

JPET #101923

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

CHUNYI ZHOU, CHENG XIAO, JOSEPH J. MCARDLE and JIANG HONG YE

Departments of Anesthesiology (CZ, CX, JJM, JHY) Pharmacology and Physiology (CZ, CX, JJM, JHY), UMDNJ, New Jersey Medical School, Newark, New Jersey

JPET #101923

Running title: Mefloquine enhances GABA release

Corresponding author:

Jiang Hong Ye,

Department of Anesthesiology, New Jersey Medical School (UMDNJ),

185 South Orange Avenue, Newark, NJ 07103-2714, USA

TEL # (973) 972-1866

FAX # (973) 972-4172

e-mail: ye@umdnj.edu

Number of text pages: 22

Number of figures: 6

Number of references: 34

Abstract: 249 words

Introduction: 352 words

Discussion: 670 words

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); DH β H, dihydro- β -erythroidine hydrobromide; GABA, γ -aminobutyric acid; MEC, mecamlamine 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

SECTION: Neuropharmacology

JPET #101923

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 μ M) robustly and reversibly increased the frequency of sIPSCs with an EC_{50} of 1.3 μ M. 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 μ M), a membrane permeable Ca^{2+} chelator. In contrast, 100 μ M 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- $\alpha 7$ 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.

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; Woollorton, 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 reticulata, 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.

JPET #101923

The object of this study was to test the hypothesis that mefloquine increases spontaneous GABA release *via* an interaction with intracellular Ca²⁺ storage and inhibition of cholinesterase.

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 μ m) with a VF-100 Slicer (Precisionary Instruments, Greenville, NC). This was done in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O₂/ 5% CO₂ (carbogen) containing: 126 mM NaCl, 1.6 mM KCl, 1.25 mM NaH₂PO₄, 1.5 mM MgCl₂, 2 mM CaCl₂, 25 mM NaHCO₃, 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 CaCl₂, 1 mM MgCl₂, 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 μ m 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

JPET #101923

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 M Ω , when filled with pipette solution containing: 140 mM CsCl, 2 mM MgCl₂, 4 mM EGTA, 0.4 mM CaCl₂, 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), mecamlamine hydrochloride (MEC), dihydro- β -erythroidine hydrobromide (DH β E), 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).

JPET #101923

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 \pm standard error of the mean (SEM). Values of $p < 0.05$ were considered significant.

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 D₂/D₃ 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 μ M) and DNQX (10 μ M), which eliminate glutamate receptor- mediated synaptic transmission. In 21 cells tested under these conditions, bicuculline (10 μ M) 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 μ M mefloquine increased sIPSC frequency by $100 \pm 10\%$ ($p < 0.001$, $n = 5$). This is further illustrated in panel B₂ 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 concentrations.. The concentration-response relationship

JPET #101923

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 $32 \pm 12\%$ ($p < 0.05$, $n = 5$). Mefloquine-induced enhancement was saturated between 3 and 10 μM . Mefloquine (10 μM) enhanced sIPSC frequency by $110 \pm 20\%$ ($p < 0.001$, $n = 5$). At concentrations of 0.1 and 1 μM , mefloquine increased sIPSC frequency by $10 \pm 10\%$ ($p > 0.05$, $n = 6$) and $43 \pm 10\%$ ($p < 0.001$, $n = 6$), 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 $130 \pm 10\%$ ($p < 0.001$). 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 $101 \pm 5\%$ 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 $113 \pm 20\%$ ($n = 5$, $p < 0.001$) in the absence of Cd^{2+} , and by $94 \pm 11\%$ ($n = 5$, $p < 0.001$) 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.

JPET #101923

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 $113 \pm 20\%$ in normal medium containing 2 mM Ca^{2+} , but only by $36 \pm 9\%$ 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 $21 \pm 11\%$ ($p = 0.3$, $n = 6$) (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 $50 \pm 15\%$ 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 $31 \pm 12\%$ ($p < 0.05$, $n = 5$). However, this increase is much smaller than the enhancement induced by mefloquine alone ($90 \pm 10\%$) ($p < 0.05$, $n = 5$). 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)

JPET #101923

is inhibited, acetylcholine accumulates and activates more AChRs. In addition, previous studies have reported the presence of several subtypes of nAChRs, including the $\alpha 7$ and non- $\alpha 7$ 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 $\alpha 7$ and non- $\alpha 7$ nAChRs to mefloquine-induced facilitation of sIPSC frequency.

