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Gamma-Hydroxybutyrate Increases a Potassium Current and Decreases the H-current in Hippocampal Neurons via $GABA_B$ Receptors

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Running title: Postsynaptic effects of GHB

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Abbreviations. GHB: Gamma-hydroxybutyrate. **GABA**: gamma-aminobutyric-acid. **aCSF**: artificial cerebrospinal fluid. **RMP**: resting membrane potential. **GIRK**: G-protein coupled inwardly rectifying potassium. ***I-V***: current-voltage.
CGP 55845: 3-N[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid. **NCS-382**: (2E)-(5-hydroxy-5,7,8,9-tetrahydro-6H-benzo[a][7]annulen-6-ylidene ethanoic acid

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Abstract

Gamma-Hydroxybutyrate (GHB) is used for the treatment of alcoholism and to induce absence seizures in animals, but also has recently emerged as a drug of abuse. In hippocampal neurons, GHB may activate its own putative receptor as well as $GABA_B$ receptors to affect synaptic transmission. We used voltage-clamp recordings of rat CA1 pyramidal neurons to characterize the postsynaptic conductances affected by GHB and further clarify the site of GHB action. Low concentrations of GHB (0.1-1 mM) did not affect postsynaptic properties, but 10 mM GHB elicited an outward current at resting potential by augmenting an inwardly rectifying potassium current and concomitantly decreased the hyperpolarization-activated h-current (I_h). Like GHB, the selective $GABA_B$-receptor agonist baclofen (20 μM) increased a potassium current and decreased I_h. In the presence of 10 mM GHB, the baclofen effects were largely occluded. The selective $GABA_B$ receptor antagonist CGP 55845 blocked the effects of both GHB and baclofen, whereas the putative GHB receptor antagonist NCS-382 was ineffective. The GHB and baclofen effects were prevented in the presence of 200 μM barium indicating that GHB augments a K^+ conductance, probably a G-protein coupled inwardly rectifying K^+ (GIRK) current. The decrease of I_h by GHB and baclofen also was prevented by barium, suggesting that the diminution of I_h is secondary to GIRK augmentation. Our results indicate that high GHB levels, which can be reached during abuse or intoxication, activate only $GABA_B$ receptors and not GHB receptors at the postsynaptic level to augment an inwardly rectifying K^+ current and decrease I_h.

Introduction

Gamma-hydroxybutyric acid (GHB) is a neurotransmitter candidate found in the mammalian central nervous system (Maitre, 1997; Bernasconi et al., 1999). GHB is released by depolarization in a calcium-dependent manner and possesses an active uptake mechanism. The GHB system possesses high-affinity binding sites that are found predominantly in neurons of the cortex, striatum and hippocampus, with the highest density found in CA1 hippocampus (Hechler et al., 1987; Maitre, 1997). The highest levels of endogenous GHB are found also in hippocampus, a brain region strongly implicated in memory formation and seizure activity (Rolls, 2000; McCormick and Contreras, 2001). Although GHB is closely related to GABA and can activate $GABA_B$ receptors at high doses, it appears to differ from the GABA transmitter system (Feigenbaum and Howard, 1996).

GHB has been used as a tool to induce a state of absence epilepsy, and several reports have suggested that GHB plays a role in absence seizures (Bernasconi et al., 1992; Banerjee et al., 1993; Maitre, 1997). This effect, as well as a sedative action of GHB that has been widely used in general anesthesia, appears to arise from an interaction between the GABA and GHB transmitter systems (Maitre, 1997). GHB also is increasingly utilized as a drug of abuse to produce euphoric and sedative states, with potential adverse effects that include memory loss, coma, seizure, and withdrawal symptoms (Galloway et al., 1997; Bernasconi et al., 1999). On the other hand, GHB can reduce voluntary ethanol drinking and withdrawal symptoms in humans, and GHB is currently used to treat alcoholism (Gallimberti et al., 2000).

In electrophysiological studies, a weak but consistent hyperpolarizing action of GHB has been described in CA1 hippocampal pyramidal neurons (Xie and Smart, 1992a). GHB

also inhibited neurons in the ventral tegmental area and neocortex (Madden and Johnson, 1998; Jensen and Mody, 2001) and concomitantly promoted oscillatory activity in substantia nigra and thalamocortical neurons (Engberg and Nissbrandt, 1993; Williams et al., 1995; Gervasi et al., 2003). These effects were prevented by a $GABA_B$ receptor antagonist. GHB also reduced excitatory and inhibitory synaptic transmission via $GABA_B$ receptors in CA1 hippocampus (Xie and Smart, 1992b; Jensen and Mody, 2001), but other studies in this brain area showed that GHB elicited similar effects via presynaptic GHB (NCS-382 sensitive) receptors, not $GABA_B$-receptors (Berton et al., 1999; Cammalleri et al., 2002). A possible participation of GHB receptors in postsynaptic mechanisms has not been tested with the selective antagonist NCS-382 (Maitre et al., 1990). Moreover, the postsynaptic conductances affected by GHB have not been characterized nor compared with $GABA_B$-mediated effects.

GHB has emerged as a drug of abuse and is used at high, intoxicating doses. The precise postsynaptic target of GHB needs to be established to further ascertain its cellular action in hippocampus, a brain region that presents the highest levels of endogenous GHB and is implicated in memory formation and seizure activity. The differing results obtained with GHB in hippocampal synaptic studies and the incomplete characterization of the GHB postsynaptic action prompted us to further investigate GHB effects and compare them to those of a $GABA_B$ receptor agonist.

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Methods

Slice preparation. We prepared hippocampal slices as previously described (Schweitzer et al., 1993). In brief, male Sprague-Dawley rats (100-150 g) were anesthetized with 3% halothane, decapitated and their brains rapidly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) gassed with 95% O_2, 5% CO_2. We isolated the hippocampus from the rest of the brain and cut transverse slices 350 μm thick with a McIlwain brain slicer, then incubated the slices in an interface configuration for about 30 min. Slices were then completely submerged and continuously superfused (2-4 ml/min) with warm (31º C), gassed aCSF of the following composition (in mM): NaCl, 130; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4$, 1.5; $CaCl_2$, 2.0; $NaHCO_3$, 24; glucose, 10. Drugs were added to this aCSF in known concentrations. NCS-382 was a gift from Dr. G.L. Gessa, CGP 55845 was a gift from Drs. W. Fröstl and A. Suter (Novartis Pharma). We obtained tetrodotoxin from Calbiochem-Novabiochem (San Diego, CA). All other drugs were obtained from Sigma Chemical Corp (St. Louis, MO).

Electrophysiological recordings. We recorded CA1 hippocampal pyramidal neurons with sharp micropipettes filled with 3M KCl using discontinuous voltage-clamp. The switching frequency was 3-4 kHz and we continuously monitored electrode settling time and capacitance neutralization at the headstage on a separate oscilloscope. Although whole-cell recordings with “patch” electrodes provide a better point clamp due to the low resistance of the electrodes, a major drawback resides in the washout (dialyse) of cytoplasmic components. Because the conductances we studied do not require reaching much depolarized potentials, we used sharp electrodes to ensure we would not compromise drug effects that might be

eliminated during whole-cell recordings. The various problems (i.e., space-clamping) always associated with voltage-clamping of neurons with extended processes are discussed elsewhere (Halliwell and Adams, 1982; Johnston and Brown, 1983), but should be minimized by the study of relative conductance changes with superfusion of drugs to equilibrium conditions. We acquired data with an Axoclamp-2A preamplifier (Axon Instruments, Foster City, CA) and stored them for later analysis using pClamp software (Axon Instruments). Graphs were constructed using Origin software (OriginLab Corporation, Northampton, MA). We subjected data to a one-way ANOVA for repeated measures, with $p < 0.05$ considered statistically significant.

