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Mefloquine enhances nigral γ-aminobutyric acid release via inhibition of cholinesterase

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Running title: Mefloquine enhances GABA release

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ABBREVIATIONS: α-BgTX, α-Bungarotoxin; ACh, acetylcholine; AChE, cholinesterase; AChR, acetylcholine receptor; BIC, bicuculline; DA, dopaminergic; DAMGO (Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol enkephalin); DHβH, dihydro-β-erythroidine hydrobromide; GABA, γ-aminobutyric acid; MEC, mecamylamine hydrochloride; mEPPS, miniature endplate potentials; mIPSCs, miniature inhibitory postsynaptic currents; sIPSCs, spontaneous inhibitory postsynaptic currents; MFQ, mefloquine; PHY, physostigmine; QP, quinpirole hydrochloride; SNc, substantia nigra pars compacta; TTX, tetrodotoxin; VGCC, voltage gated calcium channel

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ABSTRACT

Mefloquine, a widely used antimalarial drug, has many neuropsychiatric effects. While the mechanisms underlying these side effects remain unclear, recent studies show that mefloquine enhances spontaneous transmitter release and inhibits cholinesterases. In this study, we examined the effect of mefloquine on γ-aminobutyric acid (GABA) receptor mediated, spontaneous inhibitory postsynaptic currents (sIPSCs) of dopaminergic neurons, mechanically dissociated from the substantia nigra pars compacta of rats aged 6-17 postnatal days. Mefloquine (0.1 – 10 μM) robustly and reversibly increased the frequency of sIPSCs with an EC_{50} of 1.3 μM. Mefloquine also enhanced the frequency of miniature IPSCs in the presence of tetrodotoxin, but without changing their mean amplitude. This suggests that mefloquine acts presynaptically to increase GABA release. Mefloquine-induced enhancement of sIPSCs was significantly attenuated in medium containing low Ca^{2+} (0.5 mM), or following pretreatment with BAPTA-AM (30 μM), a membrane permeable Ca^{2+} chelator. In contrast, 100 μM Cd^{2+} did not alter the action of mefloquine. This suggests that mefloquine-induced facilitation of GABA release depends on extracellular and intraterminal Ca^{2+}, but not on voltage-gated Ca^{2+} channels. Mefloquine-induced enhancement of sIPSCs was significantly attenuated in the presence of the anticholinesterase agent physostigmine, or blockers of non-α7 nicotinic acetylcholine receptors. Taken together, these data suggest that mefloquine enhances GABA release through its inhibition of cholinesterase. This allows accumulation of endogenously released acetylcholine which activates neuronal nicotinic receptors on GABAergic nerve terminals. The resultant increase of Ca^{2+} entry into these terminals enhances vesicular release of GABA. This action may contribute to the neurobehavioral effects of mefloquine.

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Introduction

Mefloquine is a widely used antimalarial drug because of its effectiveness against chloroquine–resistant plasmodia. It is well known that this benefit of mefloquine is offset by many adverse side effects on both the central and peripheral nervous systems (Fuller et al., 2002; Wooltorton, 2002; Falchook et al., 2003; Kukoyi and Carney., 2003; Meier et al., 2004). The most notable adverse effects are neuropsychiatric disturbances of anxiety, confusion, dizziness, and dysphoria. The cellular basis of these effects is not understood.

Synaptic transmission is of great importance in the interplay between cells of the nervous system. Recently, several studies reported that mefloquine potently altered synaptic transmission in rodent central nervous system and peripheral synapses. Specifically, mefloquine robustly enhanced the frequency of spontaneous excitatory postsynaptic potentials in rat hippocampal slices (Cruikshank et al., 2004). Likewise, mefloquine significantly increased the frequency as well as decay time, of miniature endplate potentials (mepps) at the mouse neuromuscular junction (McArdle et al., 2005, 2006). Since the intracellular Ca^{2+} buffer BAPTA-AM prevented the effect of mefloquine on mepp frequency, it was suggested that mefloquine alters storage of Ca^{2+} within motor nerve endings. On the other hand, the prolongation of mepp decay time appeared to depend on the anticholinesterase action of mefloquine (Lim and Go, 1985). The relevance of these findings to the neuropsychiatric effects of mefloquine remains unclear.

Central dopaminergic (DA) neurons regulating cognitive and motor processes are located in the ventral mesencephalon, including substantia nigra and ventral tegmental area. The substantia nigra pars compacta (SNc) possesses a dense area of DA neurons, receiving GABAergic inhibition primarily from neurons in substantia nigra pars reticulate, pallidum, striatum, and nucleus accumbens (Giustizieri et al., 2005). The GABAergic inputs control the excitability of DA neurons (Tepper et al., 1998). The fact that SNc DA neurons can be isolated along with attached GABAergic terminal boutons (Akaike and Moorhouse, 2003; Ye et al., 2004), presents an opportunity to evaluate the effect of mefloquine on spontaneous GABA release in some detail.

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The object of this study was to test the hypothesis that mefloquine increases spontaneous GABA release *via* an interaction with intracellular Ca^{2+} storage and inhibition of cholinesterase.

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Materials and Methods

Slice preparation and mechanical dissociation. The care and use of animals, and the experimental protocol, were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey. The midbrain slices were prepared as described previously (Ye et al., 2004; Zhou et al., 2006). In brief, rats, aged 6-17 postnatal days, were decapitated, and the brain was quickly excised and coronally sliced (300 µm) with a VF-100 Slicer (Precisionary Instruments, Greenville, NC). This was done in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O_2/ 5% CO_2 (carbogen) containing: 126 mM NaCl, 1.6 mM KCl, 1.25 mM NaH_2PO_4, 1.5 mM $MgCl_2$, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM glucose. Midbrain slices were then kept in carbogen-saturated ACSF at room temperature (22-24 °C) for at least 1 hr before use.

