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Comparative Studies of Anthraquinone- and Anthracene-Tetraamines as Blockers of *N*-Methyl-D-Aspartate Receptors

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Abbreviations:

NMDA, *N*-methyl-D-aspartate; AMPA, α-aminio-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AQ, anthraquinone-2-carboxyl group; Ant, 9-

anthracenylmethyl group; AQ343, N^1-(anthraquinone-2-carbonyl)spermine; AQ444, N^1-(anthraquinone-2-carbonyl)homospermine; Ant343, N^1-(9-anthracenylmethyl)spermine; Ant444, N^1-(9-anthracenylmethyl)homospermine; MK801, dizocilpine maleate.

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ABSTRACT

Anthraquinone spermine (AQ343), anthraquinone homospermine (AQ444), anthracene spermine (Ant343) and anthracene homospermine (Ant444) were found to be potent antagonists of recombinant *N*-methyl-D-aspartate (NMDA) receptors. The effects of both anthraquinone (AQ)- and anthracene (Ant)-tetraamines were reversible and voltage-dependent. Results of experiments using mutant NR1 and NR2B subunits of NMDA receptor identified residues that influence block by AQ- and Ant-tetraamines. The results indicate that the polyamine tail is crucial for block by AQ- and Ant-tetraamines. Residues in the outer vestibule of the NR1 subunit were more strongly involved in block by AQ- and Ant-tetraamines than residues in the corresponding region of NR2B. Several amino acid residues in the inner vestibule, below the level of the selectivity filter of NR1 and NR2B, affected block by AQ444, Ant343 and Ant444, but did not affect block by AQ343. AQ-tetraamines could permeate the channel at very negative membrane potentials when the narrowest constriction of the channel was expanded by replacing the Asn residue at N616 of NR1 and NR2B with Gly, whereas Ant-tetraamines did not easily pass through the channel, apparently because of differences in the relative position of the head groups on AQ- and Ant-polyamines.

(Introduction)

N-Methyl-D-aspartate (NMDA) receptors are involved in synaptic plasticity and may also play a role in seizure activity. Overactivation of these receptors can lead to neuronal cell death. Thus, NMDA receptors are potential targets for anticonvulsants and neuroprotective agents (Choi, 1988; Rogawski, 1992). The receptors are probably tetramers composed of combinations of three types of subunits — NR1, NR2, and NR3. Most NMDA receptors in the adult central nervous system contain combinations of NR1 and NR2, with NR2A and NR2B predominating in forebrain areas such as the cerebral cortex (Hollmann and Heinemann, 1994; Dingledine et al., 1999). Each NMDA receptor subunit is suggested to have a large extracellular N-terminal domain, three membrane-spanning domains (M1, M3 and M4) and a re-entrant loop (M2) that forms part of the channel pore (Williams, 1997).

Antagonists have been discovered or developed that act at various sites on NMDA receptors, including competitive antagonists at the glutamate and glycine sites and a diverse range of organic compounds that act as channel blockers. The channel pore is also blocked by Mg^{2+} and is permeable to Ca^{2+} (Dingledine et al., 1999). The M2 loop region in NR1 and NR2 subunits is a

critical determinant of divalent cation permeability and Mg^{2+} block. In particular, asparagine residues in this region form part of a Mg^{2+} binding site and contribute to the selectivity filter of the channel (Sakurada et al., 1993; Dingledine et al., 1999). These asparagine residues have also been found to influence block by organic channel blockers such as dizocilpine (MK-801), memantine, and polyamine derivatives like N^1,N^4,N^8-tribenzylspermidine (TB34) (Benveniste and Mayer, 1993; Igarashi et al., 1997; Tai et al., 2001; Kashiwagi et al., 2002, 2004; Chen and Lipton, 2005). Residues in M1, M3 and M4, in particular M3, have also been found to affect block by MK-801 and polyamine derivatives (Kashiwagi et al., 2002; Yuan et al., 2005). These residues may contribute directly to a binding site for the blockers and/or be involved in gating of the channel (Kashiwagi et al., 2002, 2004; Yuan et al., 2005).

To study the structure of NMDA channels and to look for novel classes of antagonists, we developed several polyamine derivatives including bisethylpolyamines (Igarashi and Williams, 1995), N^1-dansylspermine (Chao et al., 1997), benzylpolyamines (Igarashi et al., 1997) and anthraquinones (Kashiwagi et al., 2004). The anthraquinone triamine AQ34 was an NMDA

channel blocker with a novel profile. However, its potency at NMDA receptors was relatively low (IC_{50} = 7 μM at NR1/NR2A receptors at –70 mV). In the present work, we tried to develop more potent polyamine blockers based on AQ34. We found that tetraamine derivatives of anthraquinone and anthracene are potent NMDA antagonists. Furthermore, we studied determinants in both NR1 and NR2B subunits that influence block by these polyamine derivatives.

