (Stell and Mody, 2002). However, competitive antagonists block synaptic currents before inhibiting tonic currents. Penicillin altered I_{rms} without any change in I_{avg}. Penicillin had a minimal effect on synaptic currents, it did not decrease peak amplitude or frequency of IPSCs, but hastened IPSCs decay, which resulted in a two-fold decrease in total charge transfer. In contrast, bicuculline completely eliminated synaptic currents and this could have contributed to changes in I_{avg}. Similarly furosemide, another noncompetitive $GABA_A$ receptor antagonist reduced I_{rms} but not I_{avg}.

High affinity $GABA_A$ receptors

Experiments with low concentrations of GABA (1 μM and 300 nM) revealed a high affinity $GABA_A$ receptor on DGCs. Low concentrations of GABA increased membrane noise, but this could have resulted from closure of channels due to desensitization. Increased I_{rms} is not simply due to $GABA_A$ receptor channel openings because the relationship between the noise variance (RMS noise) and mean current amplitude is parabolic, described by a binomial theorem $[\sigma]^2 = i^2 Np(1 - p)$, where is σ^2 is I_{rms}, N is number of channels, *i* is single channel currents and *p* is probability of single channel opening. That is, the current fluctuations at lowest and highest concentrations of a ligand are minimal because most channels are in a closed or open state, but the fluctuations are maximal at intermediate concentrations of a ligand (see review by Traynelis and Jaramillo, 1998). Several lines of evidence suggested that the augmentation of I_{rms} by 1 μM GABA was due to an opening of receptor channels and not due to closure of open channels. Comparison of membrane slope conductance in the

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presence and absence of GABA demonstrated increased conductance, suggesting that GABA opened more channels than it had shut. Furthermore, 1 μM GABA did not alter the frequency or amplitude of sIPSCs, suggesting that this concentration did not desensitize synaptic $GABA_A$ receptors. Finally, increasing the concentration of GABA to 10 μM resulted in a greater increased membrane noise than that observed with 1 μM GABA.

A number of studies report the extracellular GABA concentration to be in the range from tens of nanomoles to a few micromoles. Extracellular GABA concentration was reported to be 30 nM in neocortex, measured by HPLC (Zhang et al., 2005). Based on microdialysis studies, it has been suggested that the extracellular concentration of GABA in the hippocampus varies in the 2.1 – 3.8 μM range (Shin et al., 2002). It appears that extracellular GABA concentration *in vivo* varies in the 0.3 – 3 μM (Timmerman et al., 1997). Studies in the past used blockade of GABA uptake to modulate tonic current in DGCs (Nusser and Mody, 2002), but the exact change in extracellular concentration of GABA in these studies is unknown. The current study demonstrated that a low concentration of exogenously applied GABA could activate chloride conductance.

These studies suggested that DGCs express $GABA_A$ receptors with a high affinity to GABA. A likely explanation for high GABA affinity of receptors mediating tonic inhibition is that they contain the α_4 subunit. Recombinant $GABA_A$ receptors containing the α_4 have a higher affinity for GABA than those containing the α_1 subunit (Wafford et al., 1996). The mRNA coding for the α_4 and polypeptide has been localized to DGCs in the past. Inhibition of tonic $GABA_A$ receptor-mediated currents by furosemide suggests

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that α_4 subunit-containing $GABA_A$ receptors participate in mediating of tonic currents. Furosemide is known to inhibit α_4 subunit-containing receptors and was demonstrated to inhibit $GABA_A$ receptor currents in dentate granule cells, which express the α_4 subunit (Wafford *et al.*, 1996; Kapur and Macdonald, 1999). In addition to furosemide sensitivity, recombinant $GABA_A$ receptors containing the α_4 subunit are insensitive to diazepam and zolpidem, which was the case in our experiments with tonic currents recorded from DGCs.

Slowly desensitizing receptors on DGCs

A large residual $GABA_A$ receptor conductance was found in the equilibrium state following prolonged application of 100 μM and 1 mM GABA. This residual current was likely mediated by both synaptic and extrasynaptic receptors. Increasing the extracellular concentration of GABA inhibits synaptic $GABA_A$ receptors (Overstreet and Westbrook, 2001) by slow desensitization (Overstreet et al., 2000). Furthermore, the disappearance of synaptic currents was temporally correlated with reduction of the whole-cell conductance suggesting these receptors were being desensitized. Finally, previous studies have suggested that α_1 and γ_2 subunit-containing receptors are expressed in GABAergic synapses on DGCs. Recombinant receptors containing these subunits desensitize rapidly and to a substantial extent (90%) (Bianchi et al., 2002). In contrast, the δ subunit-containing receptors desensitize very slowly (Saxena and Macdonald, 1996; Haas and Macdonald, 1999) and to a lesser extent (35%) (Bianchi and Macdonald, 2002). However, studies with recombinant receptors have shown desensitization of γ2 subunit-containing receptors is never complete. Because γ subunit-containing receptors produce larger currents compared to δ subunit-containing receptors, even after 90% decrement of

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the current, the residual current corresponding to slow and ultraslow desensitization rates of γ subunit-containing receptors can be of similar amplitude as residual current mediated by δ subunit-containing receptors, which loose only 35% of current in response to 28 sec. application of 1 mM GABA (Bianchi and Macdonald, 2002). Therefore It is likely that in our preparation, residual current after prolonged application of 1 mM GABA is generated by mixed population of γ and δ subunit-containing receptors. However, current study does not permit to estimate the extent of participation of each subunit type containing receptors because the ratio of expression of each subunit-containing receptor is unknown. Separate study will be needed to answer this question.

Pharmacological properties of $GABA_A$ receptors mediating tonic currents

Pharmacological properties of persistently open $GABA_A$ receptors in DGCs were similar to those of recombinant receptors containing the δ subunits. Recombinant receptors containing the δ subunit are highly sensitive to neurosteroid enhancement. Enhancement of RMS noise by 10 nM allopregnanolone suggested that persistently open $GABA_A$ receptors on DGCs are sensitive to a physiological concentration of allopregnanolone. This high sensitivity to allopregnanolone is likely to be caused by the presence of δ subunit-containing $GABA_A$ receptors (Wohlfarth et al., 2002). Lack of diazepam and zolpidem sensitivity of receptors also suggested absence of γ_2 subunit and presence of δ subunit in these receptors.

We did not observe changes in mean current when low concentrations (10 and 30 nM) of allopregnanolone were applied, however the RMS noise was increased. The mean current was increased by a 300 nM concentration of allopregnanolone (see results section). In contrast, low concentrations of the neurosteroid THDOC increased mean

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current in DGCs (Stell et al., 2003), which may suggest differential sensitivity of $GABA_A$ receptors to THDOC and allopregnanolone. We have previously reported that 10 nM allopregnanolone strongly enhanced whole-cell currents in DGCs (Mtchedlishvili et al., 2001) but it was not clear whether the enhancement was due to effect of allopregnanolone on synaptic currents, tonic currents or both. The current study demonstrated that the enhancement of whole-cell currents at least in part was due to enhancement of tonic currents.