Neurons, with functional terminals, were obtained by mechanical dissociation as described previously (Akaike and Moorhouse, 2003; Ye et al., 2004; Zhou et al., 2006). Briefly, slices were transferred to a 35 mm culture dish (Falcon, Rutherford, NJ) filled with a standard external solution containing : 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose (320 mOsm, pH set to 7.3 with Tris base). The region of SNc was identified with an inverted microscope (Nikon, Tokyo, Japan). A heavily fire-polished glass pipette with a 50 µm tip in diameter was fixed on a homemade device. Then, the pipette was positioned by a manipulator to touch slightly the surface of the SNc region. The neurons in the surface of the tissue were dissociated by horizontal vibration, at a frequency of 15-20 Hz, with a range from 0.1 to 0.3 mm, for 2-5 min. The slice was then removed. Within 20 minutes, the isolated neurons adhered to the bottom of the dish for electrophysiological recording. These mechanically dissociated neurons differed from those neurons dissociated with enzyme. While the latter lost most, if not all, of the nerve terminals during the dissociation process, the former often persevered

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some functional nerve terminals (Akaike and Moorhouse, 2003; Ye et al., 2004; Zhou et al., 2006).

Electrophysiological Recording. Whole-cell and loose-patch cell attached configurations were used to record electrical activity with an Axopatch 200B amplifier (Axon Instruments, Foster city, CA), *via* a Digidata 1322A analog-to-digital converter (Axon Instruments), and pClamp 9.2 software (Axon Instruments). Data were filtered at 1 kHz and sampled at 5 kHz. The junction potential, between the pipette and the bath solutions, was nullified just before forming the Giga-seal.

The patch electrodes had a resistance of 3 - 5 MΩ, when filled with pipette solution containing: 140 mM CsCl, 2 mM $MgCl_2$, 4 mM EGTA, 0.4 mM $CaCl_2$, 10 mM HEPES, 2 mM Mg-ATP, and 0.1 mM GTP. The pH was adjusted to 7.2 with Tris - base, and the osmolarity was adjusted to 280 - 300 mOsm with sucrose. Electrophysiological recordings were performed at room temperature (22- 24 °C).

Chemicals and applications. Most of the chemicals, including bicuculline (BIC), DL-2-amino-5-phosphono-valeric acid (APV), 6,7-dinitroquinoxaline-2, 3-dione (DNQX), tetrodotoxin (TTX), (-)- quinpirole hydrochloride (QP), 1, 2-Bis (2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid tetrakis (acetoxymethyl ester) (BAPTA-AM), mecamylamine hydrochloride (MEC), dihydro-β-erythroidine hydrobromide (DHβE), α-Bungarotoxin (α-BgTX), and physostigmine were purchased from Sigma-Aldrich Inc (St. Louis, MO). All solutions were prepared on the day of experiment. Mefloquine was kindly provided by Drs. Eva-Maria Gutknecht and Pierre Weber (Hoffmann-La Roche, LTD, Basel, Switzerland). A stock solution (20 mg/ml) of the racemic salt was prepared in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Inc.). Dilution of this stock solution into physiologic solutions produced the concentrations of mefloquine studied. Chemicals were applied to dissociated neurons with a Y-tube. This exchanged the external solution surrounding the neurons within 40 ms (Zhou et al., 2006).

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Data analyses. Spontaneous inhibitory postsynaptic currents (sIPSCs) were analyzed with Clampfit 9.2 software (Molecular Devices Corporation, Sunnyvale, U.S.A.) as described previously (Zhou et al., 2006). Briefly, the sIPSCs were screened automatically using a template with an amplitude threshold of 5 pA. These were visually accepted or rejected based upon rise and decay times. More than 95% of the sIPSCs, which were visually accepted, were screened using a suitable template. The amplitudes and inter-event intervals of sIPSCs in different conditions were also obtained. Their cumulative probability distributions were constructed using Clampfit 9.2. Following this, a Kolmogorov-Smirnov (K-S) test was used for evaluating the significance of drug effects. EC_{50} was obtained with a Logistical equation: $y = y_0 + (ax^b)/ (c^b+x^b)$, where y is the drug-elicited percentage change of sIPSC frequency in the presence of concentration X of mefloquine compared to control. A is the difference between maximum effect and minimum effect. y_0, b and c denote the minimum effect, Hill coefficients and half- effective concentration (EC_{50}), respectively. Differences in amplitude and frequency were tested by Student's paired two-tailed *t*-test using their normalized values, unless indicated otherwise. Numerical values are presented as the mean ± standard error of the mean (SEM). Values of $p < 0.05$ were considered significant.

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Results

The identification of DA neurons. DA neurons in substantia nigra compacta (SNc) were identified on the basis of their well-established pharmacological and electrophysiological properties (Lacey et al., 1989; Zhou et al., 2006). Figure 1 A shows sample traces of spontaneous discharges of a DA neuron recorded in the cell-attached mode. While quinpirole (QP), a dopamine D_2/D_3 receptor agonist, inhibited ongoing discharges, DAMGO, a μ- opioid receptor agonist had no effect (data not shown). In addition, DA neurons exhibit a prominent hyperpolarization activated inward current (I_h) in response to a series of voltage steps from –60 to –130 mV (with decrement of 10 mV) when recorded under whole-cell voltage-clamp conditions (Fig. 1B, upper). These are characteristic electrophysiological properties of DA neurons. The following experiments were done on putative DA neurons identified according to the aforementioned characteristics.

Mefloquine enhances the frequency of spontaneous GABAergic IPSCs (sIPSCs) on DA neurons. Whole-cell currents were recorded from mechanically dissociated SNc DA neurons. Spontaneous IPSCs (sIPSCs) were recorded at a holding potential (V_h) of –50 mV in the presence of APV (50 μM) and DNQX (10 μM), which eliminate glutamate receptor- mediated synaptic transmission. In 21 cells tested under these conditions, bicuculline (10 μM) reversibly abolished all the spontaneous postsynaptic events, indicating that they were $GABA_A$ receptor-mediated IPSCs (Fig. 2A).

Figure 2B illustrates the effect of mefloquine on sIPSCs of SNc DA neurons. Application of 3 μM mefloquine increased sIPSC frequency by 100 ± 10% ($p < 0.001$, $n = 5$). This is further illustrated in panel B_2 by the significant leftward shift of the cumulative probability plots of the intervals between successive sIPSCs. As illustrated in Fig. 2C, mefloquine induced enhancement was fast, reversible and depended on its cocnetrations.. The concentration-response relationship

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in Fig. 2D shows that the threshold concentration of mefloquine-induced enhancement of sIPSC frequency was between 0.1 and 0.3 μM. Mefloquine (0.3 μM) significantly increased sIPSC frequency by 32 ± 12% ($p < 0.05$, n = 5). Mefloquine-induced enhancement was saturated between 3 and 10 μM. Mefloquine (10 μM) enhanced sIPSC frequency by 110 ± 20% ($p < 0.001$, n = 5). At concentrations of 0.1 and 1 μM, mefloquine increased sIPSC frequency by 10 ± 10% ($p > 0.05$, n = 6) and 43 ± 10% ($p < 0.001$, n = 6), respectively. Fit of a Logistic equation to these data yielded an estimated EC_{50} of 1.3 μM.