After more than 10 minutes of pretreatment with α -Bungarotoxin (Bgtx) (300 nM), a specific $\alpha 7$ nAChR antagonist, sIPSC frequency was not significantly altered ($95 \pm 6\%$ of control, $p = 0.21$, $n = 7$, data not shown). 3 μ M mefloquine enhanced sIPSC frequency by $113 \pm 12\%$ ($n = 7$, $p < 0.01$) in the absence of α - Bungarotoxin, and by $118 \pm 4\%$ ($p < 0.01$, $n = 6$) 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 $\alpha 7$ nAChRs.

After 5 min pre-incubation with mecamylamine (MEC) (10 μ M), a non- $\alpha 7$ nAChRs antagonist, sIPSC frequency was depressed by $20 \pm 4\%$ ($p < 0.01$, $n = 6$). Subsequent application of mefloquine increased sIPSC frequency by only $30 \pm 9\%$ ($p < 0.01$, $n = 6$), which was significantly less than that in the absence of MEC (Fig. 6B, D). Similarly, the application of dihydro- β -erythroidine hydrobromide (DH β E) (100 nM), an antagonist for nAChRs containing $\alpha 4\beta 2$ subunits, depressed sIPSC frequency by $30 \pm 4\%$ ($p < 0.01$, $n = 6$). In the absence of DH β E, mefloquine (3 μ M) enhanced sIPSC frequency by $113 \pm 12\%$ ($p < 0.01$, $n = 6$). After a 5 min pre-incubation in DH β E (100 nM), mefloquine (3 μ M) –induced enhancement was significantly smaller, only by $28 \pm 8\%$ ($p < 0.05$, $n = 6$) (Fig. 6C, D). These results indicate that presynaptic nAChRs containing $\alpha 4\beta 2$ subunits are involved in mefloquine-induced potentiation of sIPSC frequency.

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 EC₅₀ 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 increase GABA release.

The role of Ca²⁺ in mefloquine enhanced GABA release. Ca²⁺ 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 Cd²⁺ under our experimental conditions. This indicates that VGCCs are not involved in mefloquine-induced enhancement of sIPSCs. Interestingly, a decrease in extracellular Ca²⁺ attenuated mefloquine-induced facilitation of GABA release. This indicates that the action of mefloquine depends on extracellular Ca²⁺. The residual Ca²⁺ 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 Ca²⁺ chelator BAPTA-AM, which can efficiently buffer intraterminal Ca²⁺, 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 Ca²⁺ entry into GABAergic terminals, *via* pathways independent of

JPET #101923

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- $\alpha 7$ and $\alpha 7$ -nAChRs are expressed in midbrain. However, in SNc, $\alpha 4\beta 2$ -nAChRs express at high density. In contrast, $\alpha 7$ -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- $\alpha 7$ nAChRs antagonist, and DH β E, a selective antagonist of $\alpha 4\beta 2$ nAChRs, but not α -Bungarotoxin, a selective antagonist of $\alpha 7$ -nAChRs, depressed basal sIPSC frequency. These findings suggest the non- $\alpha 7$ 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 DH β E, but not α -Bungarotoxin, reduced mefloquine-induced

JPET #101923

facilitation of sIPSC frequency. Therefore, the presynaptic nAChRs involved in the action of mefloquine are likely to correspond to a class of heterooligomers containing $\alpha 4\beta 2$ 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 $\alpha 4\beta 2$ 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.

JPET #101923

References

- Akaike N and Moorhouse AJ (2003) Techniques: applications of the nerve-bouton preparation in neuropharmacology. *Trends Pharmacol Sci* **24**:44-47.
- Augustine GJ, Charlton MP and Smith SJ (1987) Calcium action in synaptic transmitter release. *Annu Rev Neurosci* **10**:633-693.
- Bolam JP, Francis CM and Henderson Z (1991) Cholinergic input to dopaminergic neurons in the substantia nigra: a double immunocytochemical study. *Neuroscience* **41**:483-494.
- Clarke PBS, Hommer DW, Pert A and Skirboll LR (1985) Electrophysiological actions of nicotine on substantia nigra single units. *Br J Pharmacol* **85**:827-835.
- Creager R, Dunwiddie T and Lynch, G (1980) Paired-pulse and frequency facilitation in the CA1 region of the in vitro rat hippocampus. *J Physiol (London)* **299**:409-424.
- Cruikshank SJ, Hopperstad M, Younger M, Connors BW, Spray DC and Srinivas M (2004) Potent block of Cx36 and Cx50 gap junction channels by mefloquine. *Proc Natl Acad Sci U S A* **101**:12364-12369.
- Debanne D, Guerineau NC, Gahwiler BH and Thompson SM (1996) Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release. *J Physiol* **491**:163-176.
- Emmett SR and Greenfield SA (2005) Correlation between dopaminergic neurons, acetylcholinesterase and nicotinic acetylcholine receptors containing the $\alpha 3$ or $\alpha 5$ - subunit in the rat substantia nigra. *J Chemical Neuroanatomy* **30**:34-44.
- Falchook GS, Malone CM, Upton S and Shandera WX (2003) Postmalaria neurological syndrome after treatment of Plasmodium falciparum malaria in the United States. *Clin Infect Dis* **37**:e22-24.
- Fuller SJ, Naraqi S and Gilessi G. (2002) Paranoid psychosis related to mefloquine antimalarial prophylaxis. *P N G Med J* **45**:219-221.