The loreclezole inhibition of tonic currents suggested the expression of β_1 subunit in receptors mediating tonic currents. Loreclezole is an anticonvulsant that enhances peak $GABA_A$ receptor currents elicited by subsaturating concentrations of GABA by acting at an allosteric regulatory site on β_2 and β_3 subunits (Fisher and Macdonald, 1997; Wafford et al., 1994). Peak currents elicited from receptors containing the β_1 subunit were not enhanced by loreclezole because the receptors lack of the positive modulatory site present on the β_2 and β_3 subunits. In addition to potentiation of peak currents, loreclezole inhibited steady state $GABA_A$ receptor currents by acting at a site distinct from the positive modulatory site on β_2 and β_3 subunits and increased the apparent desensitization. This inhibitory action of loreclezole occurred regardless of the β subunit. Thus, $GABA_A$ receptors containing the β_1 subunit may be inhibited by loreclezole or stay unaffected by it. Spontaneously opening $GABA_A$ receptors are present in hippocampal neurons (Macdonald et al., 1989; Birnir et al., 2000). It is possible that spontaneously opening $GABA_A$ receptors participate in the tonic current described in this paper.

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Conclusions

Ligand-gated ion channels can mediate at least three kinds of tonic currents: currents mediated by spontaneously open channels in the absence of the ligand, slowly desensitizing currents mediated by ligand-bound channels, and residual equilibrium currents recorded after desensitization of channels. These three forms of "tonic" currents may be mediated by overlapping pools of receptors demonstrated in the current study. The current study demonstrated that on DGCs some $GABA_A$ receptors are persistently open. These channels have high affinity for GABA therefore may be ligand bound open channels. Properties of persistently open $GABA_A$ receptors on DGCs were similar to recombinant receptors containing α_4, β_1 and δ subunits. A persistent current could be recorded after desensitization of $GABA_A$ receptors on DGCs.

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Footnotes

Support: RO1 NS 040337 (JK), RO1 NS 044370 (JK)

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Legends for figures

Figure 1) Bicuculline (100 μM) inhibits sIPSCs mean baseline current (I_{avg}). (A) Current trace recorded from a dentate granule cell. 100 mM bicuculline (solid line) eliminated synaptic currents and decreased I_{avg}. Decrease of I_{avg} was accompanied by decrease of baseline noise (I_{rms}). Averaged 5 msec epochs of I_{avg} are scattered wider before application of bicuculline. (B) Competitive $GABA_A$ receptor antagonist SR 95531 (100 μM, solid line) inhibited synaptic currents with no effect on I_{avg} in a DGC. Note that unlike bicuculline, SR 95531 did not affect $I_{avg.}$ (C) SR 95531 inhibited I_{rms} in DGC. The traces display baseline noise during a 50 msec epoch before application of SR 95531 (top trace) and in the presence of 100 μM SR 95531 (bottom trace). I_{rms} (shown by arrows between two solid lines) was defined as a square root of the average of squares of the deviation of individual points from the average value over 50 msec period. Note larger I_{rms} in the bottom trace compared to the top trace in panel B.

Figure 2) 1 μM GABA did not change I_{avg} but increased I_{rms}. (A) Current trace recorded from a dentate granule cell voltage-clamped to -65 mV in the presence of 50 μM DL-AP5 and 20 μM DNQX, before (baseline recording) and during application of 1 μM GABA (solid line); the current trace did not appear to change. (B) 1 μM GABA did not change I_{avg}. Measurements of I_{avg} in 30 epochs (1 epoch = 100 msec) at 1 sec intervals during the baseline period (solid line) and in the presence of 1 μM GABA (dotted line). Note that the I_{avg} did not change after application of 1 μM GABA. (C) Current traces from the same DGCs display increased I_{rms} after application of 1 μM GABA. The top trace shows a 10 second recording before and the bottom trace shows 10 second recording in the presence

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of 1 μM GABA. (D) The current traces display baseline noise during a 50 msec epoch before application of GABA (top trace) and in the presence of 1 μM GABA (bottom trace). I_{rms} (shown by arrows between two solid lines) was defined as a square root of the average of squares of the deviation of individual points from the average value over a 50 msec period. Note larger I_{rms} in the bottom trace compared to the top trace in panel B. (E) Kolmogorov – Smirnov (KS) test was used to compare I_{rms} before and after application of GABA in a neuron. I_{rms} in 60 epochs (1 epoch = 50 msec) at a 500 msec interval in the presence of 1 μM GABA (dotted line) was more than that during baseline period (solid line). (F) Distribution of RMS noise in cell during the baseline period (black columns) and in the presence of 1 μM GABA (white columns) were compared using a frequency distribution histogram with a bin size of 0.2 pA to demonstrate the rightward shift of I_{rms} in the presence of 1 μM GABA. (G) Membrane slope conductance before and after application of 1 μM GABA was compared. DGCs were voltage clamped to -65, -35 and -15 mV, and 60 epochs of I_{rms} were obtained at each holding potential during the baseline period and in the presence of 1 μM GABA, and I_{rms} values were plotted against holding potential. During the baseline period, the slope of the I_{rms} (current) voltage relationship was 18.1 pS and application of 1 μM GABA increased it to 26.2 pS.

Figure 3) The open channel blocker penicillin inhibits I_{rms}, synaptic currents but does not affect I_{avg}. (A) Current trace recorded from a DGC. 300 μM penicillin (solid line) did not change I_{avg}. (B) Expanded current traces before application of penicillin (upper trace) and 5 minutes after application of penicillin (lower trace). I_{rms} (shown by arrows between

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solid and dashed lines) was smaller in the bottom trace compared to the top trace. (C) Penicillin (300 μM) did not change peak amplitude of sIPSCs but sharply decrease decay time constant. Current traces recorded from a DGC before application of penicillin (left panel) and 5 minutes after application of penicillin (right panel). Note faster decay in the presence of penicillin.

Figure 4) 10 μM GABA augmented baseline RMS noise and mean current. (A) A current trace recorded from DGC before (baseline period) and during application of 10 μM GABA (solid line). Note that 10 μM GABA resulted in an inward current, which reached a maximum and then decayed to persistent current, which did not return to the baseline. (B) Expanded fragments from current trace in A, lower trace recorded in the presence of 10 μM GABA demonstrated larger noise than the upper trace recorded during baseline period. (C). Plot of KS test of I_{avg} in 30 epochs before and in 30 epochs after application of 10 μM GABA were compared with KS test. Note the significant increase of I_{avg} after application of 10 μM GABA (dotted line). (D) Normalized sIPSCs (average of approx. 500 sIPSCs) from the current trace recording shown on A. Thin line represents sIPSCs recorded in the baseline and the thick line represents sIPSCs after application of 10 μM GABA. Note the reduction of peak amplitude after application of 10 μM GABA.