Mefloquine increases the frequency of miniature IPSCs (mIPSCs). To determine the location where mefloquine acts, we examined the effect of mefloquine on mIPSCs, in the presence of tetrodotoxin (TTX, 1 μM) to eliminate action potential-induced spontaneous events. As shown in figure 3A, 3 μM mefloquine robustly increased mIPSC frequency. This is further illustrated in figure 3C, by the significant leftward shift of the cumulative probability plot of the intervals between successive mIPSCs, as well as by the accompanying histogram (K - S test, $p < 0.01$). In 5 neurons tested, 3 μM mefloquine increased the frequency of mIPSCs by 130 ± 10% ($p < 0.001$). In contrast, 3 μM mefloquine did not change the mean amplitude of the mIPSCs (Fig. 3C, right, K - S test, $p = 0.8$). The mean amplitude of mIPSCs in the presence of mefloquine was 101 ± 5% of control ($p > 0.05$, n = 5).

Role of Ca^{2+} in mefloquine-induced enhancement of sIPSC frequency. To assess the contribution of voltage-gated calcium channels (VGCCs), we compared the effects of mefloquine (3 μM) in the absence and presence of Cd^{2+} (100 μM), a nonselective VGCC blocker. Mefloquine enhanced sIPSC frequency by 113 ± 20% (n = 5, $p < 0.001$) in the absence of Cd^{2+}, and by 94 ± 11% (n = 5, $p < 0.001$) in the presence of Cd^{2+}. Since these two values are equivalent ($p > 0.05$, n = 5) (Fig. 4A, D), mefloquine induced potentiation of GABA release was not dependent on VGCCs.

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To determine whether Ca^{2+} influx was required in the action of mefloquine on sIPSCs, we compared the effect of mefloquine (3 μM) in normal medium and in medium containing lower Ca^{2+} concentration. Mefloquine (3 μM) enhanced sIPSC frequency by 113 ± 20% in normal medium containing 2 mM Ca^{2+}, but only by 36 ± 9% in medium containing 0.5 mM Ca^{2+} ($p < 0.05$, n = 5) (Fig. 4B, D). This indicates that mefloquine induced potentiation of GABA release was dependent on extracellular Ca^{2+}.

To test whether intraterminal Ca^{2+} contributes to the facilitation of mefloquine on sIPSC frequency, we examined the effect of BAPTA-AM, a membrane permeable Ca^{2+} chelator. About 60-80 min after pretreatment with 30 μM BAPTA-AM, 3 μM mefloquine increased sIPSC frequency by 21 ± 11% ($p = 0.3$, n = 6) (Fig. 4C, D). Thus, mefloquine failed to increase sIPSC frequency after pretreatment with BAPTA-AM. These observations suggest that mefloquine induced potentiation of IPSC frequency requires an increase in Ca^{2+} concentration within the presynaptic terminals.

Physostigmine attenuates mefloquine-induced potentiation of sIPSC frequency. It has been reported that mefloquine inhibits cholinesterase (Lim and Go, 1985; McArdle et al., 2005). Therefore, we next explored whether inhibition of cholinesterases with physostigmine can attenuate mefloquine-induced potentiation of sIPSC frequency. As shown in figure 5, 30 μM physostigmine (PHY) alone increased sIPSC frequency by 50 ± 15% of control ($p < 0.05$, n = 5). After the response to physostigmine had stabilized, the application of mefloquine continued to significantly enhance sIPSC frequency, by 31 ± 12% ($p < 0.05$, n = 5). However, this increase is much smaller than the enhancement induced by mefloquine alone (90 ± 10%) ($p < 0.05$, n = 5). This suggests that mefloquine-induced enhancement of sIPSC frequency partially depends upon its anticholinesterase action. Additional mechanisms, including mobilization of intracellular Ca^{2+}, are likely to mediate the remainder of the increase of sIPSC frequency (McArdle et al., 2006).

Presynaptic nicotinic acetylcholine receptors (nAChRs) are involved in mefloquine-induced potentiation of sIPSC frequency. It is known that when acetyl-cholinesterase (AChE)

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is inhibited, acetylcholine accumulates and activates more AChRs. In addition, previous studies have reported the presence of several subtypes of nAChRs, including the α7 and non- α7 nAChRs in the presynaptic sites of midbrain DA neurons (Mansvelder and McGehee, 2000; Wonnacott, 1997; MacDermott et al., 1999). To further test whether mefloquine enhanced GABA release by its anticholinesterase action, we examined the contribution of α7 and non- α7 nAChRs to mefloquine-induced facilitation of sIPSC frequency.

After more than 10 minutes of pretreatment with α-Bungarotoxin (Bgtx) (300 nM), a specific α7 nAChR antagonist, sIPSC frequency was not significantly altered (95 ± 6% of control, $p = 0.21$, $n = 7$, data not shown). 3 μM mefloquine enhanced sIPSC frequency by 113 ± 12% ($n = 7$, $p < 0.01$) in the absence of α- Bungarotoxin, and by 118 ± 4% ($p < 0.01$, $n = 6$) in the presence of 300 nM α- Bungarotoxin (Fig. 6A, D). Since these two values are equivalent ($p > 0.5$, $n = 6$), mefloquine induced potentiation of GABA release was independent of α7 nAChRs.