Materials and Methods

NMDA Clones and Site-Directed Mutagenesis. The NR1 clone used in these studies is the NR1A variant (Moriyoshi et al., 1991), which lacks the 21-amino acid insert encoded by exon-5. This clone, and some of the NR1 mutants in the M2 and M1-M2 linker region (Sakurada et al., 1993), were gifts from Dr. S. Nakanishi (Osaka Bioscience Institute, Japan). The wild-type mouse and rat NR2B clones (Kutsuwada et al., 1992; Monyer et al., 1992) were gifts from Drs. M. Mishina (Graduate School of Medicine, University of Tokyo, Japan) and P. H. Seeburg (Center for Molecular Biology, University of Heidelberg, Germany). The mouse NR2C and NR2D clones (Ikeda et al., 1992; Kutsuwada et al., 1992), and the mouse GluR1 clone (Sakimura et al., 1990) were also gifts from Dr. M. Mishina. The preparation of most other NR1 and NR2B mutants has been previously described (Kashiwagi et al., 1997, 2002, 2004; Williams et al., 1998; Masuko et al., 1999). Site-directed mutagenesis to construct NR2B F550L, F554L, M562S, M565S, G597E, T601G, V620E, L643A, A644S, A648G, L650A, A651T, Q812C and D814A was carried out by the method of Ho et al. (1989) using the polymerase chain reaction. Amino acids are numbered from the initiator methionine in each subunit. This differs

from the numbering system used in some laboratories, in which residues are numbered from the start of the mature peptide. In the case of NR1, there is an 18-amino acid signal peptide, for example, residue NR1(N616) described in this study corresponds to residue NR1(N598) using the alternative numbering scheme (Kuner et al., 1996).

Expression in Oocytes and Voltage-Clamp Recording. The preparation of capped cRNAs and the preparation, injection, and maintenance of oocytes were carried out as described previously (Williams, 1993). Oocytes were injected with NR1 plus NR2 cRNAs in a ratio of 1:5 (0.1 – 4 ng of NR1 plus 0.5 – 20 ng of NR2) or GluR1 cRNA (20 ng). Macroscopic currents were recorded with a two-electrode voltage clamp using a GeneClamp 500 amplifier (Axon Instruments, Union City, CA). Electrodes were filled with 3 M KCl and had resistances of 0.4 to 4 MΩ. Oocytes were continuously superfused with a saline solution (100 mM NaCl, 2 mM KCl, 1.8 mM $BaCl_2$, and 10 mM HEPES, pH 7.5), and in most experiments, oocytes were injected with 40 mM K^+-BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid) (100 nL, pH 7.0 – 7.4) on the day of recording. Receptors were activated by superfusion of glutamate and glycine (10 μM).

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Data analysis and curve fitting were carried out using Axograph (Axon Instruments) or SigmaPlot (SPSS Inc., Chicago, IL). To obtain IC_{50} values of polyamine derivatives, concentration-inhibition curves were fit to eq. 1.

$$I_{Glu+blocker}/I_{Glu} = 1/[1 + ([blocker]/IC_{50})^{nH}] \quad (1)$$

in which I_{Glu} is the response to glutamate and $I_{Glu+blocker}$ is the response to glutamate measured in the presence of the blocker. We used this equation, which is constrained to 100% inhibition of the response to the agonist, based on the assumption that the blockers produce a complete inhibition at the various receptors studied.

To study the voltage dependence of block, voltage ramps were constructed by ramping the command signal from –150 to +40 mV over 6 s. Leak currents, measured in the absence of agonist and blockers, were digitally subtracted. We chose concentrations of blockers that gave a 60 to 90% inhibition at –70 mV with a particular mutant.

Synthesis of Anthraquinone- and Anthracene-Tetraamines. The anthraquinone-spermine and homospermine (AQ343 and AQ444; structures shown in Fig. 1) were prepared by coupling of anthraquinone *p*-nitrophenyl ester and tetraamines (spermine and homospermine) in $CHCl_3$ (Zhang et al., 1987; Hidai et al., 2000). The anthracene [N^1-(9-anthracenylmethyl)] derivatives (Ant343 and Ant444; structure shown in Fig. 1) were synthesized as described previously (Wang et al., 2003). The structures of each compound were confirmed by spectral data and elemental analysis.