Figure 5) Saturating concentration of GABA revealed a slowly desensitizing conductance. (A) Current trace from a DGC showing baseline recording followed by application of 1 mM GABA (solid line), which evoked a large whole-cell current that decayed to a persistent, slowly-desensitizing current. Note that sIPSCs gradually disappeared in during application of 1 mM GABA and were completely eliminated in the persistent slowly desensitizing component, suggesting that synaptic $GABA_A$ receptors

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were desensitized by 1 mM GABA. The difference between the baseline and the persistent, slowly desensitizing component (dashed lines) was measured. (B) (i) Current trace from a DGC voltage-clamped to 4 mV (near reversal potential for Cl^-) with application of conductance pulses (5 mV, 2 msec, 1 Hz), before (baseline period) and during application of 1 mM GABA (solid line). (ii) Expanded potions of a current trace from (i) demonstrated a large increase in conductance in response to GABA by reduction of conductance (the trace on the right), but the conductance remained increased compared to the baseline period. (c) Histograms demonstrating membrane resistance (in MΩ) at baseline (black), at maximally increased conductance evoked by 1 mM GABA (white) and in persistently increased conductance (dashed).

Figure 6) Effect of allopregnanolone on $GABA_A$ receptor mediated tonic current. (A). Typical recording of current trace before (baseline) and during bath application of 10 nM allopregnanolone (solid line) to a DGC demonstrating no change in I_{avg}. (B) Expanded fragments from current trace in A, lower trace recorded in the presence of 10 nM allopregnanolone demonstrated larger noise than the upper trace recorded during baseline period. (C) Frequency distribution histogram of I_{rms} in 60 epochs during the application of 10 nM allopregnanolone (white columns, bin size 0.2 pA) demonstrated rightward shift compared to epochs in the baseline period (black columns). (D) Typical recording of current traces from a DGC before and after application of 300 nM allopregnanolone. Presence of tonic $GABA_A$ receptor-mediated current was confirmed by application of 100 μM bicuculline. Note the downward-deflection of the holding current.

Figure 7) Furosemide (300 μM) decreased $GABA_A$ receptor-mediated I_{rms} in DGCs. (A) A typical recording of a current traces from a DGC before (baseline) and during

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application of 300 μM furosemide indicated by a solid line. Note that 300 μM furosemide did not change I_{avg} and bicuculline (100 μM) applied at the end of the experiment demonstrated the presence of tonic inhibition. (B, C) Expanded fragments from the trace shown in panel A show diminished I_{rms} during the furosemide application (lower traces) than in the baseline period (upper trace). Note decreased I_{rms} in the lower trace compared to the upper trace in C (distance between solid and dashed lines). (D) Frequency distribution histogram of I_{rms} during the furosemide application (white columns bin size, 0.2 pA) was shifted to the left compared to that in the baseline period (black columns). Loreclezole (30 μM) decreased $GABA_A$ receptor mediated I_{rms} in DGCs.

Figure 8) Typical recording of current trace from a DGC before (baseline) and during application of 30 μM loreclezole (solid line) (A). Note that 30 μM loreclezole did not change I_{avg}. (B,C) Expanded fragments from the trace shown in the panel A show diminished I_{rms} during the loreclezole application (lower traces) as compared to that in the baseline period (upper trace). Note decreased I_{rms} in lower trace compared to upper trace in C (distance between solid and dashed lines). (D) Frequency distribution histograms of I_{rms} during loreclezole application, (white columns bin size, 0.2 pA) was shifted to the left as compared to that in the baseline period (black columns).

Figure 9) Diazepam (100 nM) did not affect $GABA_A$ receptor-mediated tonic current in DGCs. (A) A current trace from a DGC before (baseline) and during application of 100 nM diazepam indicated by solid line. (B, C) Expanded fragments from the trace shown in panel A show unchanged I_{rms} during diazepam application (lower traces) compared to the baseline period (upper trace). (D) Frequency distribution histograms of I_{rms} during

diazepam application, (white columns bin size, 0.2 pA) was unchanged compared to that in the baseline period (black columns).

Figure 10) Zolpidem (100 nM) did not affect $GABA_A$ receptor mediated tonic current in DGCs. (A) A current trace from a DGC before (baseline) and during application of 100 nM zolpidem indicated by a solid line. 100 μM bicuculline was applied in the presence of zolpidem (solid line) to confirm presence of tonic inhibition (B,C) Expanded fragments from the trace shown in panel A show unchanged I_{rms} during zolpidem application (lower traces) compared to the baseline period (upper trace). (D) Frequency distribution histograms of I_{rms} during zolpidem application, (white columns bin size, 0.2 pA) was unchanged compared to that in the baseline period (black columns).

Figure 1.