JPET #101923

- Giustizieri M, Bernardi G, Mercuri NB and Berretta N (2005) Distinct mechanisms of presynaptic inhibition at GABAergic synapses of the rat substantia nigra pars compacta. *J Neurophysiol* **94**:1992-2003.
- Henderson Z and Greenfield SA (1984) Ultrastructural localization of acetylcholinesterase in substantia nigra: a comparison between rat and guinea pig. *J Comp Neurol* **230**:278-286.
- Kollaritsch H, Karbwang J, Wiedermann G, Mikolasek A, NaBangchang K and Wernsdorfer WH (2000) Mefloquine concentration profiles during prophylactic dose regimens. *Wien Klin Wochenschr* **112**:441-447.
- Kukoyi O and Carney CP (2003) Curses, madness, and mefloquine. *Psychosomatics* **44**:339-341.
- Lacey MG, Mercuri NB and North RA (1989) Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. *J Neurosci* **9**:1233-1241.
- Lee HS and Go ML (1996) Effects of mefloquine on Ca²⁺ uptake and release by dog brain microsomes. *Arch Int Pharmacodyn Ther* **331**:221-231.
- Lichtensteiger W, Hefti F, Felix D, Huwyler T, Melamed E and Schlumpf M (1982) Stimulation of nigrostriatal dopamine neurons by nicotine. *Neuropharmacology* **21**:963-968.
- Lim LY and Go ML (1985) The anticholinesterase activity of mefloquine. *Clin Exp Pharmacol Physiol* **12**:527-531.
- MacDermott AB, Role LW and Siegelbaum SA (1999) Presynaptic ionotropic receptors and the control of transmitter release. *Annu Rev Neurosci* **22**:443-485.
- Mansvelder HD and McGehee DS (2000) Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* **27**:349-357.
- McArdle JJ, Sellin LC, Coakley KM, Potian JG and Hognason K (2006) Mefloquine selectively increases asynchronous acetylcholine release from motor nerve terminals. *Neuropharmacology*. **50**:345-353.

JPET #101923

- McArdle JJ, Sellin LC, Coakley KM, Potian JG, Quinones-Lopez MC, Rosenfeld CA, Sultatos LG and Hognason K (2005) Mefloquine inhibits cholinesterases at the mouse neuromuscular junction. *Neuropharmacology* **49**:1132-1139.
- McGehee DS, Heath MJ, Gelber S, Devay P and Role LW (1995) Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science* **269**:1692-1696.
- Meier CR, Wilcock K and Jick SS (2004) The risk of severe depression, psychosis or panic attacks with prophylactic antimalarials. *Drug Saf* **27**:203-213.
- Mennerick S and Zorumski CF (1995) Presynaptic influence on the time course of fast excitatory synaptic currents in cultured hippocampal cells. *J Neurosci* **15**:3178-3192.
- Simpson JA, Price R, ter Kuile F, Teja-Isavatharm P, Nosten F, Chongsuphajaisiddi T, Looareesuwan S, Aarons L and White NJ (1999) Population pharmacokinetics of mefloquine in patients with acute falciparum malaria. *Clin Pharmacol Ther* **66**:472-484.
- Sullivan JM (1999) Mechanisms of cannabinoid-receptor-mediated inhibition of synaptic transmission in cultured hippocampal pyramidal neurons. *J Neurophysiol* **82**:1286-1294.
- Swanson LW, Simmons DM, Whiting PJ and Lindstrom J (1987) Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. *J Neurosci* **7**:3334-3342.
- Tepper JM, Paladini CA and Celada P (1998) GABAergic control of the firing pattern of substantia nigra dopaminergic neurons. *Adv Pharmacol* **42**:694-699.
- Wonnacott S (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci* **20**:92-98.
- Wooltorton E (2002) Mefloquine: contraindicated in patients with mood, psychotic or seizure disorders. *CMAJ* **167**:1147.
- Wooltorton JR, Pidoplichko VI, Broide RS and Dani JA (2003) Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci* **23**:3176-3185.