Preferred Conformation of AQ343, AQ444, Ant343 and Ant444. The conformational stability of the four compounds was determined by molecular dynamics (MD) simulation (Sander molecule in AMBER8 package, University of California, San Francisco, 2004) at 310K (37 °C) in water phase using CPU: intel® Xeon™ 1.70 Ghu 2 CPU, OS; Red Hat Linux 7.3.2. The MD simulation was started by the minimization of 100 steps with the steepest decent method. The temperature was raised to 310 K at 80-ps, and was maintained at 310 K. The simulation continued for 10-ns. The coordinates were stored in the output file every 1-ps, and total 10,080 conformers were obtained. The analysis of conformational stability was executed with the 9,000

conformers obtained at 310 K. The conformation whose appearance probability was the highest was adopted as the most stable conformation with MMTSB tool set (Feig, M., Karanicolas, J., and Brooks III, C. L., NIH Research Resource, 2001). The "gaff.dat" force field parameters (Wang et al., 2004) were used for bond, angle, torsion and van der Waals parameters, and Gaussian 03 program (Revision C.02., 2004, Gaussian Inc., Wallingford, CT) for calculation of the electrostatic potentials.

Results

Voltage-dependent Block by AQ343, AQ444, Ant343 and Ant444. We previously reported that the triamine AQ34 is a blocker of NMDA receptors. However, its potency was relatively low (IC_{50} at –70 mV = 7 μM at NR1/NR2A). Thus, we studied the effects of tetraamine derivatives of AQ34 and also the effects of replacing the anthraquinone (AQ) moiety with anthracene (Ant). As shown in Fig. 1, AQ343, AQ444, Ant343 and Ant444 inhibited NR1/NR2A receptors, and the inhibition was reversible and voltage-dependent. The IC_{50} values for AQ343, AQ444, Ant343 and Ant444 at NR1/NR2A receptors were 0.4, 0.6, 0.06 and 0.02 μM, respectively (Table 1). The results suggest that the potency of inhibition depends on the number of positive charges because the NH moiety of the amide (-CONH-) group in the AQ derivatives is not protonated at pH 7.5 (Cox et al., 1981) (thus, they have only three positive charges) whereas all four amines of the Ant derivatives are protonated at this pH (thus, they have four positive charges).

Subtype-Selective Inhibition. To determine the subunit selectivity and potency of block by AQ343, AQ444, Ant343 and Ant444, we measured

concentration-inhibition curves at NR1/NR2 receptors containing the NR2A, NR2B, NR2C and NR2D subunits and at the α-aminio-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor expressed from the GluR1 subunit. For AQ343 and AQ444, the polyamines were more potent at NR1/NR2A, NR1/NR2B and NR1/NR2D receptors than at NR1/NR2C and GluR1 receptors (Fig. 2A, B). However, the profile was different with Ant343 and Ant444. For Ant343, potency was in the order NR1/NR2A > NR1/NR2D ≈ NR1/NR2B >> NR1/NR2C > GluR1, and for Ant444, it was NR1/NR2A ≈ NR1/NR2B > NR1/NR2D >> GluR1 ≈ NR1/NR2C (Fig. 2 and Table 1). Thus, both AQ- and Ant-tetraamines are more potent at NR1/NR2A, NR1/NR2B and NR1/NR2D than at NR1/NR2C and GluR1.

Identification of Amino Acid Residues That Influence Block by AQ- and Ant-Tetraamines. We carried out experiments to identify the amino acid residues in NR1 and NR2B that influence block by the tetraamines using a series of NR1 and NR2B mutants (Figs. 3 and 4). We previously identified residues that differentially affect block by memantine, MK-801, tribenzylspermidine and AQ34 (Kashiwagi et al., 2002, 2004). For the present study, we made an additional 14 mutants in NR2B. Thus, the effects of the tetraamines on 24

NR1 mutants and 27 NR2B mutants were examined with two different concentrations of each blocker.

In Figs. 3 and 4, residues at which mutations reduce block by AQ- and Ant-tetraamines by more than 15% compared to wild-type are highlighted by an open box; mutations that increase block by a particular polyamine are highlighted by a black box. As shown in Figs. 3 and 5, block by AQ343, AQ444, Ant343 and Ant444 shared many common determinants in the NR1 subunit. These residues were located in the outer vestibule and at the selectivity filter/narrowest constriction of the channel (N616 in NR1). Eight mutations, at NR1 F558, W563, N616, N650, A652, L655, D669 and T807, reduced block by the four tetraamines and also by AQ34 and tribenzylspermidine (Kashiwagi et al., 2002, 2004). This suggests that these residues are involved in the recognition of all of these compounds. With regard to residues in the inner vestibule below the level of the selectivity filter, several residues affected block by Ant343 and Ant444. In this region, only E621 in NR1 influenced block by AQ343 and AQ444. The results support the idea that the polyamine tail passes through the selectivity filter and can interact with residues below that level whereas the head group cannot easily permeate

the narrow constriction. The results also suggest that the structure of the head group and/or the angle between the head group and the polyamine tail shown in Fig. 6 influence the interaction of the tail with the residues below the selectivity filter.