We routinely added 1 μM tetrodotoxin in the aCSF to block Na^+ channels and prevent synaptic transmission. Neurons were held near resting potential for stability but were briefly depolarized to deliver current-voltage (*I-V*) protocols, then repolarized near resting potential. We generated *I-V* curves by holding neurons at -63.3 ± 0.3 mV (n = 41) and applying hyperpolarizing and depolarizing voltage steps (1.5 sec duration, 7 sec apart). Steady-state currents were measured at the end of the voltage steps. To study I_h, we further analyzed the hyperpolarizing voltage steps (15 mV increments, 1.5 sec duration) obtained upon delivery of the *I-V* protocols. We quantified I_h as the difference between the peak current of the relaxation (best fit using pClamp) observed at onset of hyperpolarizing voltage steps (following the capacitance artifact, no extrapolation) and the current measured at the end of the voltage step. These and other measures (e.g. current-voltage curves) were taken before (control), during (5-10 min), and after (washout) drug superfusion. All experimental protocols were consistent with guidelines issued by the National Institutes of Health and were approved by The Scripps Research Institute's Institutional Animal Care and Use Committee.

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Results

We recorded from 50 CA1 pyramidal neurons that had a mean resting membrane potential (RMP) of -68 ± 1 mV and action potential amplitude of 105 ± 1 mV. All experiments were conducted in the presence of 1 μM tetrodotoxin to prevent synaptic transmission.

GHB affects postsynaptic properties across the voltage range

GHB dose-dependently elicited an outward current associated with an increased input conductance at resting potential. Superfusion of 0.1 mM (n = 2) or 1 mM (n = 5) GHB had little or no effect on neuronal properties (Fig.1A). On average, 1.0 mM GHB elicited an outward steady-state current of 12 ± 9 pA near RMP (n = 5). A higher concentration of GHB (10 mM) elicited a marked outward steady-state current associated with increased input conductance (Fig 1B). Current values fully recovered to control levels upon washout. On average, 10 mM GHB elicited an outward steady state current of 83 ± 16 pA at RMP (n = 15).

We generated current-voltage (*I-V*) relationships to study the effects of GHB on steady-state membrane properties at depolarized and hyperpolarized potentials. GHB elicited an outward current at depolarized potentials and an inward current at hyperpolarized potentials (Fig. 2A). Current values recovered to near control values on washout. Subtraction of control values from those observed in the presence of GHB revealed the net current effect, which appeared to rectify inwardly (n = 15, Fig. 2B). The reversal potential of the GHB component was determined individually for each neuron from their respective *I-V* graph and then averaged across all neurons, yielding a mean reversal potential of -104 ± 4 mV. Such a reversal potential is close to the theoretical value of the K^+ equilibrium potential calculated

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using the Nernst equation, -96 mV in our experimental conditions (3.5 mM extracellular K^+ and assuming 135 mM intracellular K^+). The observed shift of -8 mV could be due to GHB affecting a conductance not carried solely by K^+, for example the cationic I_h.

GHB has effects similar to those of baclofen and acts at $GABA_B$ receptors

GHB can affect neuronal activity by activating $GABA_B$ receptors. We first assessed the effect of the $GABA_B$ receptor agonist baclofen on CA1 pyramidal neurons. Superfusion of 15-20 μM baclofen elicited an inwardly rectifying component that recovered upon washout (n = 12, Fig. 3A). The baclofen component had a reversal potential of -103 ± 3 mV and appeared to have characteristics similar to the component elicited by GHB. We then performed sequential applications of GHB and baclofen to investigate whether the two transmitters share similar sites of action. In this neuronal sample, 10 mM GHB alone elicited a steady-state current that had inward rectifying properties and reversed at -103 mV (n= 6, Fig. 3B). After the GHB response reached equilibrium, we added 20 μM baclofen in the continued presence of GHB. Baclofen elicited a small additional effect that presented similar characteristics of voltage-dependency and reversed at -97 mV (Fig. 3B, 4A). The GHB and baclofen effects were not significantly different ($p > 0.05$). Thus, GHB mostly occluded the baclofen effects suggesting that these compounds act through a common mechanism to inhibit CA1 pyramidal neurons.

We assessed the involvement of $GABA_B$ receptors by using receptor antagonists. The putative GHB receptor antagonist NCS-382 has been reported to prevent GHB effects in several preparations. NCS-382 applied alone (1 or 4 mM, n = 4) had no effect on membrane properties. In the presence of NCS-382, 10 mM GHB still elicited a pronounced outward current associated with increased input conductance, and further superfusion of baclofen

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elicited an additional outward current (Fig. 4A). These results indicate that the GHB effect we observed is not mediated by NCS382-sensitive receptors. To ascertain that GHB acted via $GABA_B$ receptors, we applied GHB in slices pretreated with the selective $GABA_B$ receptor antagonist CGP 55845. Superfusion of 1 μM CGP 55845 alone did not alter neuronal properties but completely prevented the effects of 10 mM GHB (n = 5; Fig 4B). The effect of GHB was blocked at all potentials (Fig. 4C), demonstrating that GHB acted via $GABA_B$ receptors to inhibit CA1 neurons.

GHB and baclofen decrease the h-current

The I_h is a mixed K^+/Na^+ current active in the hyperpolarized range. This current is observed in CA1 pyramidal neurons as a slow relaxation that develops upon hyperpolarization, usually tested from a holding potential negative to -60 mV to avoid contamination by the M-current relaxation. We held neurons at -64 ± 1 mV (n = 14) and delivered 5 hyperpolarizing voltage steps (-15 mV increments) to study the modulation of I_h by baclofen and GHB. Baclofen (20 μM) decreased I_h, concomitant with an outward steady-state current around RMP and an inward steady-state current at hyperpolarized potentials (Fig. 5A). On average, superfusion of baclofen significantly ($p < 0.001$, n = 14) decreased I_h amplitude to 78 ± 3% of control with recovery to 92 ± 3% upon washout (Fig. 5B). Thus baclofen concomitantly decreased I_h and augmented a K^+ conductance.

We then assessed the effect of GHB on I_h. We held neurons at -63 ± 1 mV (n = 17). Application of 1 mM GHB had no significant effect on I_h (97 ± 3% of control; $p > 0.05$, n = 5; data not shown), but 10 mM GHB altered steady-state currents and decreased I_h in a manner similar to baclofen (Fig. 5C). On average, GHB significantly ($p < 0.001$, n = 17) decreased I_h to 83 ± 3% of control, with recovery to 94 ± 3% upon washout (Fig. 5D). To

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ascertain that GHB decreased I_h via $GABA_B$ receptors, we repeated the experiments in slices pre-treated with the selective $GABA_B$ receptor antagonist CGP 55845 (1 μM). With $GABA_B$ receptors blocked, 10 mM GHB did not significantly alter I_h, which remained at 101 ± 2% of pre-drug level ($p > 0.05$, n = 5; Fig. 5E). Thus, 10 mM GHB decreased I_h via activation of $GABA_B$ receptors, an effect similar to that of baclofen.