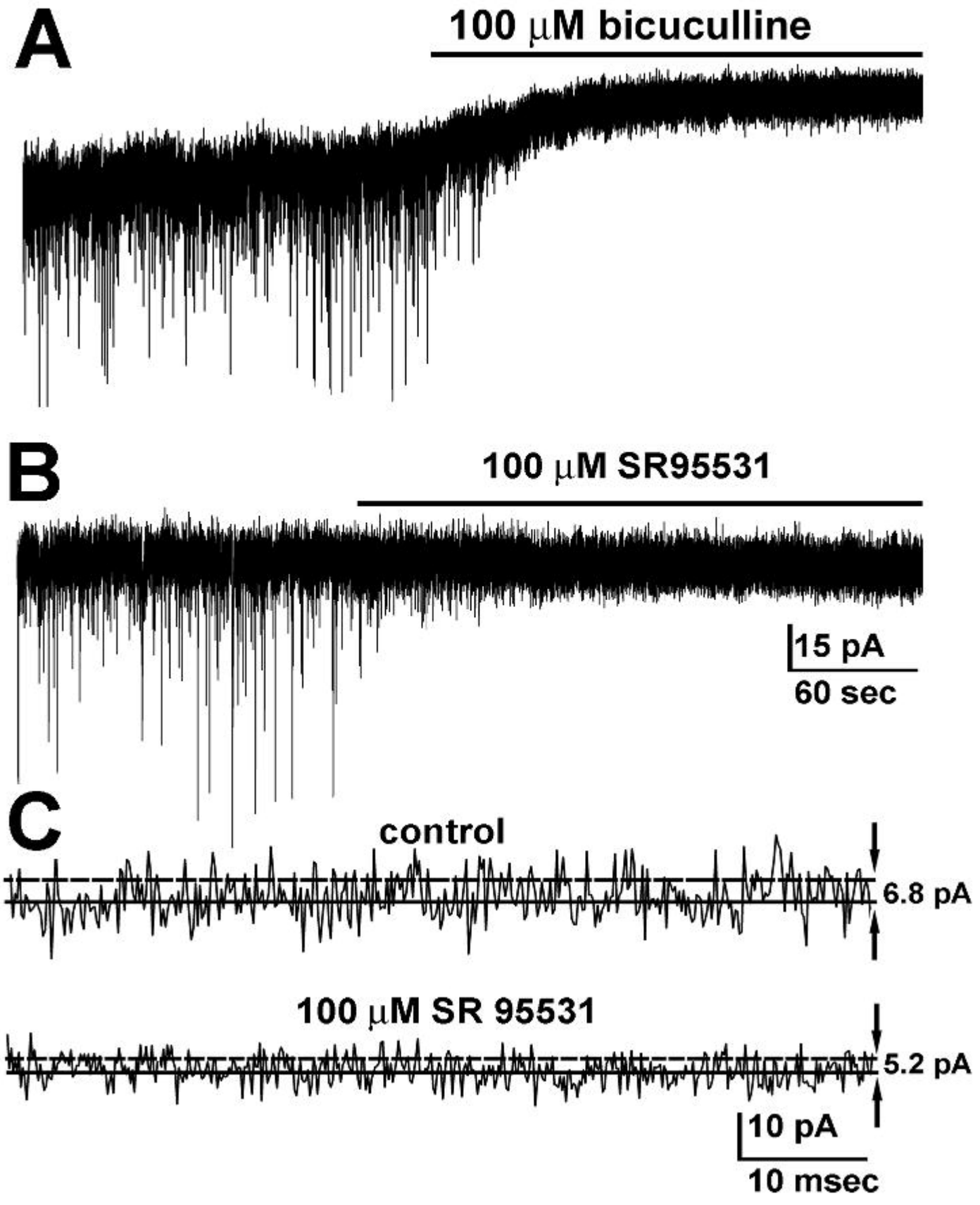

A
100 μM bicuculline
B
100 μM SR95531
15 pA
60 sec
C
control
6.8 pA
100 μM SR 95531
5.2 pA
10 pA
10 msec

Figure 2.

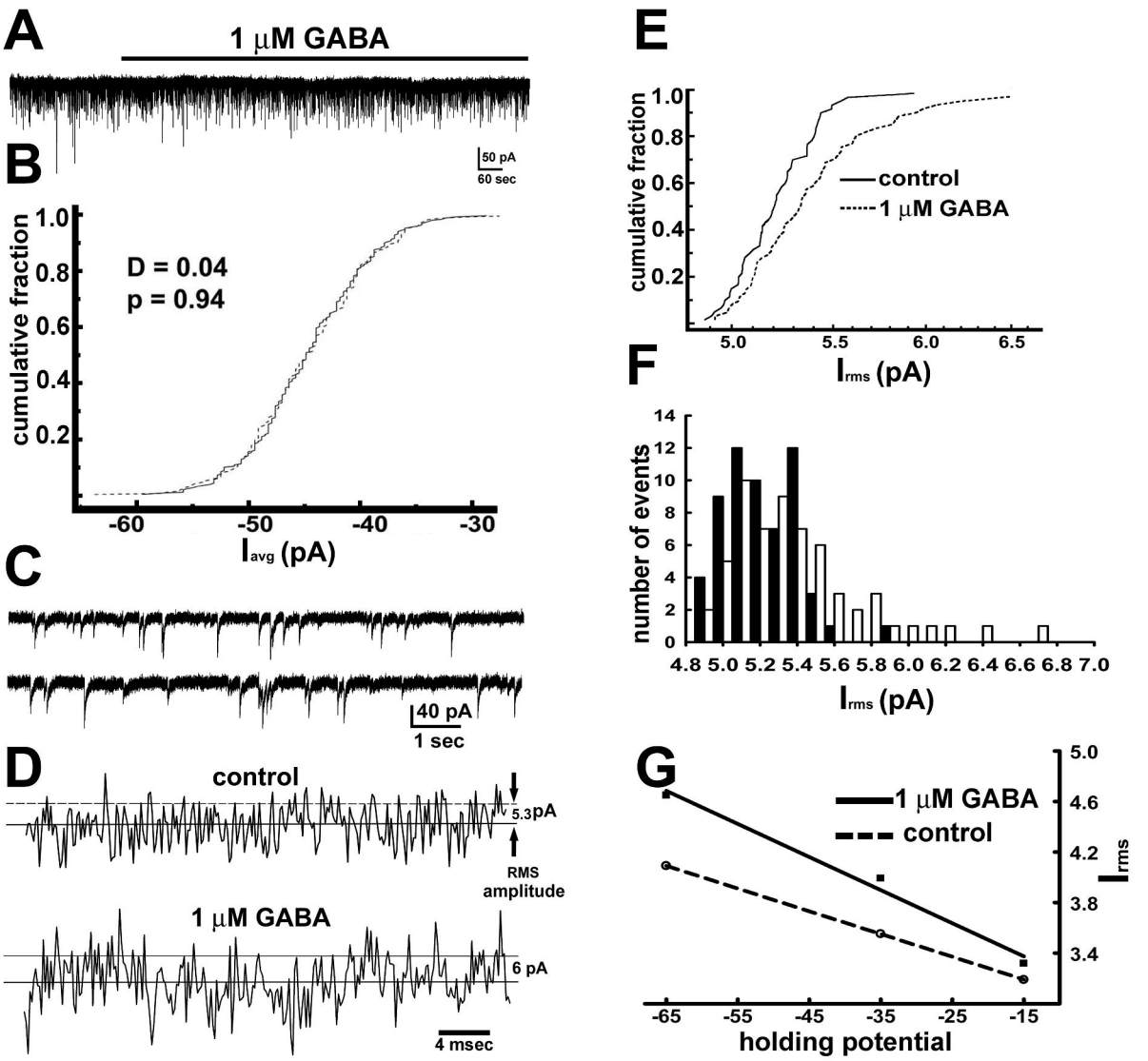

A
1 μM GABA
50 pA
60 sec
B
cumulative fraction
D = 0.04
p = 0.94
I avg (pA)
C
40 pA
1 sec
D
control
5.3pA
RMS amplitude
1 μM GABA
6 pA
4 msec
E
cumulative fraction
control
1 μM GABA
I rms (pA)
F
number of events
I rms (pA)
G
1 μM GABA
control
I rms
holding potential

Figure 3.