After 5 min pre-incubation with mecamylamine (MEC) (10 μM), a non-α7 nAChRs antagonist, sIPSC frequency was depressed by 20 ± 4% ($p < 0.01$, $n = 6$). Subsequent application of mefloquine increased sIPSC frequency by only 30 ± 9% ($p < 0.01$, $n = 6$), which was significantly less than that in the absence of MEC (Fig. 6B, D). Similarly, the application of dihydro-β-erythroidine hydrobromide (DHβE) (100 nM), an antagonist for nAChRs containing α4β2 subunits, depressed sIPSC frequency by 30 ± 4% ($p < 0.01$, $n = 6$). In the absence of DHβE, mefloquine (3 μM) enhanced sIPSC frequency by 113 ± 12% ($p < 0.01$, $n = 6$). After a 5 min pre-incubation in DHβE (100 nM), mefloquine (3 μM) –induced enhancement was significantly smaller, only by 28 ± 8% ($p < 0.05$, $n = 6$) (Fig. 6C, D). These results indicate that presynaptic nAChRs containing α4β2 subunits are involved in mefloquine-induced potentiation of sIPSC frequency.

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Discussion

Mefloquine enhances GABA release onto midbrain DA neurons. Our major finding is that mefloquine enhanced $GABA_A$ receptor-mediated synaptic transmission *via* the inhibition of AChE. Mefloquine concentration-dependently enhanced sIPSC frequency with an EC_{50} of 1.3 μM. It significantly enhanced sIPSC frequency at a concentration of 0.3 μM. The maximum enhancement reached 110%. It should be emphasized that this effect of mefloquine is very potent, considering that the plasma concentrations are estimated to be 3.8 -23 μM during mefloquine therapy (Simpson et al., 1999; Kollaritsch et al., 2000). Furthermore, mefloquine raises the frequency of spontaneous mIPSCs in the presence of tetrodotoxin, without altering their mean amplitude. These data suggest that mefloquine acts at the presynaptic site to increases GABA release.

The role of Ca^{2+} in mefloquine enhanced GABA release. Ca^{2+} influx into the terminals through voltage-gated calcium channels (VGCCs) is a common mechanism of modulation of transmitter release. Mefloquine blocks L-type VGCCs as well as volume- and calcium- activated chloride channels in crude microsomes prepared from brain (Lee and Go, 1996). However, mefloquine-induced enhancement of sIPSC frequency did not change in the presence of Cd^{2+} under our experimental conditions. This indicates that VGCCs are not involved in mefloquine-induced enhancement of sIPSCs. Interestingly, a decrease in extracellular Ca^{2+} attenuated mefloquine-induced facilitation of GABA release. This indicates that the action of mefloquine depends on extracellular Ca^{2+}. The residual Ca^{2+} in nerve terminals is known to influence transmitter release (Creager et al., 1980; Augustine et al., 1987; Debanne et al., 1996; Mennerick and Zorumski, 1995; Sullivan, 1999). In the presence of the high affinity membrane permeable Ca^{2+} chelator BAPTA-AM, which can efficiently buffer intraterminal Ca^{2+}, mefloquine-induced enhancement of GABA release was almost eliminated. This is consistent with previous findings at the neuromuscular junction (McArdle et al., 2006). Taken together, mefloquine enhances GABA release by increasing Ca^{2+} entry into GABAergic terminals, *via* pathways independent of

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VGCCs. However, because in low Ca^{2+} medium mefloquine still significantly enhanced GABA release, other pathways independent of extracellular Ca^{2+}, such as inhibition of Ca^{2+} uptake into mitochondria (McArdle et al., 2006; Lee and Go, 1996) may also be involved in the action of mefloquine.

Anticholinesterase activity and presynaptic nAChRs mediate mefloquine-induced enhancement of GABA release. The SNc DA neurons receive cholinergic input from the pedunculopontine nucleus (Lichtensteiger et al., 1982; Clarke et al., 1985; Swanson et al., 1987; Bolam et al., 1991). Both AChE and nAChRs are expressed in SNc (Henderson and Greenfield, 1984; Emmett and Greenfield, 2005). ACh, released from cholinergic terminals activates nAChRs, induces influx of cations and excitation of dopaminergic neurons in SNc. AChE hydrolyses ACh, and terminates the action of ACh.

Both non-α7 and α7-nAChRs are expressed in midbrain. However, in SNc, α4β2-nAChRs express at high density. In contrast, α7-nAChRs are at low density (Wooltorton et al., 2003). Nicotinic AChRs present on presynaptic terminals facilitate the release of many neurotransmitters, such as GABA, glutamate, serotonin and dopamine (McGehee et al., 1995; Wonnacott, 1997; MacDermott et al., 1999). In the present study in SNc DA neurons, MEC, a non-α7 nAChRs antagonist, and DHβE, a selective antagonist of α4β2 nAChRs, but not α-Bungarotoxin, a selective antagonist of α7-nAChRs, depressed basal sIPSC frequency. These findings suggest the non-α7 nAChRs on GABAergic terminals are tonically activated. Because of its anticholinesterase action (Lim and Go, 1985; McArdle et al., 2005), mefloquine may enhance GABA release *via* the activation of presynaptic nAChRs. In support of this hypothesis, mefloquine-induced enhancement of GABA release was attenuated in the presence of physostigmine. Thus, it is conceivable that mefloquine inhibits AChE which allows accumulation of ACh. The resultant activation of nAChRs on GABAergic terminals facilitates GABA release. We attempted to identify the possible combinations of nAChR subunits on GABAergic terminals on rat SNc DA neurons. MEC and DHβE , but not α- Bungarotoxin, reduced mefloquine-induced

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facilitation of sIPSC frequency. Therefore, the presynaptic nAChRs involved in the action of mefloquine are likely to correspond to a class of heteroligomers containing α4β2 subunits.

In conclusion, our data suggest that mefloquine enhances GABA release through its inhibition of cholinesterase. This allows accumulation of endogenously released acetylcholine which activates neuronal nicotinic receptors, probably the α4β2 nAChRs on GABAergic nerve terminals. The resultant increase of Ca^{2+} entry into these GABAergic terminals enhances vesicular release of GABA. This action may contribute to the neurobehavioral effects of mefloquine, given that, this action of mefloquine occurred at the concentrations (0.3-10 μM) equivalent to, or even below the plasma concentrations (3.8 – 23 μM) during mefloquine therapy.

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Footnote:

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Legends for Figures

Fig. 1. Dopamine neurons have distinct properties**.** A, cell-attached recordings show that 100 nM quinpirole (QP, dopamine D_2/D_3 receptor agonist) reversibly depressed a putative DA neuron. B, upper panel, typical whole cell current traces illustrate I_h evoked in a putative DA neuron by a series of hyperpolarizing voltage pulses (from –70 mV to –130 mV, in steps of 10 mV, as shown below). For this and the following figures, all records were recorded from putative DA neurons mechanically dissociated from SNc.