The profiles measured with mutations in NR2B were different from those in NR1 (Figs. 4 and 5). Mutations at NR2B W559, N616 and N649 decreased the block by all four tetraamines and also by AQ34, AQ33b and tribenzylspermidine (Kashiwagi et al., 2002, 2004). Mutations at only a few residues in the outer vestibule in NR2B reduced block by AQ343, AQ444, Ant343 and Ant444 compared with residues in the corresponding region of NR1. On the other hand, mutations at several residues, especially to the smaller residues Ala or Gly, enhanced block by AQ343 and Ant343 but did not affect block by AQ444 and Ant444. This may reflect differences in the space occupied by these compounds (Fig. 6). The "width" of the polyamine derivatives was estimated in Fig. 6 and revealed that AQ343 (8.3 Å) and Ant343 (8.2 Å) were greater than those of AQ444 (6.5 Å) and Ant444 (7.3 Å). The differences between NR1 and NR2B are consistent with the idea that the M3 segments from the two subunits are staggered relative to each other in the

vertical axis of the channel (Sobolevsky et al., 2002).

Mutations at G597, T601, W607, and V620 in the inner vestibule of NR2B affected block by AQ444, but only a mutation at T601 reduced block by AQ343. This may be due to a difference of the structure of AQ343 and AQ444 in which AQ444 is straighter than AQ343 as well as having a longer polyamine tail (Fig. 6). With regard to Ant343 and Ant444, residues D668 and Q812 in the outer vestibule, and T601 and V620 in the inner vestibule were more strongly involved in block by Ant444 than by Ant343. It may be that the longer homospermine tail (444) penetrates deeper into the inner vestibule than the spermine (343) tail. This idea is supported by the observation that block by Ant343 was enhanced if G597 or V620 in NR2B were replaced by a Glu residue, which is larger than Gly or Val and could potentially interact with the terminal amine of Ant343 in these mutants (Figs. 4 and 5).

Effects of Mutations at the Selectivity Filter. An Asn to Gln mutation at the critical asparagine in the M2 loop of NR1 (N616Q) reduced block by AQ343, AQ444, Ant343 and Ant444 (Fig. 3); however, substitution of Gly for Asn enhanced the block by Ant343, but not by AQ343, AQ444 and Ant444.

There are two asparagine residues (N615 and N616) at a similar position in the M2 loop of NR2B, which also contribute to the selectivity filter and Mg^{2+} binding site (Dingledine et al., 1999). We also reported data consistent with the proposal that NR1 N616 and NR2B N616 make the narrowest constriction of the channel (Kashiwagi et al., 2002). An N616G mutation in NR2B increased block by AQ343 and Ant343, did not influence block by AQ444, and decreased block by Ant444. An N616Q mutation at this position decreased block by all four compounds. The results may be explained as follows: Narrowing of the channel by substitution of Gln for Asn at N616 in NR1 or NR2B decreased the block by these compounds, maybe due to the disturbance of penetration of polyamine tail into the inner vestibule. Expanding of the channel (NR1 N616G, NR2B N615G and NR2B N616G) enhanced block by AQ343 and Ant343 preferentially. This may reflect occupancy of a wider space by AQ343 and Ant343 than by AQ444 and Ant444 (Fig. 6).

We carried out experiments to determine whether the AQ and Ant polyamines could permeate wild-type and mutant NMDA channels (Fig. 7). To do this, we measured block and looked for relief of block at extreme negative membrane potentials (Chao et al., 1997). At wild-type receptors, block was

voltage-dependent and was almost complete from -100 to -150 mV (Fig. 7). However, by expanding the size of channel pore with NR1(N616G) and NR2B(N615G) or NR2B(N616G), AQ343 and, in particular, AQ444 showed significant permeation of the channel manifest as a partial relief of block at very negative membrane potentials (Fig. 7). In contrast, there was little or no permeation of Ant343 and Ant444 at the mutant channels (Fig. 7).

Discussion

NMDA receptor-mediated neurotoxicity contributes to a variety of neurological disorders as well as cell death following trauma and stroke. Thus, there are continuing efforts to develop selective NMDA channel blockers for clinical use. NMDA channels are blocked by a large number of structurally dissimilar organic compounds including ketamine, phencyclidine, MK-801, memantine, and various spider toxins and polyamine derivatives (Huettner and Bean, 1988; Rodríguez-Paz et al., 1995; Igarashi et al., 1997; Kashiwagi et al., 2004; Chen and Lipton 2005). Among these blockers, memantine has been used clinically in the treatment of Alzheimer's disease (Reisberg et al., 2003). Memantine is a readily reversible and selective channel blocker (Lipton, 2005) that may have better clinical utility and fewer side-effects than the very high affinity and slowly reversible blockers such as MK-801 (Huettner and Bean, 1988). In this study, we looked for new, rapidly reversible and selective NMDA channel blockers with a higher potency than memantine. It was found that AQ343, AQ444, Ant343 and Ant444 are reversible, voltage-dependent NMDA blockers, particularly at NR1/NR2A and NR1/NR2B receptors, and only weakly block GluR1 AMPA receptors. Both the polyamine tail and the head

group are important for the activity of these compounds. By changing the structure of both the polyamine tail and the head group, we would like to develop rapidly reversible and more selective NMDA channel blockers for potential clinical application. Because of the relatively slow exchange time of the bath solution using oocyte recordings, we did not attempt to measure the dissociation of these various blockers or to compare their rates of dissociation with other blockers such as MK-801, ketamine, and phencyclidine. Nonetheless, it is clear that the Ant- and AQ-polyamines dissociate much faster than MK-801.