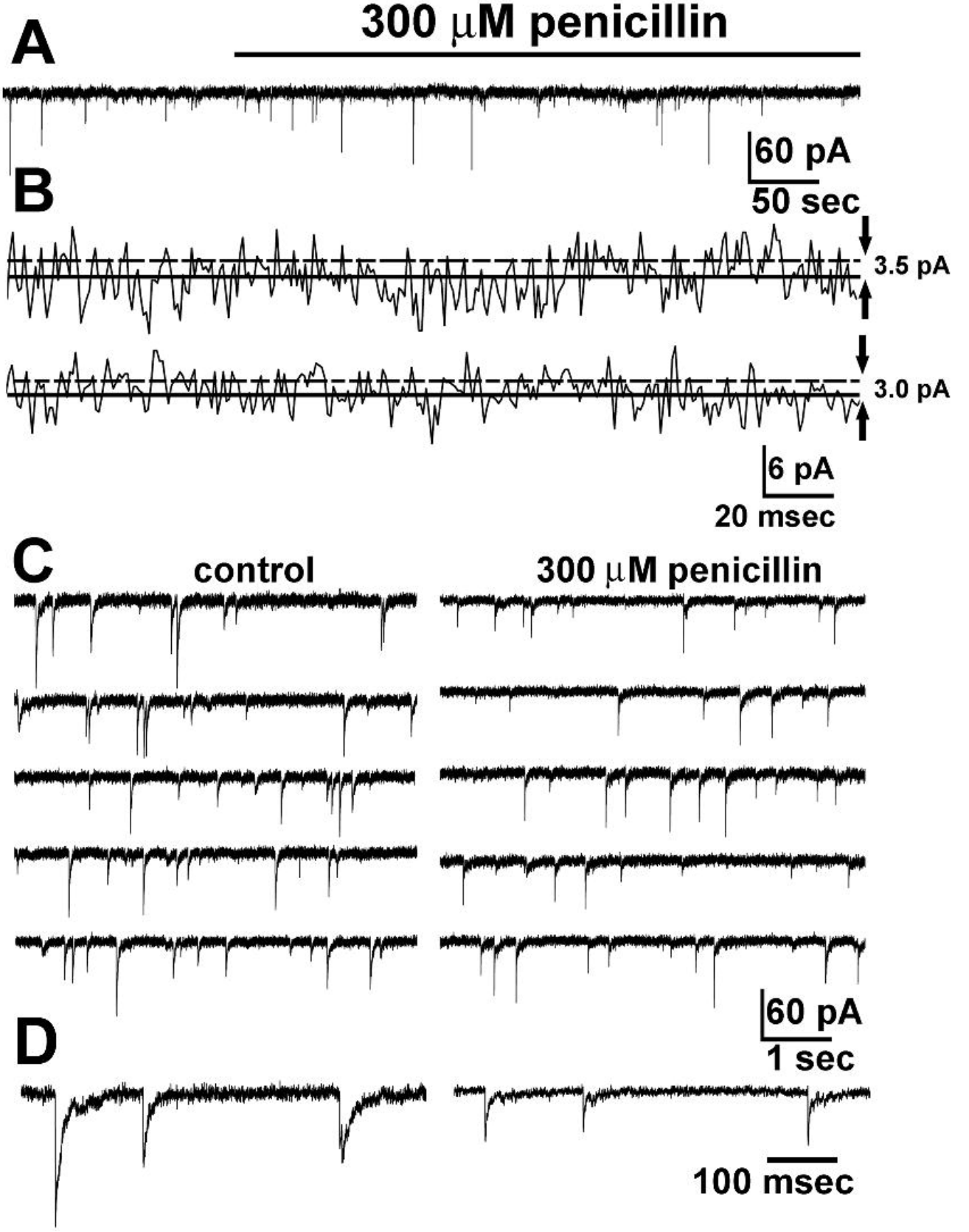

A
300 μM penicillin
60 pA
50 sec
B
3.5 pA
3.0 pA
6 pA
20 msec
C
control
300 μM penicillin
60 pA
1 sec
D
100 msec

Figure 4.

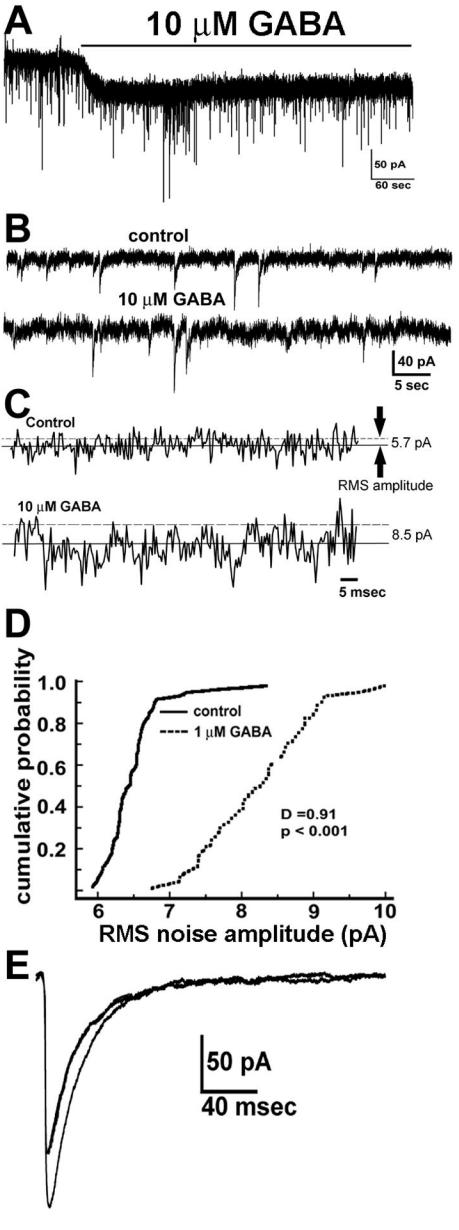

A
10 μM GABA
50 pA
60 sec
B
control
10 μM GABA
40 pA
5 sec
C
Control
5.7 pA
RMS amplitude
10 μM GABA
8.5 pA
5 msec
D
cumulative probability
1.0
0.8
0.6
0.4
0.2
control
1 μM GABA
D =0.91
p < 0.001
6
7
8
9
10
RMS noise amplitude (pA)
E
50 pA
40 msec

Figure 5.