Fig. 2. Mefloquine potentiates sIPSCs. A, sIPSCs recorded from a putative DA neuron were completely (but reversibly) blocked by 10 µM bicuculline (BIC). For this and the following figures, all IPSCs were recorded in whole cell configuration at a holding potential of –50 mV, in the presence of APV (50 µM) and DNQX (10 µM). $B_{1,}$ Application of 3 µM mefloquine dramatically and reversibly enhances the frequency of sIPSCs. $B_{2,}$ Cumulative probability plots show much increased incidence of short sIPSC interevent intervals (K – S test, $p < 0.001$; 3 µM mefloquine vs. control). C, Time course of increases in sIPSC frequency by 10, 0.3, and 3 µM mefloquine (in 1 cell). For this and the following figures, the open bars above indicate the time courses of the application of the chemicals indicated. D, Dose – dependent potentiation of sIPSC frequency by mefloquine (0.1 – 10 µM; with an EC_{50} of 1.3 µM). Numbers of cells are indicated in brackets. Values are mean and SEM. The smooth curve is produced by the fitting of the Logistic equation to the data points.

Fig. 3. Mefloquine increases the frequency of miniature IPSCs (mIPSCs). A, Sample traces of GABAergic mIPSCs show that were recorded before, during and after application of 3 µM mefloquine in the presence of 1 µM TTX. B, Time course of increase in mIPSC frequency induced by 3 µM mefloquine (in 1 cell). C, 3 µM mefloquine caused a significant leftward shift of the cumulative probability plot for inter- event interval (left, **$p < 0.001$, K - S test), but not for the amplitude (right, $p > 0.05$, K - S test) of GABAergic mIPSCs. Insets: pooled data from 5 neurons show that mefloquine increases mIPSC frequency but not amplitude.

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Fig. 4. Intra- and extra-cellular Ca^{2+}, but not VGCCs, are involved in mefloquine-induced potentiation of sIPSC frequency. GABAergic sIPSCs were recorded before, during and after application of 3 μM mefloquine in presence of Cd^{2+} (A_1) or Ca^{2+} (0.5 mM) (B_1) and pretreated with BAPTA-AM (30 μM) for 60-80 min (C_1). Cumulative probability plots for inter-event intervals are shown in A_2 ($p < 0.001$, K - S test), B_2 ($p < 0.05$, K - S test) and C_2 ($p > 0.05$, K - S test). D, Summary of the effects of 3 μM mefloquine on sIPSC frequency in medium containing 2 mM Ca^{2+} (MFQ), 0.5 mM Ca^{2+}, in medium added with 100 μM Cd^{2+}, and with 30 μM BAPTA-AM. * $p < 0.05$ or ** $p < 0.001$ compared with the control group.

Fig. 5. Mefloquine-induced facilitation of sIPSCs involves in the inhibition of cholinesterases. A, Sample traces show that cholinesterase inhibitor physostigmine (PHY, 30 μM) enhances sIPSC frequency but attenuated mefloquine (MFQ, 3 μM)-induced enhancement of sIPSC frequency. Cumulative probability plots for inter-event interval of IPSCs from one cell, show the effect of mefloquine applied alone (B) and in the presence of physostigmine (C). D, Time course of the changes in sIPSC frequency-induced by mefloquine, in the absence and presence of 30 μM physostigmine from one cell. E, Summary of 3 μM mefloquine-induced enhancement of sIPSC frequency in the absence (MFQ) and presence of 30 μM physostigmine (PHY, n = 6). Note that in the presence of physostigmine, the effect of mefloquine is significantly attenuated. **$p < 0.001$.

Fig. 6. Mefloquine-induced potentiation of sIPSC frequency involves presynaptic nAChRs. GABAergic sIPSCs were recorded before, during and after application of 3 μM mefloquine in the presence of α -Bugarotoxin (BgTX, 300 nM) (A_1), mecamylamine hydrochloride (MEC, 10 μM) (B_1), and dihydro-β-erythroidine hydrobromide (DHβE, 100 nM) (C_1). Cumulative probability plots for inter-event interval are shown in A_2 ($p < 0.05$, K - S test), B_2 ($p < 0.05$, K - S test) and C_2 ($p < 0.001$, K - S test). D, Summary of the effect of 3 μM mefloquine on sIPSC frequency in the absence (MFQ) and presence of α -BgTX, MEC and DHβE (n = 6-7).

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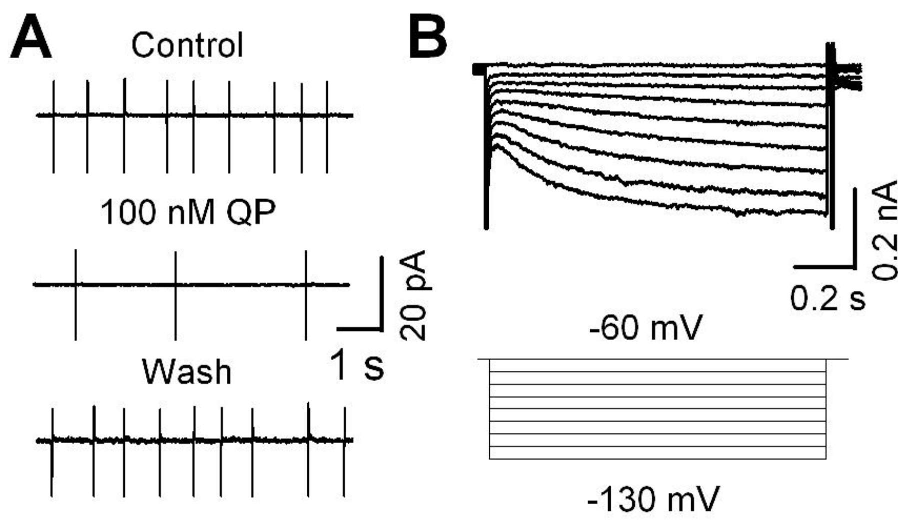