JPET #101923

Ye JH, Wang F, Krnjevic K, Wang W, Xiong ZG and Zhang J (2004) Presynaptic glycine receptors on GABAergic terminals facilitate discharge of dopaminergic neurons in ventral tegmental area. *J Neurosci* **24**:8961-8974.

Zhou C, Xiao C, Commissiong JW, Krnjevic K and Ye JH (2006) Mesencephalic astrocyte-derived neurotrophic factor enhances nigral gamma-aminobutyric acid release. *Neuroreport* **17**:293-297.

JPET #101923

Footnote:

This publication was made possible by Grant AA-11989 from the National Institute of Alcohol Abuse and Alcoholism (NIAAA), and AT-001182 from the National Center for Complementary and Alternative Medicine (NCCAM), and an award from UMDNJ Foundation to JH Ye as well as NINDS (NS045979) and an award from the Kirby Foundation to JJ McArdle.

Send all reprint requests to:

Jiang Hong Ye,

Department of Anesthesiology, New Jersey Medical School (UMDNJ),

185 South Orange Avenue, Newark, NJ 07103-2714, USA

e-mail: ye@umdnj.edu

JPET #101923

Legends for Figures

Fig. 1. Dopamine neurons have distinct properties. A, cell-attached recordings show that 100 nM quinpirole (QP, dopamine D₂/D₃ 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). B₁, Application of 3 μM mefloquine dramatically and reversibly enhances the frequency of sIPSCs. B₂, 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 EC₅₀ 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.

JPET #101923

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 (DH β E, 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 DH β E ($n = 6-7$).