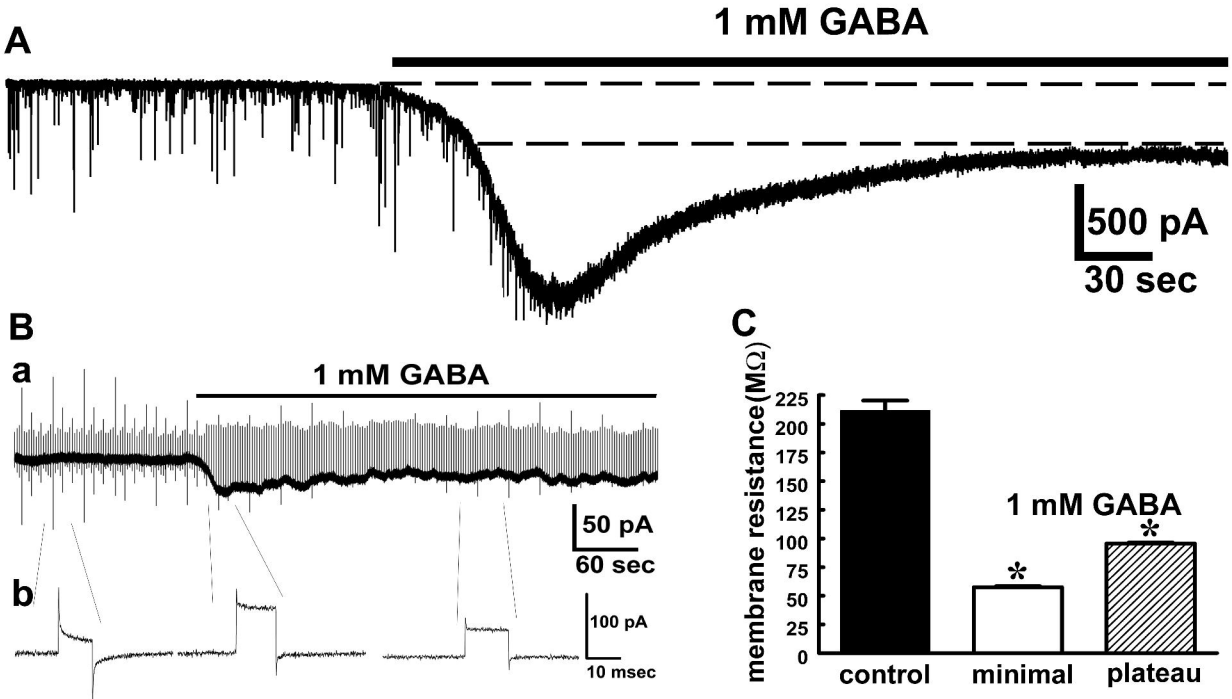

A
1 mM GABA
500 pA
30 sec
B
a
1 mM GABA
50 pA
60 sec
b
100 pA
10 msec
C
membrane resistance(MΩ)
225
200
175
150
125
100
75
50
25
0
1 mM GABA
*
*
control
minimal
plateau

Figure 6.

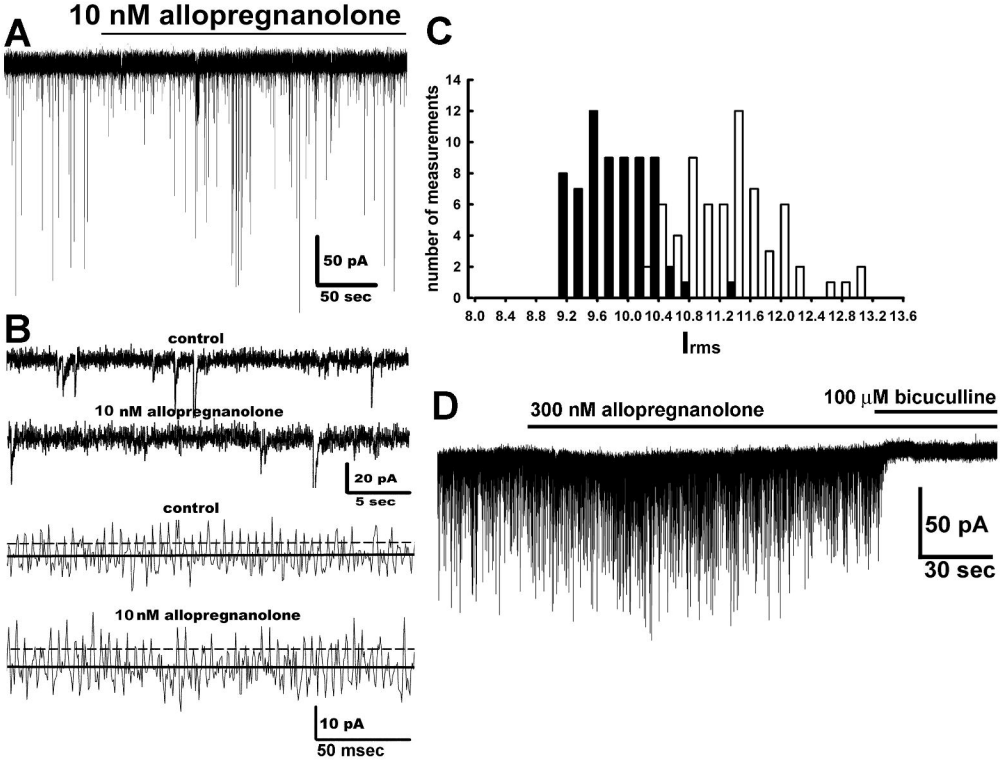

A
10 nM allopregnanolone
50 pA
50 sec
B
control
10 nM allopregnanolone
20 pA
5 sec
control
10 nM allopregnanolone
10 pA
50 msec
C
number of measurements
0
2
4
6
8
10
12
14
8.0 8.4 8.8 9.2 9.6 10.0 10.4 10.8 11.2 11.6 12.0 12.4 12.8 13.2 13.6
rms
D
300 nM allopregnanolone
100 μM bicuculline
50 pA
30 sec

Figure 7.

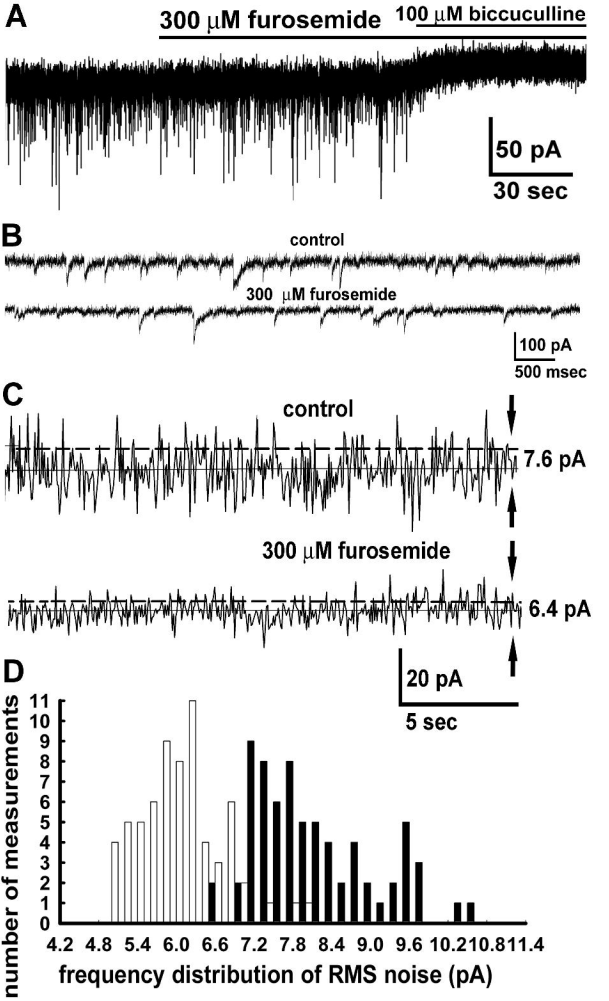

A
300 μM furosemide
100 μM biccuculline
50 pA
30 sec
B
control
300 μM furosemide
100 pA
500 msec
C
control
7.6 pA
300 μM furosemide
6.4 pA
20 pA
5 sec
D
number of measurements
0
1
2
3
4
5
6
7
8
9
10
11
4.2 4.8 5.4 6.0 6.6 7.2 7.8 8.4 9.0 9.6 10.2 10.8 11.4
frequency distribution of RMS noise (pA)

Figure 8.