Fig.1

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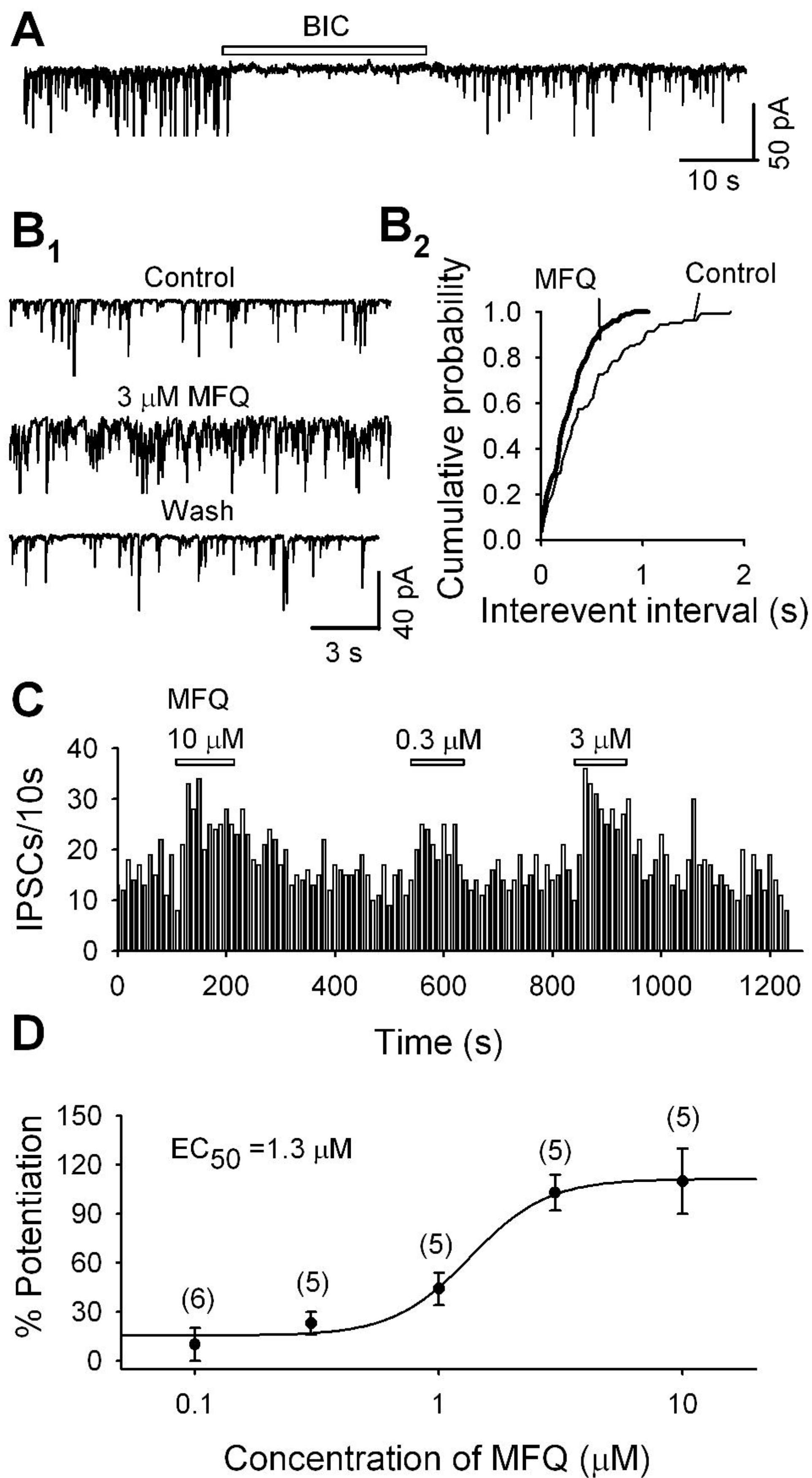

Fig 2

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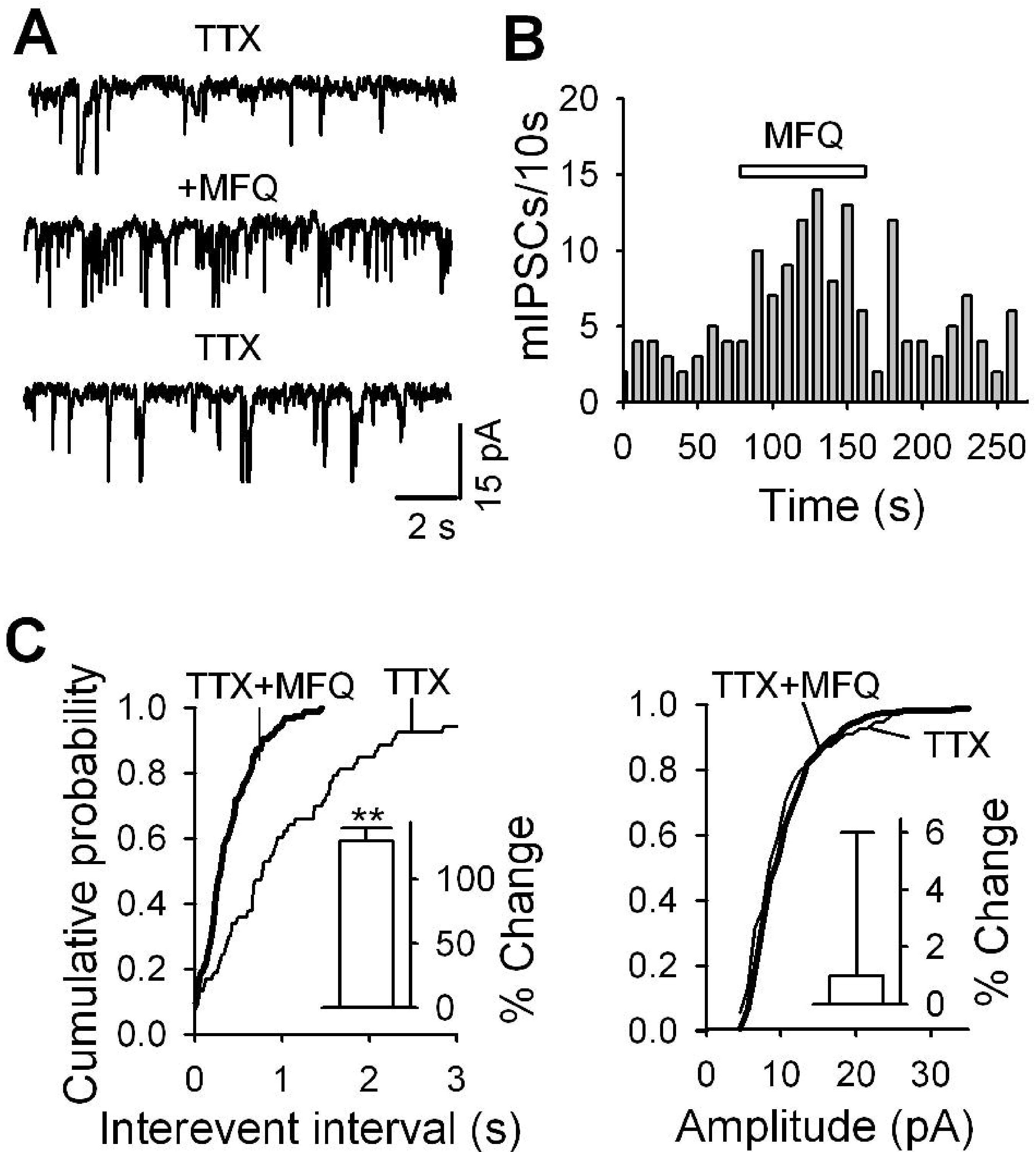