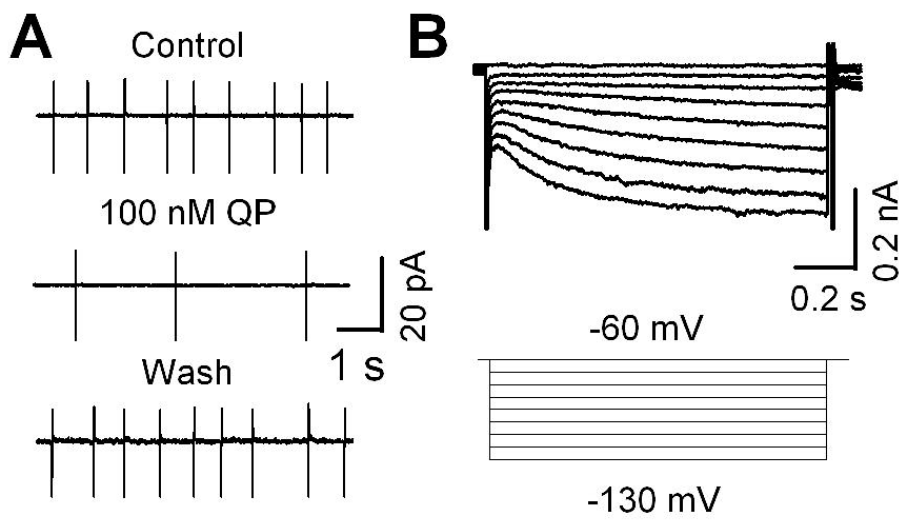


Fig.1

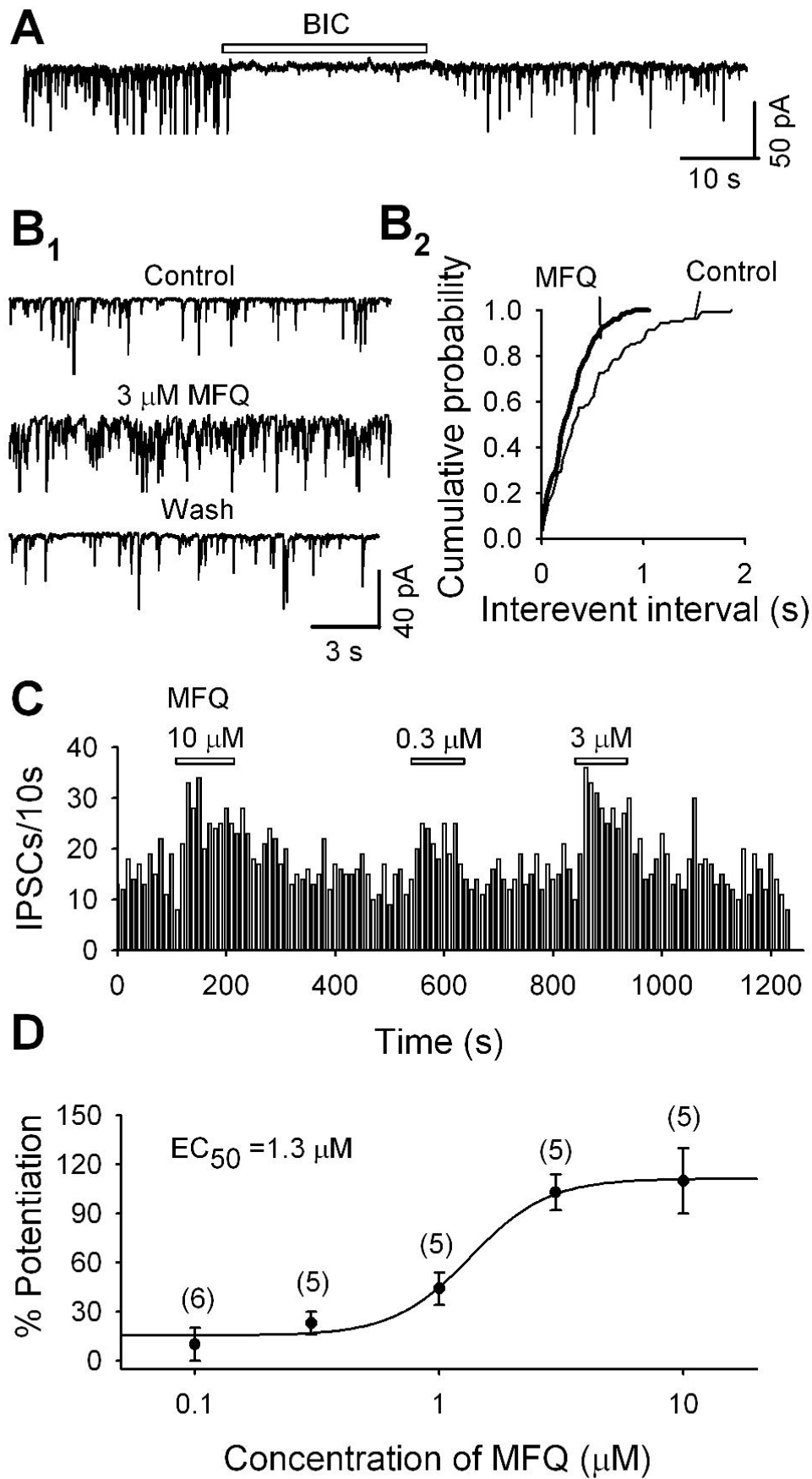