We also investigated a possible effect of GHB on the M-current (I_M), a voltage-dependent K^+ current active at depolarized potentials in CA1 pyramidal neurons. Neurons were held at -44 ± 1 mV and subjected to 5 hyperpolarizing voltage steps (-5 mV increments) to deactivate I_M, which then appeared as a slow inward relaxation. Upon superfusion of 10 mM GHB, I_M remained unaffected at 101 ± 5% of control (n = 6, data not shown).

Barium prevents GHB and baclofen effects

We further characterized GHB and baclofen effects by using the K^+ channel blocker barium. At a concentration of 200 μM, barium selectively inhibits inwardly rectifying K^+ conductances such as GIRK currents (Sodickson and Bean, 1996; Takigawa and Alzheimer, 1999). In neurons pretreated with 200 μM barium, the effect of GHB on steady-state currents was mostly prevented. GHB only elicited a small steady-state outward component that showed no voltage-dependence and appeared to be carried by K^+ (n = 6; Fig. 6A). Further addition of baclofen in the superfusate did not alter steady-state current values, indicating that barium also prevented baclofen effects. These results are consistent with the augmentation of a GIRK current by GHB and baclofen in control condition.

We assessed the modulation of I_h by GHB and baclofen in the presence of barium. The I_h is insensitive to this ion (Pape, 1996). Surprisingly, the diminution of I_h by GHB or baclofen was largely prevented in neurons pre-exposed to 200 μM barium. While GHB and

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baclofen decreased I_h in control superfusate (by 17% and 22%, respectively), GHB only decreased I_h by 4 ± 3% in the presence of barium ($p > 0.05$, n = 6; Fig. 6B). Further addition of baclofen did not decrease I_h. Thus, blockade of GIRK currents by barium prevented the action of GHB on I_h, suggesting that the modulation of I_h is secondary to the increase of the K^+ conductance. A summary of the reported effects is presented on Figure 7.

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Discussion

Characterization of the postsynaptic effects of GHB

Our results indicate that GHB alters the postsynaptic properties of CA1 pyramidal neurons by two ionic mechanisms. Current-voltage relationships show that the GHB-induced steady-state current reverses at -104 mV, suggesting that the overall effect is principally carried by K^+. The inwardly rectifying profile of the GHB effect points to a GIRK current as the most likely candidate. In the presence of a low concentration of barium to selectively block GIRK currents (Takigawa and Alzheimer, 1999), the GHB-elicited effect was markedly decreased, consistent with the involvement of a GIRK conductance.

GHB also decreased the hyperpolarization-activated cationic I_h, an effect consistent with the reversal potential for the overall GHB effect (-104 mV) slightly more negative than the theoretical reversal for K^+ (-96 mV in our experimental conditions). Because I_h is a mixed Na^+/K^+ conductance that reverses at about -30 mV (Maccaferri et al., 1993), a reduction of this current elicits an outward steady-state current at about -100 mV and therefore negatively shifts the reversal potential of the overall effect. Thus, GHB affects the postsynaptic properties of CA1 pyramidal neurons by two inhibitory mechanisms, the augmentation of an inwardly rectifying K^+ current (likely GIRK) and the decrease of I_h.

GHB and baclofen effects are similar and occur via $GABA_B$ receptors

In this study, we compared the effects of GHB with those of the $GABA_B$ receptor agonist baclofen. Like GHB, baclofen elicited an outward current near resting potential and decreased I_h. The current-voltage relationship profile of the baclofen effect showed inward rectification, reversed at -103 mV, and was prevented by barium. Our results are in accord with previous studies performed in CA1 hippocampal pyramidal neurons showing that

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baclofen principally augments an inwardly rectifying K^+ conductance of the GIRK type (Lüscher et al., 1997) and concomitantly decreases I_h (Takigawa and Alzheimer, 2003). Thus GHB elicits baclofen-like postsynaptic effects in CA1 hippocampus. Accordingly, a selective $GABA_B$ receptor antagonist completely prevented GHB actions, conclusively involving $GABA_B$ receptors to mediate GHB effects. In contrast, the putative GHB receptor antagonist NCS-382 did not alter GHB or baclofen effects. Moreover, GHB mostly occluded the baclofen action, suggesting a common cellular mechanism for the two agonists. Notably, GHB is reportedly a weak (but selective) $GABA_B$ receptor agonist (Mathivet et al., 1997), explaining the incomplete occlusion of the baclofen effect by GHB.

The implication of $GABA_B$ receptors to mediate GHB effects is in agreement with most electrophysiological studies (Xie and Smart, 1992a; Xie and Smart, 1992b; Engberg and Nissbrandt, 1993; Williams et al., 1995; Emri et al., 1996; Madden and Johnson, 1998; Jensen and Mody, 2001; Gervasi et al., 2003). Still, GHB is believed to be a neurotransmitter with its own binding site different from $GABA_B$ receptors (Feigenbaum and Howard, 1996; Maitre, 1997) and GHB can alter synaptic activity with $GABA_B$ receptors blocked, an effect prevented by the putative GHB receptor antagonist NCS-382 (Berton et al., 1999; Cammalleri et al., 2002; Brancucci et al., 2004). This latter action occurs at low concentrations of GHB, while the modulation of postsynaptic conductances requires high (millimolar) concentrations. GHB interaction with $GABA_B$ receptors may occur only at high doses, whereas GHB effects via a specific binding site take place at lower concentrations (Maitre, 1997; Bernasconi et al., 1999).

Modulation of the H-current

We did not observe a decrease of I_h by GHB or baclofen in the presence of barium. Such preventive effect of barium on the baclofen-elicited decrease of I_h has been reported in substantia nigra and hippocampal neurons where it was concluded that the diminution of I_h was secondary to the activation of GIRK currents (Watts et al., 1996; Takigawa and Alzheimer, 2003). In ventral tegmental neurons, however, baclofen appeared to directly inhibit I_h (Jiang et al., 1993). In the CA1 hippocampal study, the authors argued that although the modulation of I_h by baclofen was secondary to activation of GIRK, such shunting inhibition has important physiological significance (Takigawa and Alzheimer, 2003). That is, the interplay between the activation of GIRK and the deactivation of I_h dynamically alters the processing of incoming excitatory signals to modify the temporal integration of excitatory inputs. Thus, GHB acting at $GABA_B$ receptors may modulate the temporal integration of synaptic activity by affecting both GIRK and I_h.

Interestingly, the GABA-like compound gabapentin also acts at $GABA_B$ receptors in hippocampus but augments I_h (Ng et al., 2001; Surges et al., 2003), and whereas gabapentin is used in the treatment of partial seizures, GHB is used to elicit absence seizures. In thalamic neurons, where I_h regulates rhythmic activity (McCormick and Pape, 1990), GHB promotes oscillatory cellular bursting via $GABA_B$ receptors (Williams et al., 1995) and administration of GHB in this region induces absence seizures (Snead, 1996). A reduction of I_h in inferior olivary neurons results in hyperpolarization and increased oscillations (Bal and McCormick, 1997). Thus, GHB reduction of I_h via $GABA_B$ receptors could modulate ensemble oscillations or seizure-like bursting.