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Fig. 3

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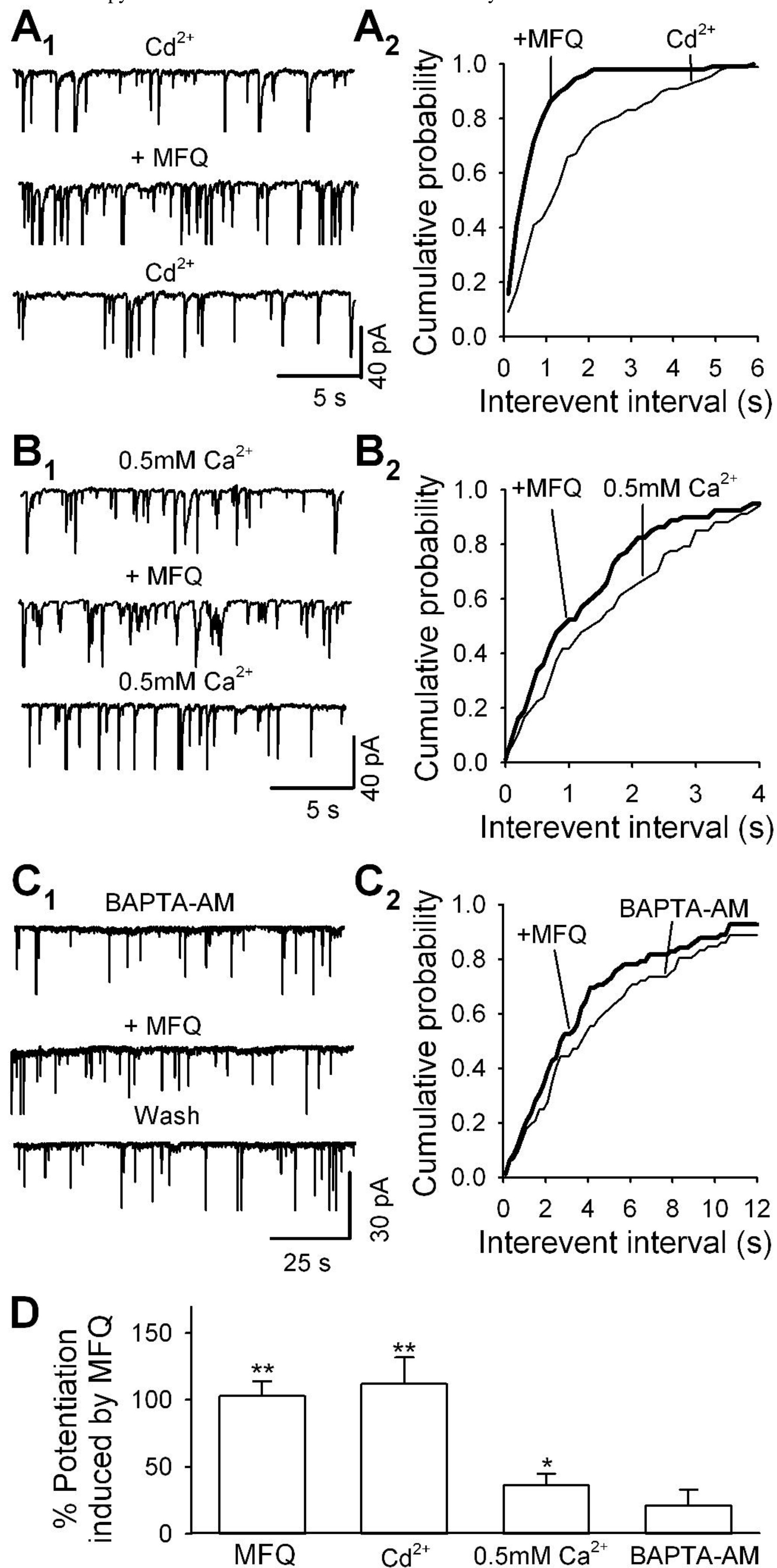