Fig 2

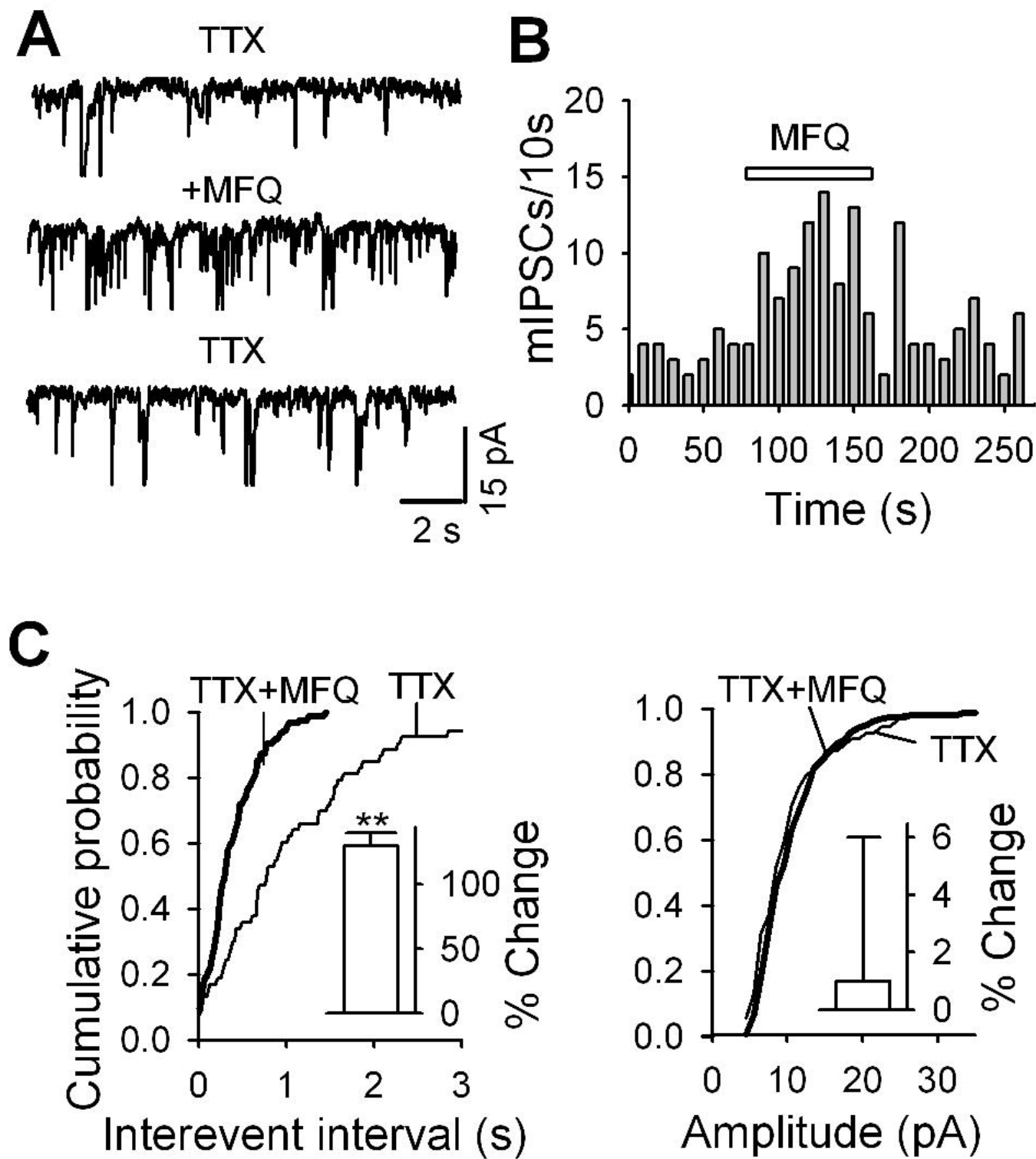


Fig. 3