Memantine is very weakly selective for NMDA receptor subtypes, with a rank order of potency NR1/NR2D ≈ NR1/NR2C > NR1/NR2B > NR1/NR2A (Parsons et al., 1999). It has been reported that NR2A is expressed widely in the brain, and NR2B in forebrain. However, NR2C is predominantly in the cerebellum, and NR2D in neonatal stages (Watanabe et al., 1992). AQ- and Ant-tetraamines preferentially blocked NR1/NR2A, NR1/NR2B and NR1/NR2D. Tribenzylspermidine (Igarashi et al., 1997) preferentially blocked NR1/NR2A or NR1/NR2B receptors compared to those containing NR2C or NR2D. Thus, these types of blockers may have different *in vivo* profiles

compared to memantine because of their somewhat different subtype selectivity.

We probed the interaction of the AQ- and Ant-tetraamines with the NMDA channel by using a large series of NR1 and NR2B mutants. We found that mutations in the M3 region in the outer vestibule of NR1 generally had greater effects on the blockers than mutations in the equivalent region of NR2B. The polyamine tail may pass through the narrowest constriction of the channel, and its interaction with the M2 loop and inner vestibule may be dependent on the angle of head group and polyamine tail. In this regard, AQ444 and Ant444, which have a longer polyamine tail than AQ343 and Ant343, were influenced by residues deeper in the inner vestibule. The data are consistent with the proposal that NR1 N616 and NR2B N616 form the narrowest constriction of the channel, with the NR1 and NR2 subunits arranged asymmetrically and that the M3 region in the outer vestibule of NR1 is strongly involved in the recognition of blockers (Wollmuth et al., 1996; Dingledine et al., 1999; Kashiwagi et al., 2002). However, some amino acid residues in M3 of NR2B also affected block by AQ343 and Ant343, but not AQ444 and Ant444. This may be due to the difference of space occupied by these compounds, because substitution of larger amino acid residues with smaller ones enhanced block by AQ343 and Ant343,

but not by AQ444 and Ant444.

With regard to the potencies of the four compounds, one of the important factors is the number of positive charges - the degree of inhibition was greater with Ant343 and Ant444 than with AQ343 and AQ444. Furthermore, AQ343 was more potent than AQ444, and Ant444 was more potent than Ant343. The results suggest that the distance and the angle between the head skeleton and the NH_2-group in the polyamine tail are important for interactions with the NMDA channel (see Fig. 6). In conclusion, subtle molecular shape differences involving the angle between the polycyclic ring and the linear polyamine tail as well as the length of the polyamine tail itself are key parameters to be considered in the design of polyamine-derived NMDA receptor antagonists.

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Footnotes

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Legends for figures

Fig. 1. Effects of AQ343 (A), AQ444 (B), Ant343 (C), and Ant444 (D) at NR1/NR2A receptors. The structures of the compounds are shown at the top of each panel. Representative traces are shown to illustrate the effects of 0.1 to 1 μM of each compound on currents activated by glutamate and glycine (Glu+Gly) in oocytes voltage-clamped at -70 mV. I-V curves for Glu+Gly-activated currents were measured by voltage ramps in the absence (control) and presence of the AQ and Ant compounds. Leak currents have been subtracted. The I-V curves are representative of results in four similar experiments.

Fig. 2. Effects of AQ343, AQ444, Ant343 and Ant444 at NMDA and AMPA receptors. Concentration-inhibition curves were determined at NR1/NR2 receptors containing NR2A, NR2B, NR2C and NR2D subunits and at AMPA receptors expressed from the GluR1 subunit in oocytes voltage-clamped at -70 mV. Data are expressed as a percentage of the control current measured in the absence of blocker. Values are mean ± S. E. M. from four oocytes for each subunit combination.

Fig. 3. Effects of AQ and Ant tetraamines at NR1/NR2B receptors containing NR1 mutants. The effects of AQ343, AQ444, Ant343 and Ant444, each at two concentrations, were determined in oocytes expressing wild-type (WT) and mutant receptors and voltage-clamped at -70 mV. Values are mean ± S. E. M. from twenty oocytes for wild-type and four oocytes for each mutant. Mutations that reduced block by ≥ 15% compared to wild-type are highlighted by an open box. Mutations that enhanced block compared to wild-type are indicated by white letters in black boxes.