Pharmacological implications

Our results show that high concentrations of GHB activate $GABA_B$ receptor-mediated mechanisms, in accord with the current literature. The concentration of GHB that elicited a postsynaptic effect is higher than GHB levels described in normal physiological conditions (Maitre, 1997). But most behavioral responses induced by GHB occur at high concentrations when GHB sites are saturated and GHB interacts with other systems, especially GABAergic transmission where GHB and $GABA_B$ receptors appear to be involved in a functional interplay (Bernasconi et al., 1999). The postsynaptic effects of GHB that we characterized occur via $GABA_B$ receptors and parallel behavioral effects such as anesthesia and coma elicited by $GABA_B$ receptor agonists. Plasma concentrations in the low millimolar range induce moderate anesthesia in humans (Kleinschmidt et al., 1999) and reach several millimolars in abuse situations (Galloway et al., 1997). GHB levels might be especially high in “acquaintance rape” settings where victims loose consciousness after intoxication. Thus, although this GHB concentration may not be physiological, it is consistent with pharmacological actions of GHB.

Further, the actual concentration of GHB surrounding the recorded neuron into the slice is likely lower than the nominal concentration delivered in the superfusion system, and active mechanisms of GHB uptake (Muller et al., 2002) may reduce the potency of GHB in the slice preparation. The relevance of high GHB concentrations has also been discussed in other studies (Madden and Johnson, 1998; Jensen and Mody, 2001). Thus, while the physiological and therapeutic roles of GHB appear to involve high micromolar concentrations of GHB, the millimolar range tested in the present study compares to GHB levels obtained during anesthesia, abuse and intoxication in humans. The implication of

postsynaptic effects that involve $GABA_B$ receptor mechanisms may therefore only occur in these latter circumstances and be treated with $GABA_B$ receptor antagonists, whereas the transmitter role of GHB may not implicate postsynaptic mechanisms.

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Figure Legends

Figure 1. GHB elicits an outward current at resting potential. **A.** Continuous current record from a CA1 pyramidal neuron held at -70 mV. The addition of 1 mM GHB in the superfusate (arrow) had no effect on membrane properties. Downward deflections are current responses to 15 mV hyperpolarizing steps, and occasional groups of larger deflections (truncated) are current responses elicited by the *I-V* protocols; RMP was -70 mV. **B.** In another cell held at -68 mV, superfusion of 10 mM GHB elicited an outward current and increased input conductance, with recovery to control level (dashed line) upon washout. RMP was -68 mV.

Figure 2. GHB affects steady-state currents throughout the voltage range. **A.** Current traces of a representative neuron held at -61 mV and subjected to 3 different voltage steps sequentially applied and superimposed at each condition (voltage protocol bottom left). GHB (10 mM) elicited an outward current at depolarized potentials and an inward current at hyperpolarized potentials. Dotted lines indicate control levels; RMP was -70 mV. **B.** Net steady-state currents (subtracted from control) elicited by 10 mM GHB (n = 15). The GHB effect showed inward rectification and reversed at -104 mV. Curve obtained by polynomial fit.

Figure 3. The $GABA_B$ receptor agonist baclofen has an effect similar to GHB. **A.** Averaged net steady-state currents elicited by 15-20 μM baclofen (n = 12). Similarly to GHB, baclofen elicited an effect that showed inward rectification and reversed at -103 mV. **B.** Net steady-state currents obtained from 6 neurons exposed to 10 mM GHB followed by 20 μM baclofen in the continued presence of GHB. GHB mostly (but not completely) occluded the baclofen

response. Baclofen elicited a further effect ("difference") that had comparable properties. All curves obtained by polynomial fit.

Figure 4. GHB exerts its effect via $GABA_B$ receptors, not GHB receptors. **A.** Continuous current record from a neuron held at -70 mV. The GHB receptor antagonist NCS-382 (1 mM) did not prevent the outward current elicited by 10 mM GHB nor the additional effect elicited by further addition of baclofen. RMP was -69 mV. **B.** In the presence of 1 μM CGP 55845, a $GABA_B$ receptor antagonist, 10 mM GHB had no effect on membrane properties. RMP was -68 mV, holding potential was -68 mV. **C.** Net steady-state current elicited by 10 mM GHB in the presence of 1 μM CGP 55845 (n = 5). The GHB effect was prevented at all potentials.

Figure 5. Baclofen and GHB decrease I_h. **A.** Current recording of a neuron stepped from -66 to -125 mV to activate I_h (relaxation at step onset). The lower panel shows the I_h relaxations (identified with letters) magnified and superimposed. Baclofen (20 μM) decreased I_h by 26%. Dotted lines indicate control levels; RMP was -69 mV. **B.** Plot of the I_h relaxation amplitude (n = 14; polynomial fit). On average, baclofen decreased I_h by 22%. **C.** GHB also decreases I_h. In this neuron, 10 mM GHB reduced I_h by 24%. The right panel shows the magnified I_h relaxations. RMP was -68 mV. **D.** Plot of the I_h relaxation amplitude (n = 17; polynomial fit). On average, 10 mM GHB decreased I_h by 17%. **E.** In the presence of 1 μM CGP 55845 to block $GABA_B$ receptors, 10 mM GHB had no effect on I_h.

Figure 6. The blockade of GIRK currents by barium prevents the GHB effects. **A.** Net steady-state currents elicited by GHB followed by baclofen in the presence of 200 μM barium (n = 6). GHB (10 mM) only induced a small voltage-independent component, and

further addition of baclofen (20 μM) was without effect. **B.** Graph of I_h amplitude. In the presence of 200 μM barium, neither GHB nor baclofen decreased I_h.

Figure 7. Summary of results. **A.** Net (control-subtracted) effect on steady-state current level at -64 mV, close to resting potential (average RMP was -68 mV). GHB had no effect at 1 mM, whereas 10 mM GHB or 15-20 μM baclofen elicited a pronounced outward current. The GHB effect persisted in the presence of the GHB receptor antagonist NCS-382 (1-4 mM), but was abolished by the $GABA_B$ receptor antagonist CGP 55845 (1 μM) or 200 μM barium to block inwardly rectifying K^+ currents. **B.** Percentile of I_h decrease (pre-drug application is 100%) at about -135 mV (maximum I_h activation). Again 1 mM GHB had no effect, but 10 mM GHB or 15-20 μM baclofen decreased I_h. The effect of 10 mM GHB was unaffected by 1-4 mM NCS-382 but prevented by 1 μM CGP 55845 or 200 μM barium.