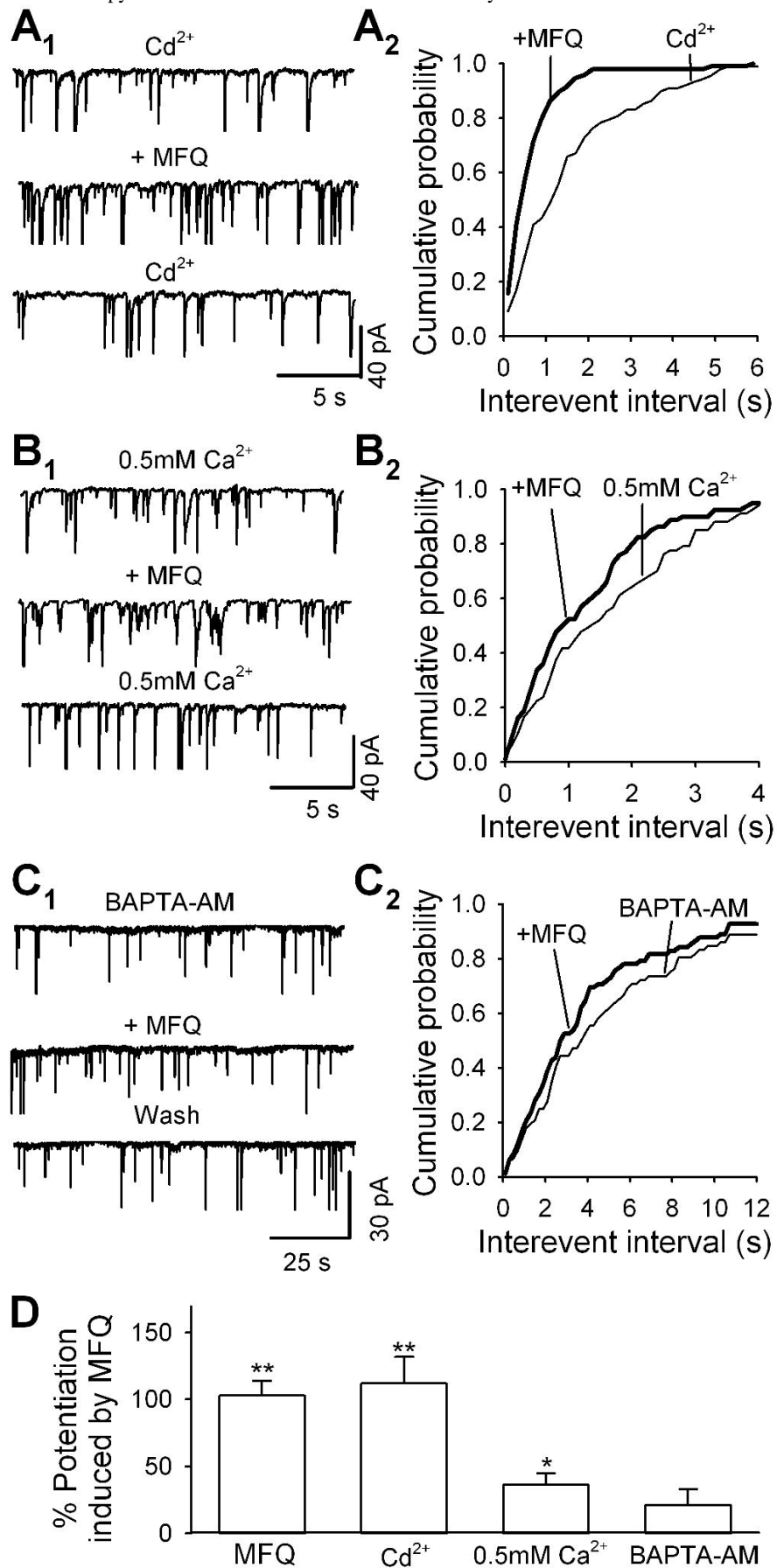


Fig. 4

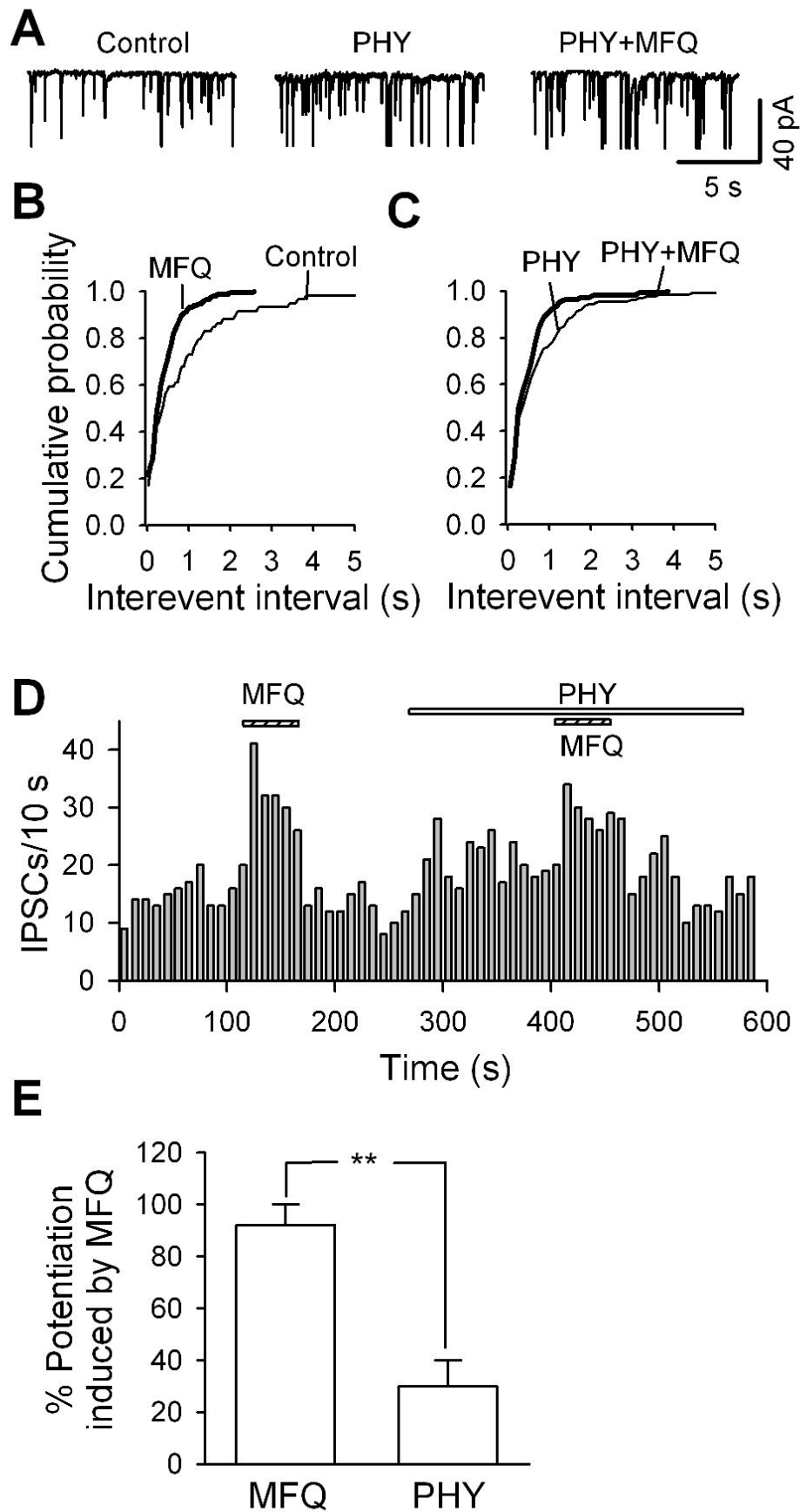