Fig. 4. Effects of AQ and Ant polyamines at receptors containing NR2B mutants. Experiments were carried out as described in the legend of Fig. 3. Values are mean ± S. E. M. from twenty oocytes for wild-type and four oocytes for each mutant. Mutations that reduced block by ≥ 15% compared to wild-type are highlighted by an open box. Mutations that enhanced block compared to wild-type are indicated by white letters in black boxes.

Fig. 5. Modeling residues that affect block by AQ343, AQ444, Ant343 and Ant444 in the pore and vestibule of the NMDA receptor. The M1-M2-M3 region is depicted as a helix-pore-loop helix similar to the structure reported for

the KcsA potassium channel from *Streptomyces lividans* (Doyle et al., 1998). The M2 region contains a helix followed by a random coil structure. Red circles indicate residues at which mutations reduce block by ≥ 15% compared to wild-type as shown in Figs. 3 and 4. Blue circles indicate residues at which mutations enhance block compared to wild type. Yellow circles indicate residues at which mutations affect block differently depending on the size of mutated amino acid. Grey circles indicate residues at which mutations have little or no effect.

Fig. 6. Preferred conformations of AQ343, AQ444, Ant343 and Ant444. The most stable conformation of each compound was determined by MD simulation as described in Materials and Methods. Histograms show the frequency of relative location of polyamine backbone against anthraquinone or anthracene backbone. Horizontal and vertical length of each compound is shown in the figure.

Fig. 7. Block and permeation of NMDA channels by AQ and Ant compounds. I-V curves were constructed by voltage ramps from -150 to +40 mV in oocytes expressing wild-type and mutant receptors. Responses to 10 μM glutamate

(with 10 μM glycine) were measured in the absence and presence of the blocker. Leak currents have been subtracted. The experiments were repeated four times with similar results.

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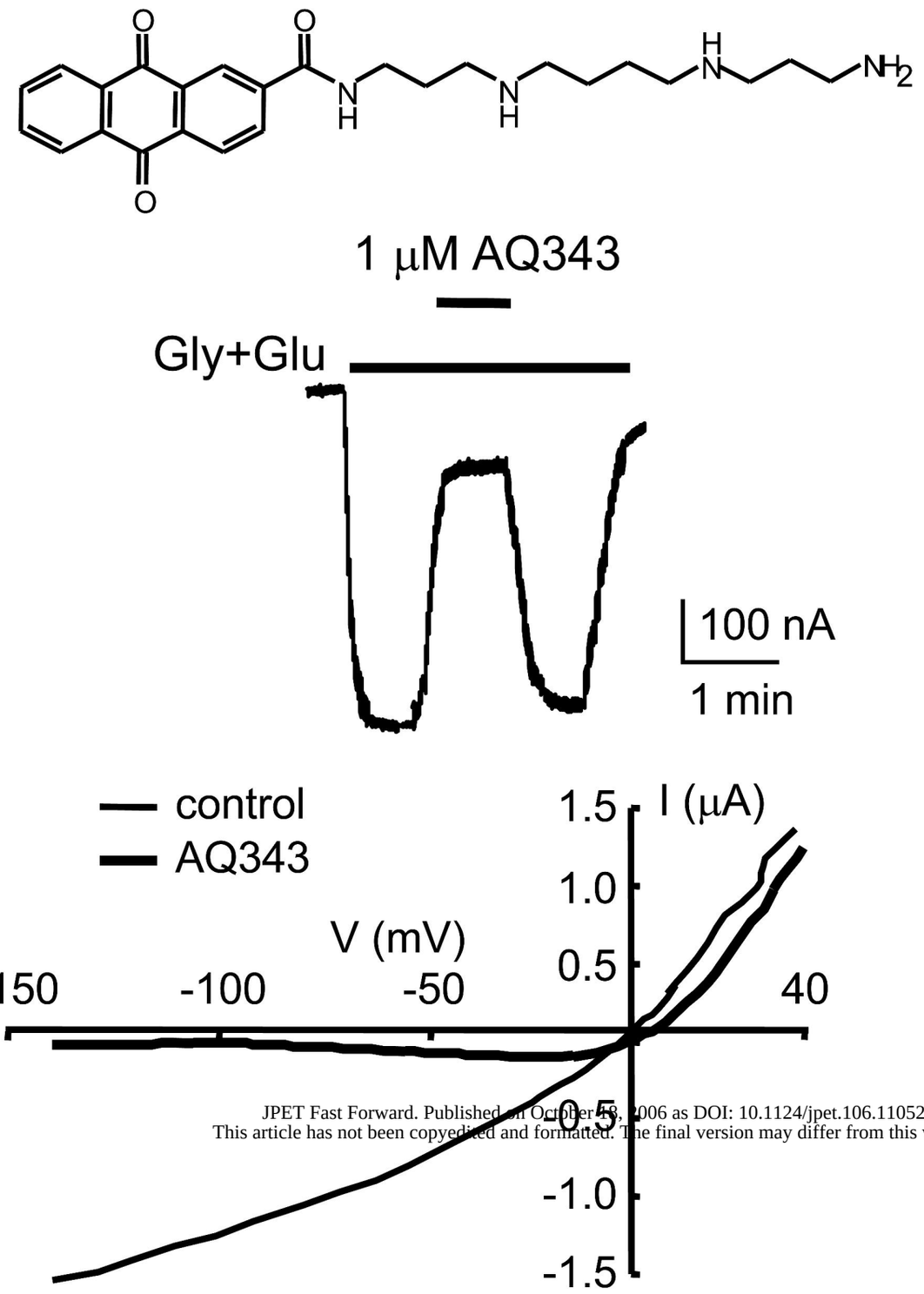
Table 1. IC_{50} values of AQ343, AQ444, Ant343 and Ant444 on NMDA and AMPA receptors