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Figure 1

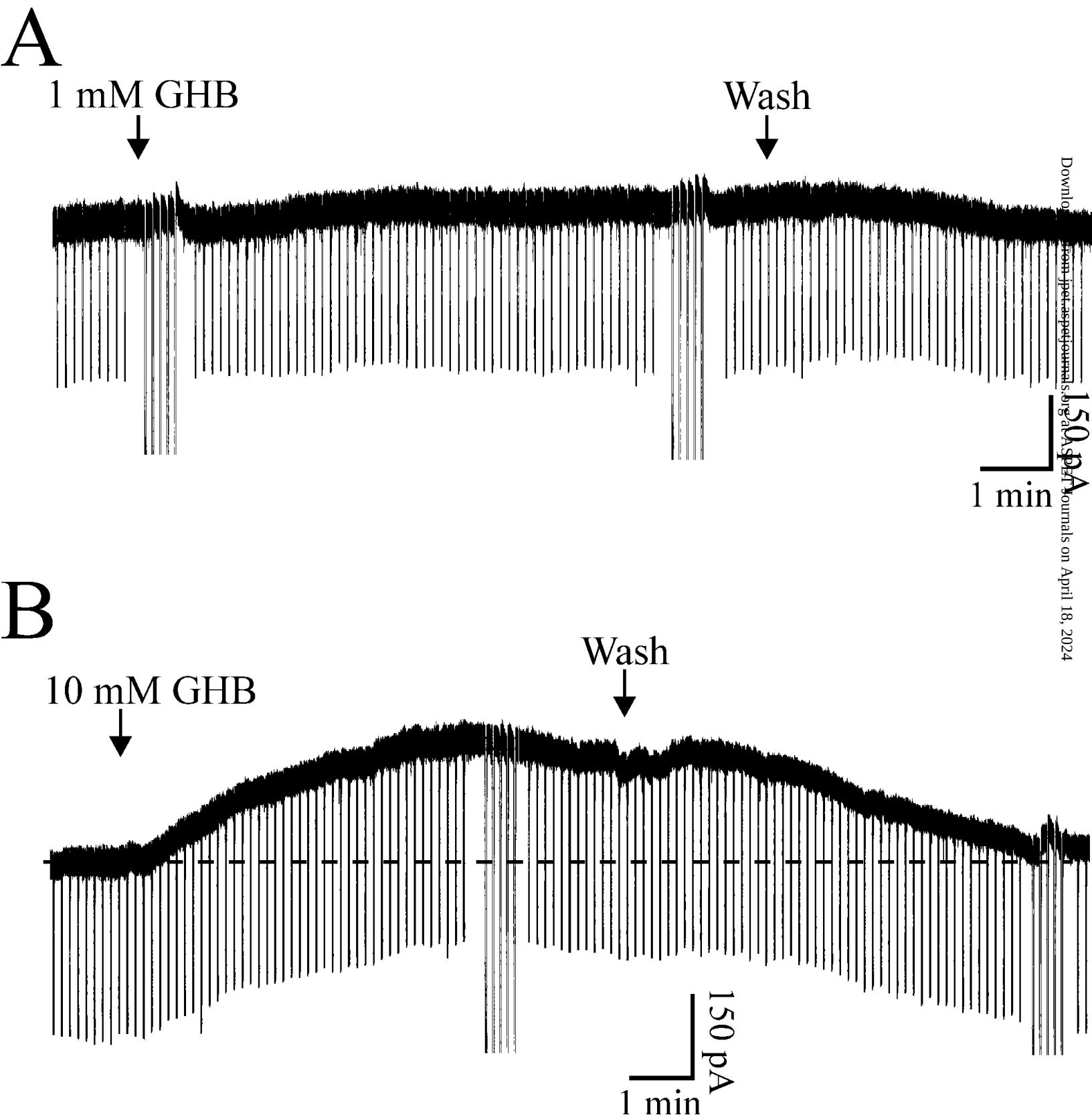

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Figure 2

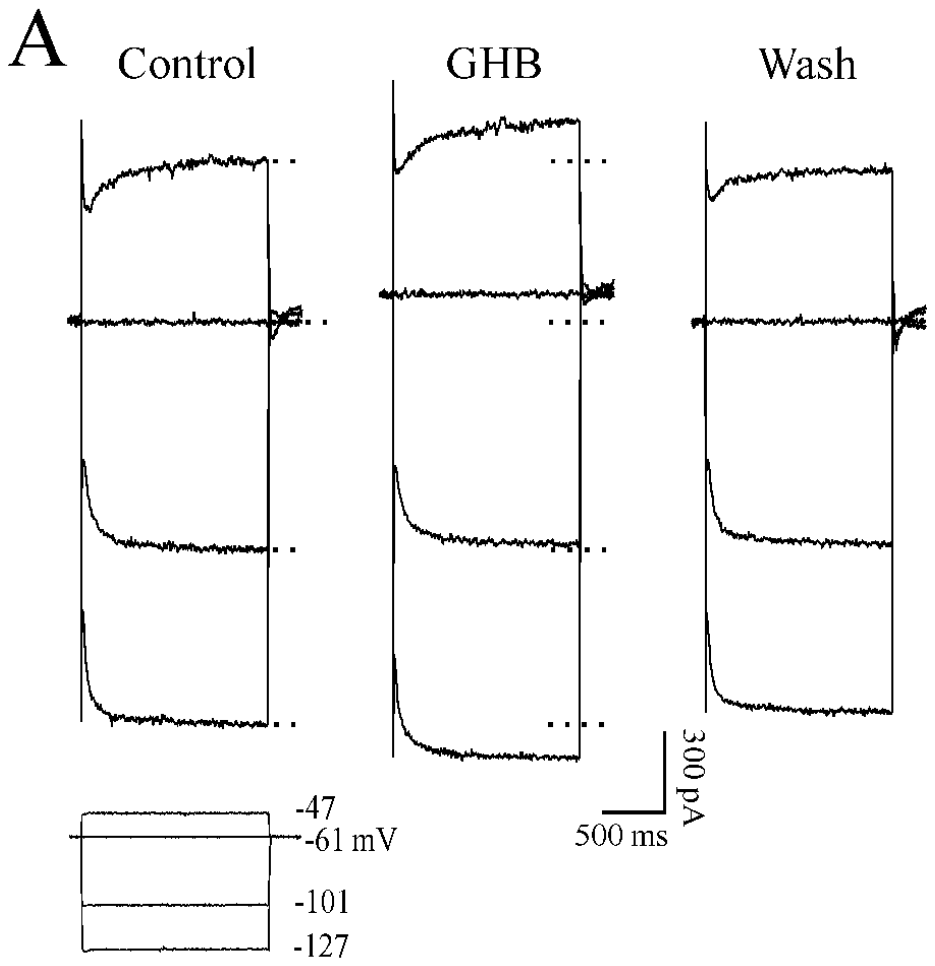