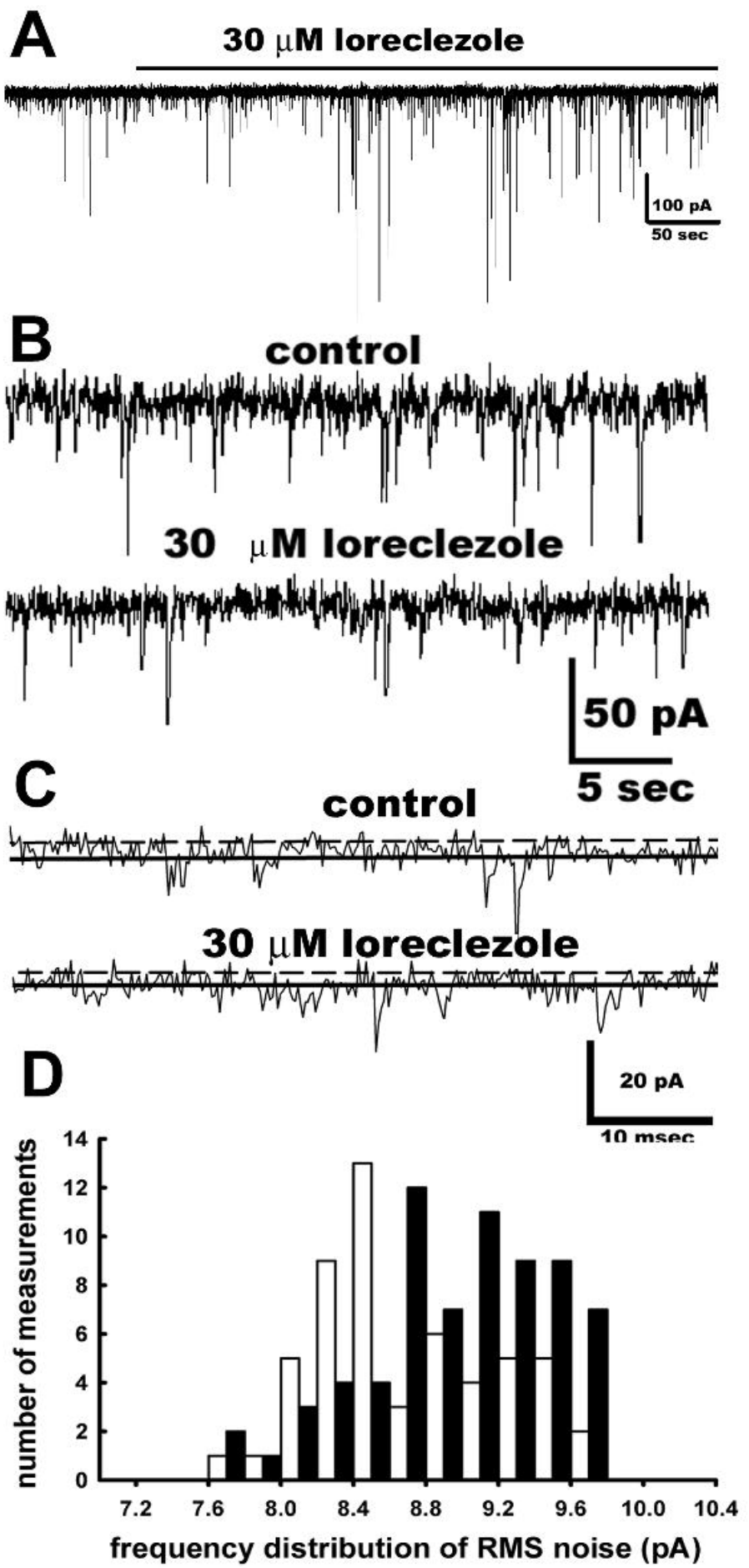

A
30 μM loreclezole
100 pA
50 sec
B
control
30 μM loreclezole
50 pA
5 sec
C
control
30 μM loreclezole
20 pA
10 msec
D
number of measurements
0
2
4
6
8
10
12
14
7.2
7.6
8.0
8.4
8.8
9.2
9.6
10.0
10.4
frequency distribution of RMS noise (pA)

Figure 9.