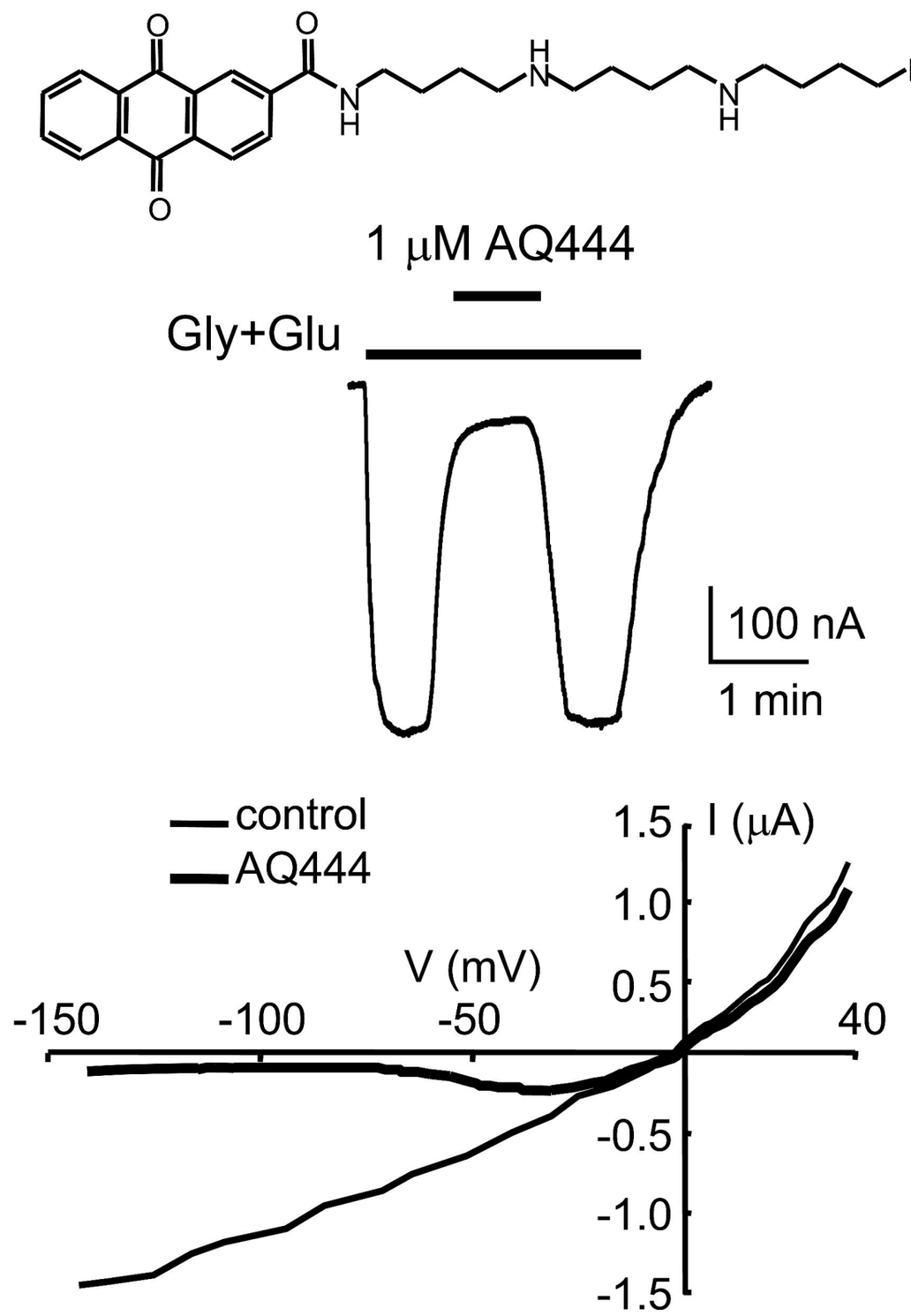
Values of the IC_{50} were determined from concentration-inhibition curves shown in Fig. 2 according to equation 1 in Materials and Methods. Values are mean ± S. E. M. from four oocytes for each subunit combination.