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Figure 3

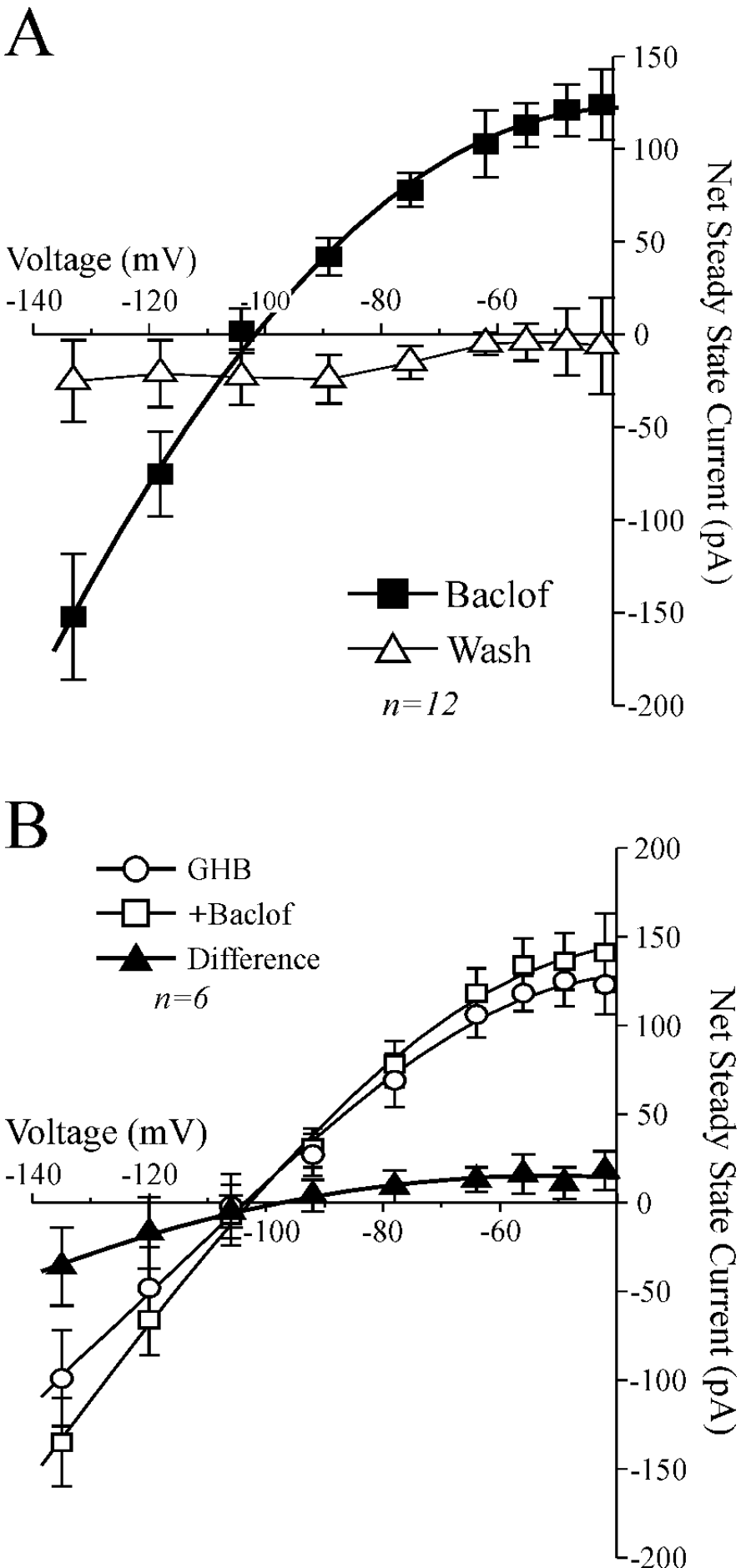

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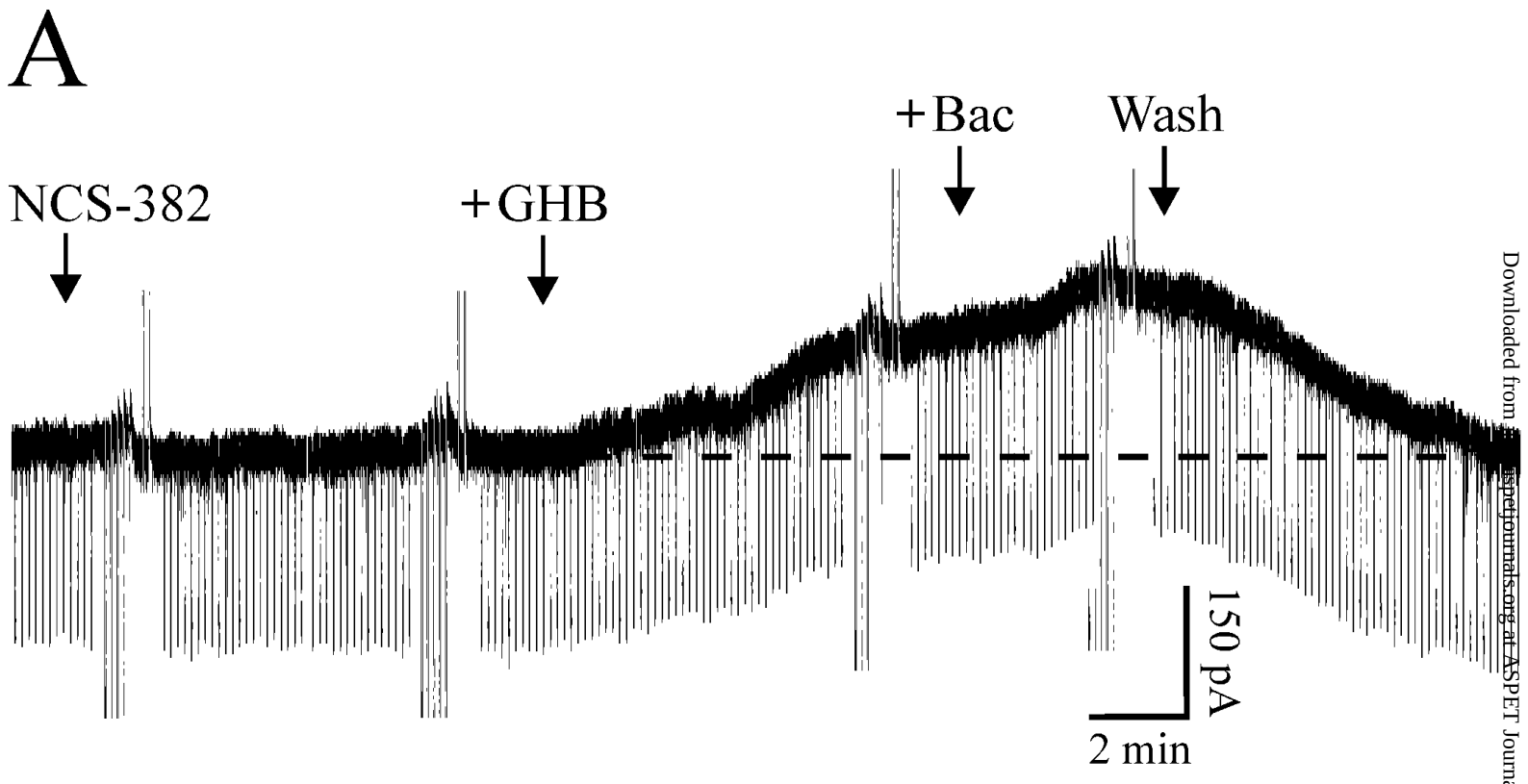
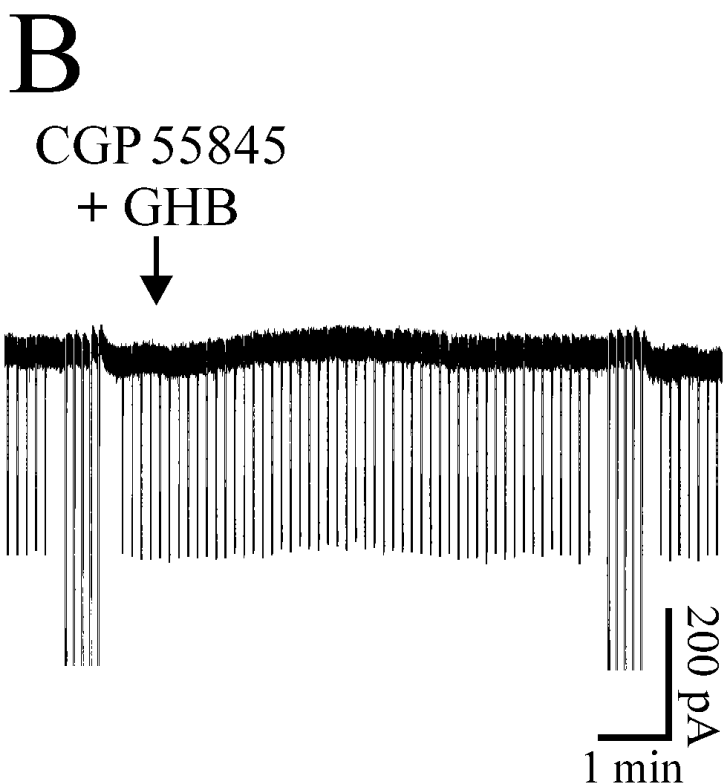
Figure 4