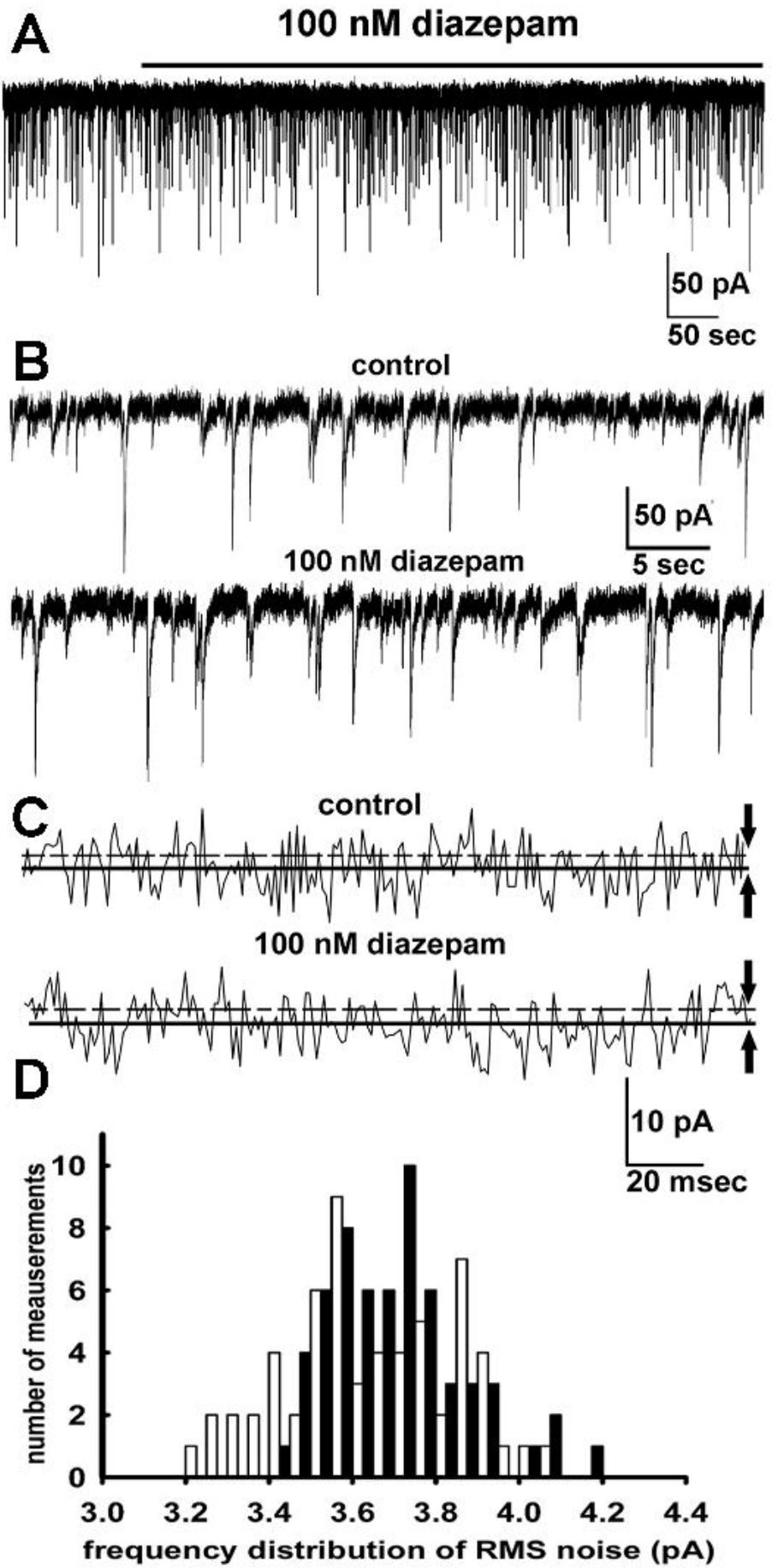
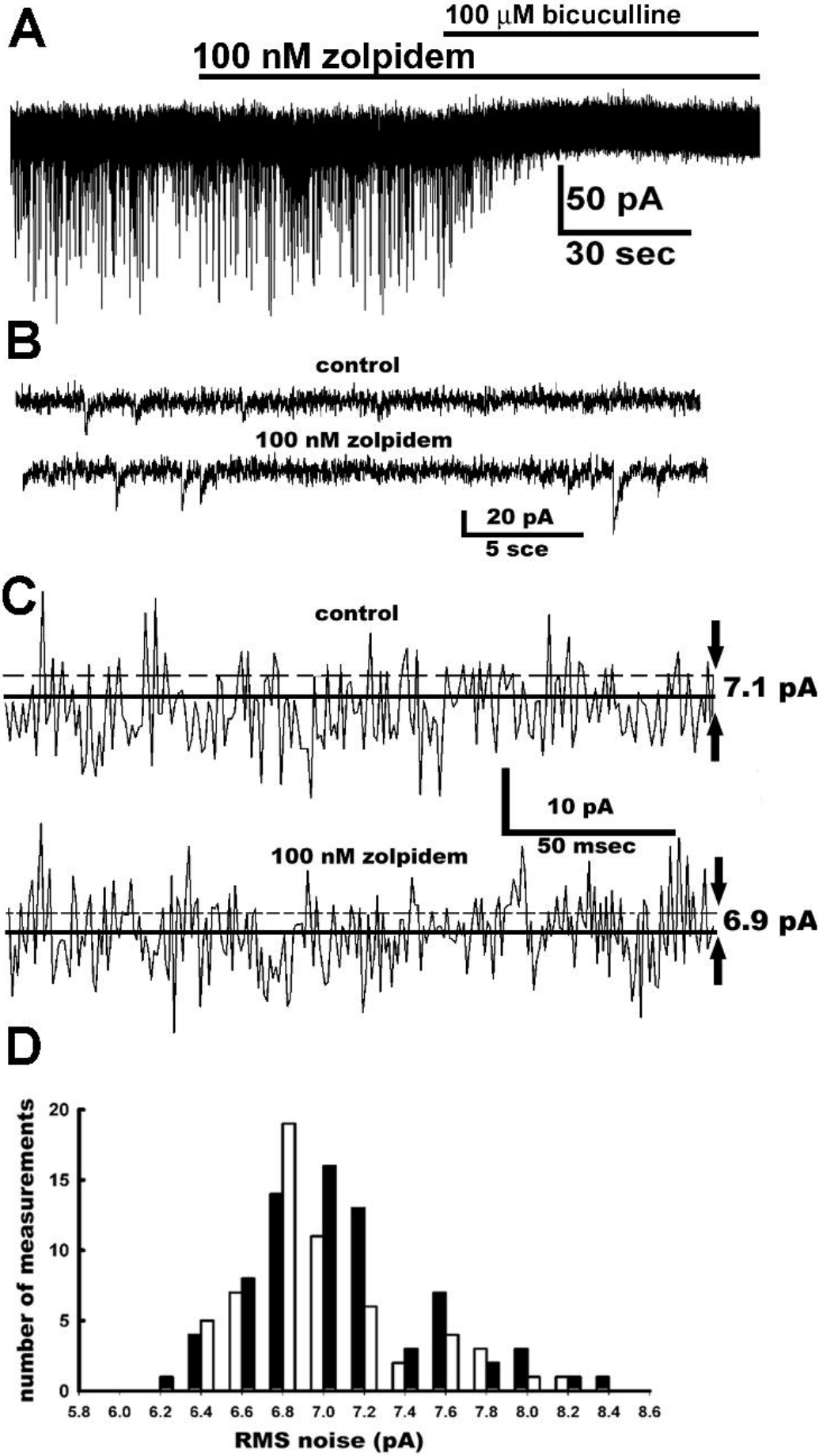

A
100 nM diazepam
50 pA
50 sec
B
control
50 pA
5 sec
100 nM diazepam
C
control
100 nM diazepam
D
10 pA
20 msec
number of meauserements
0
2
4
6
8
10
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
frequency distribution of RMS noise (pA)

Figure 10.


A
100 μM bicuculline
100 nM zolpidem
50 pA
30 sec
B
control
100 nM zolpidem
20 pA
5 sce
C
control
7.1 pA
10 pA
50 msec
100 nM zolpidem
6.9 pA
D
number of measurements
0
5
10
15
20
5.8
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
7.8
8.0
8.2
8.4
8.6
RMS noise (pA)