Fig. 5

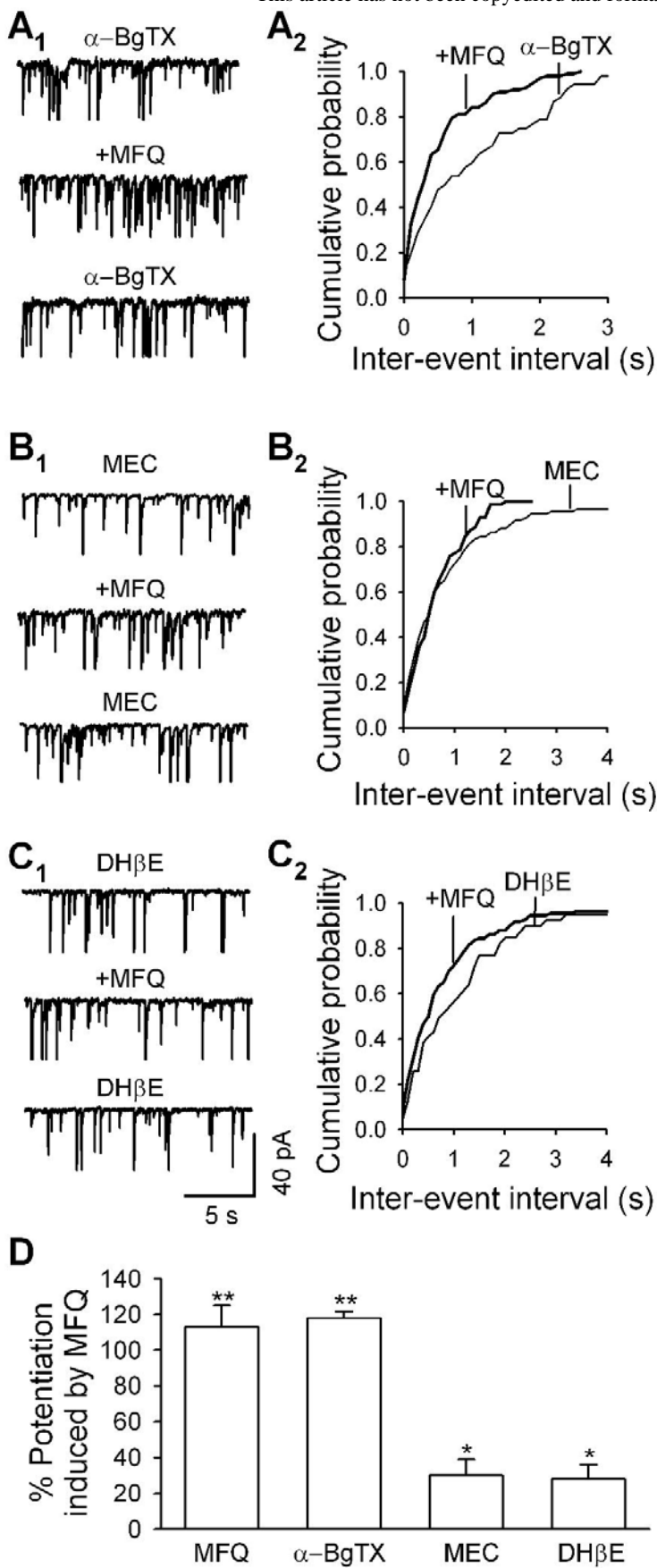


Fig. 6