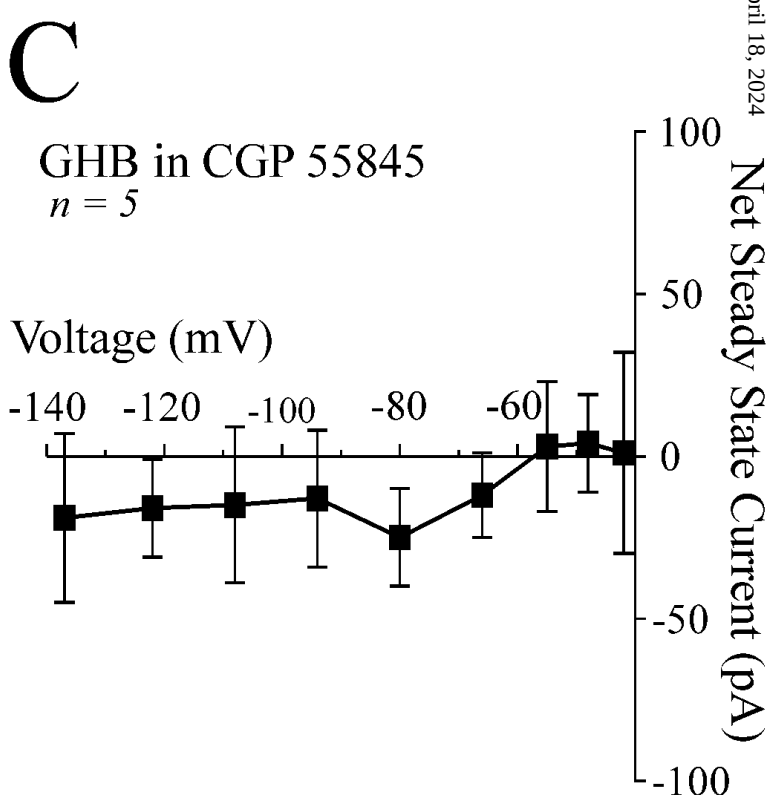


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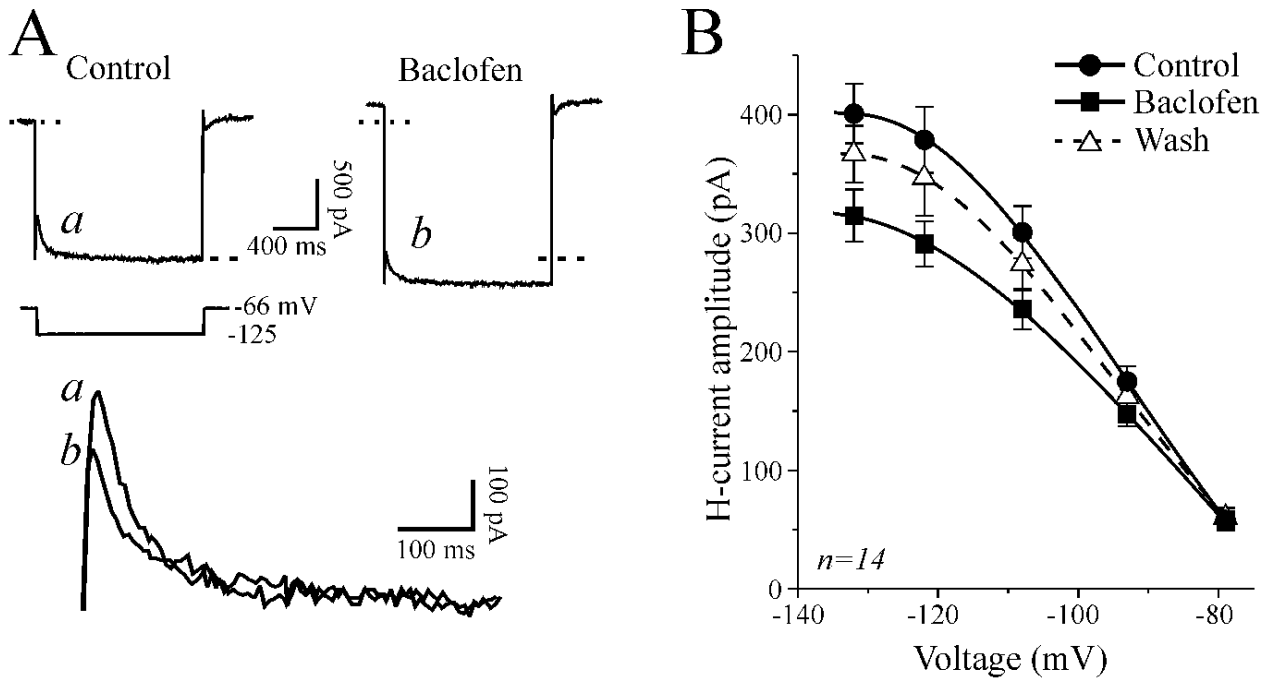
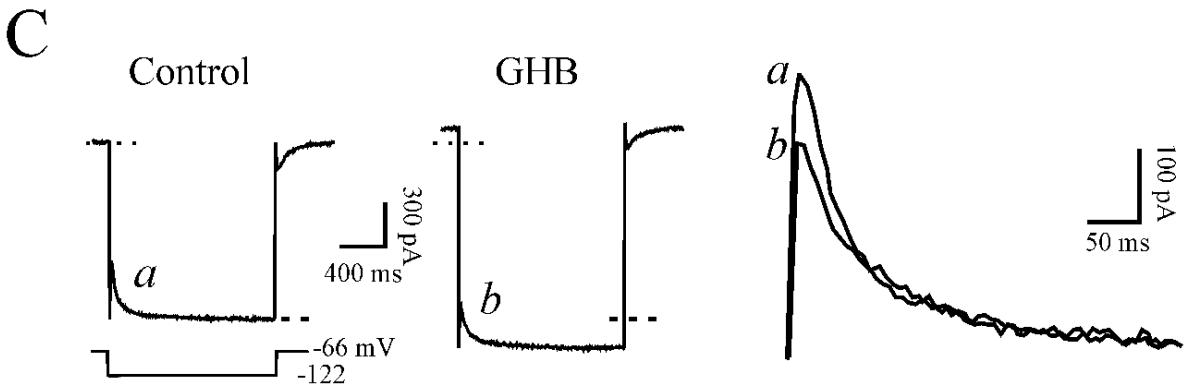
Figure 5