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Fig. 4

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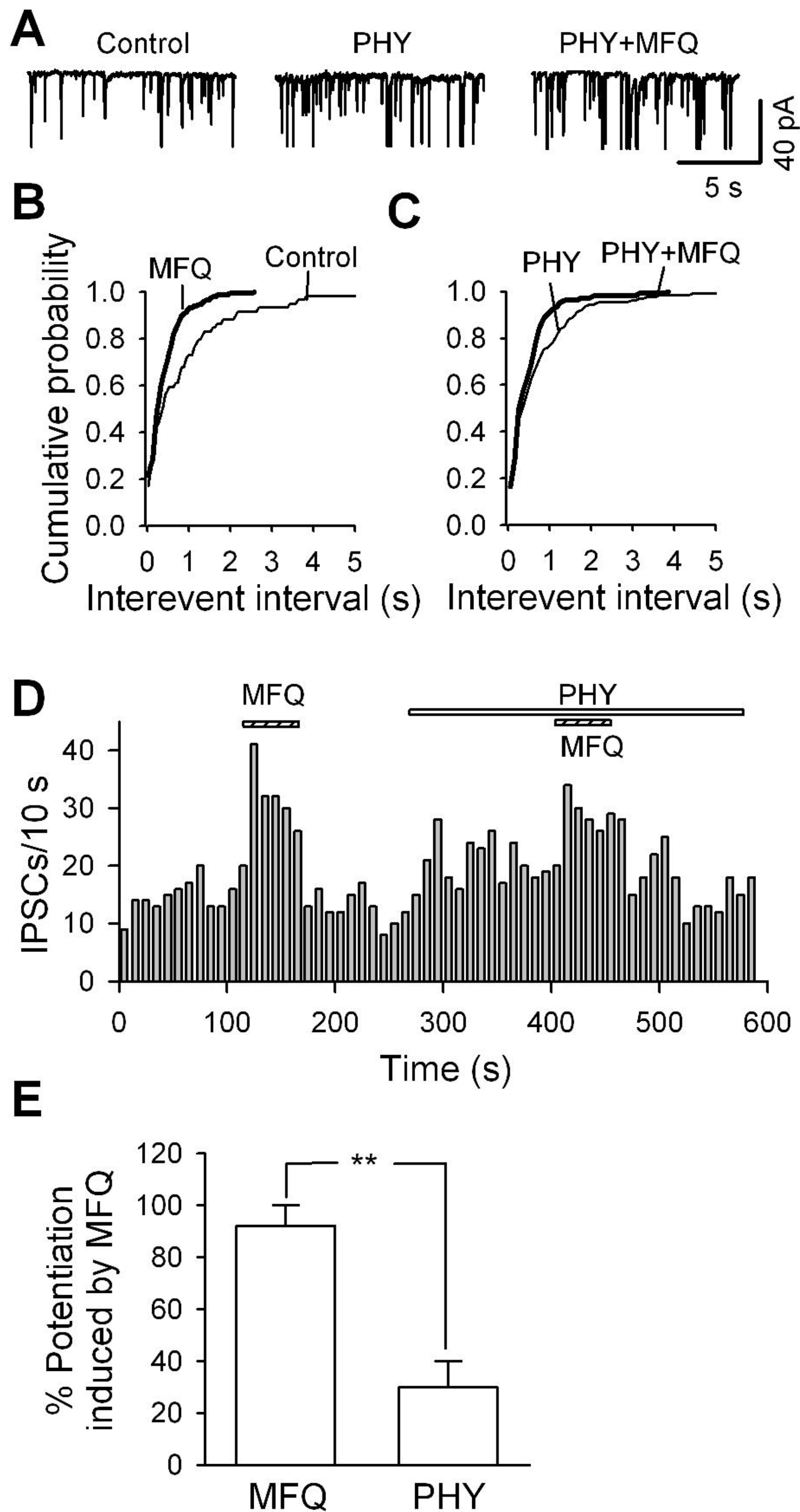

Fig. 5

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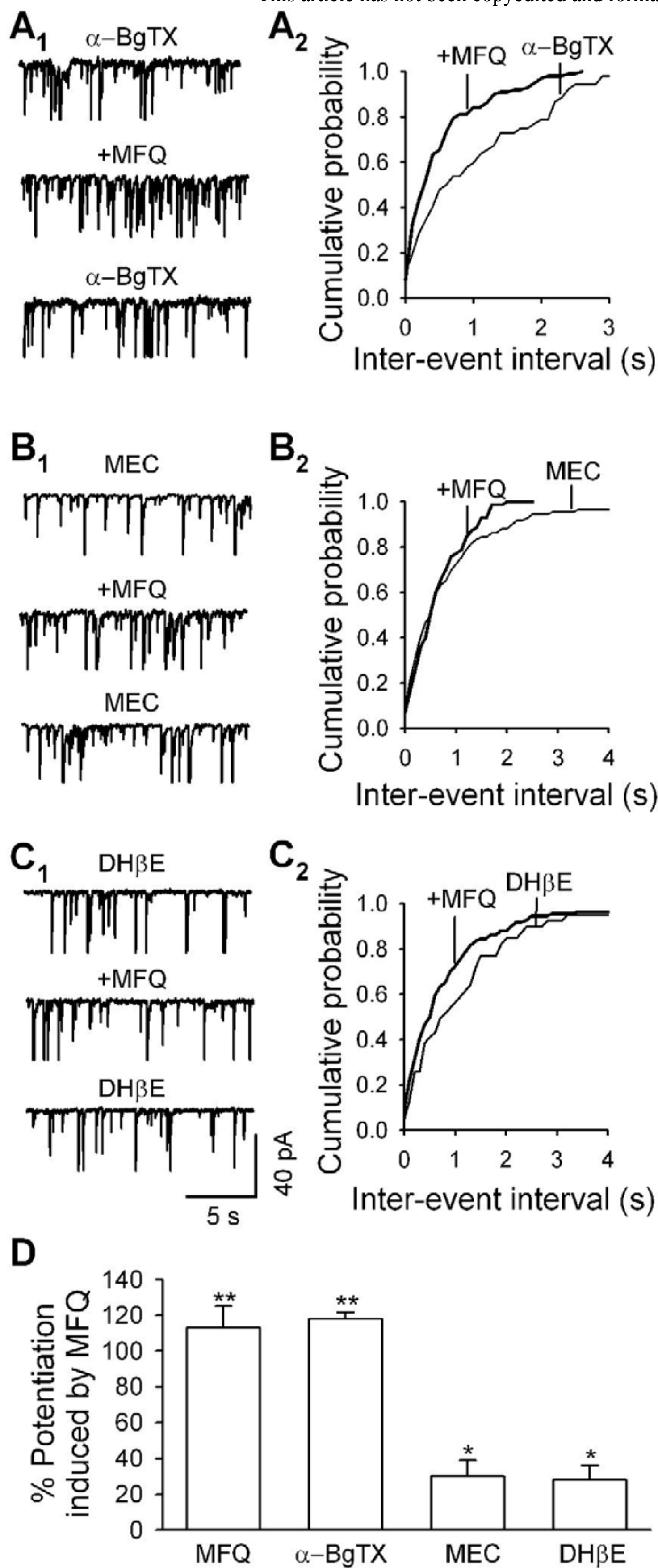

Fig. 6

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