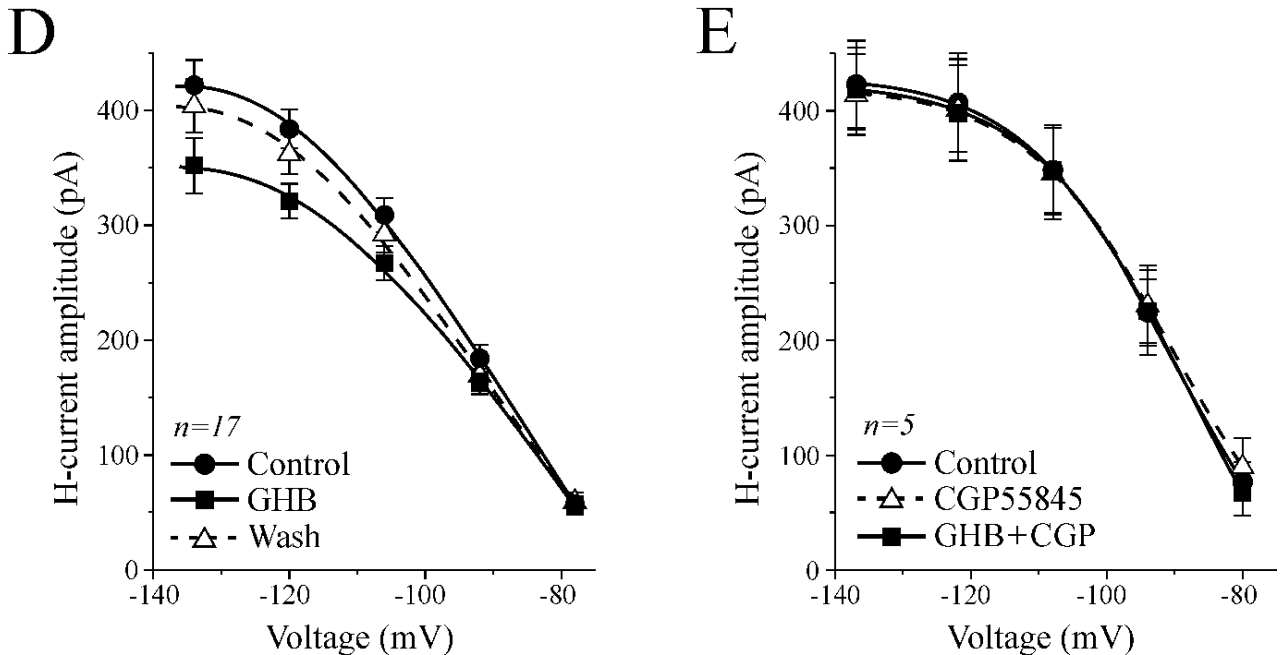


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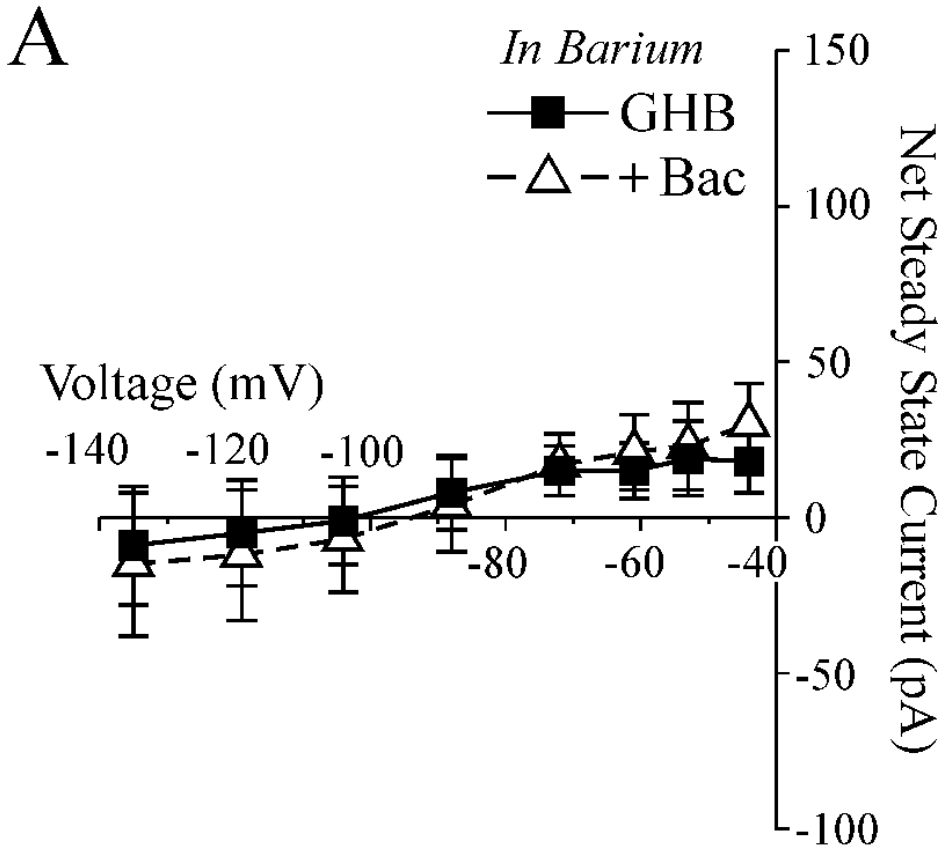
Figure 6



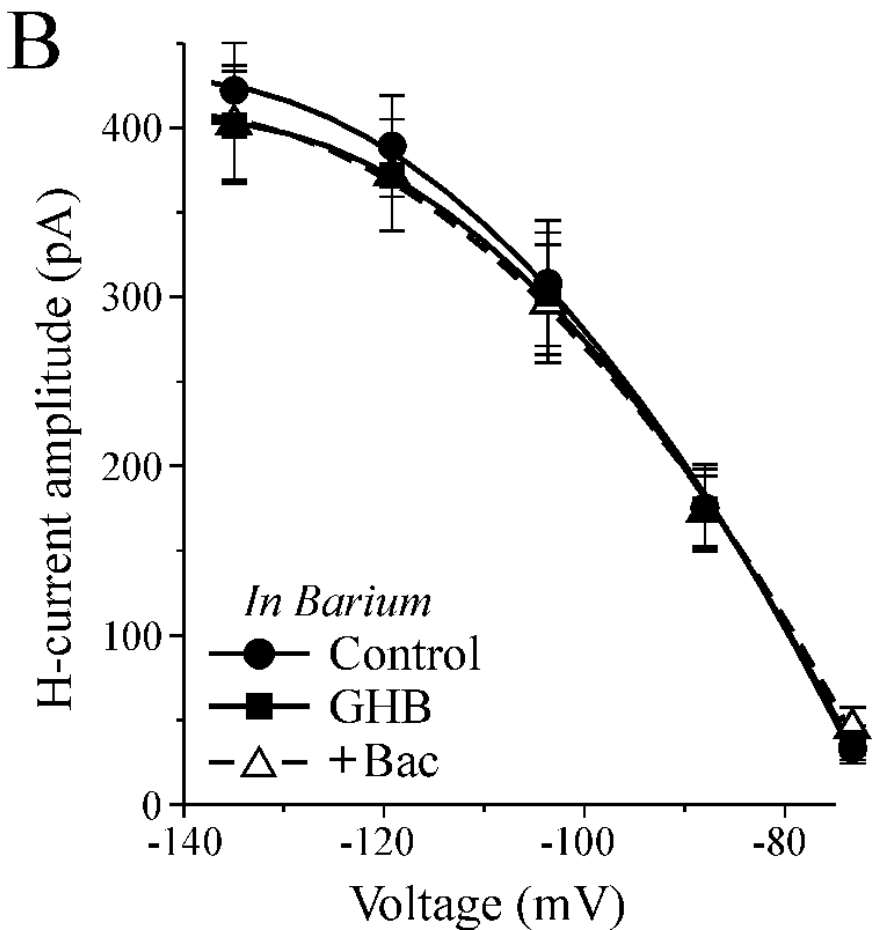


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Figure 7