Receptor	IC_{50} (μM)			
	AQ343	AQ444	Ant343	Ant444
NR1/NR2A	0.39 ± 0.04	0.57 ± 0.06	0.057 ± 0.012	0.018 ± 0.003
NR1/NR2B	0.49 ± 0.06	0.57 ± 0.06	0.19 ± 0.01	0.029 ± 0.004
NR1/NR2C	5.19 ± 0.49	5.02 ± 0.48	1.60 ± 0.19	2.18 ± 0.26
NR1/NR2D	0.34 ± 0.06	0.50 ± 0.06	0.16 ± 0.02	0.15 ± 0.016
GluR1	32.1 ± 2.85	2.95 ± 0.23	2.65 ± 0.31	1.42 ± 0.17

A. AQ343



B. AQ444



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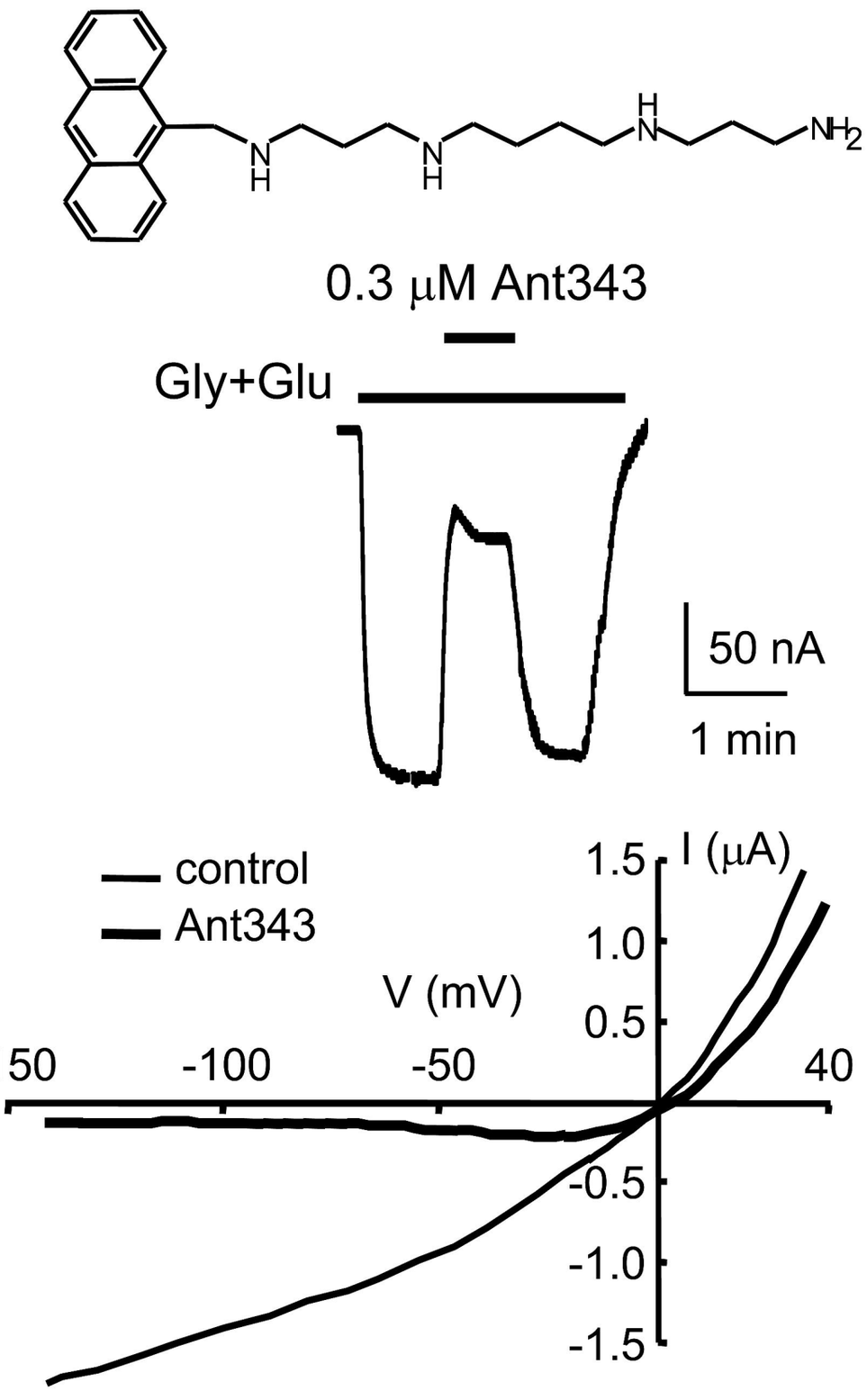
C. Ant343



D. Ant444

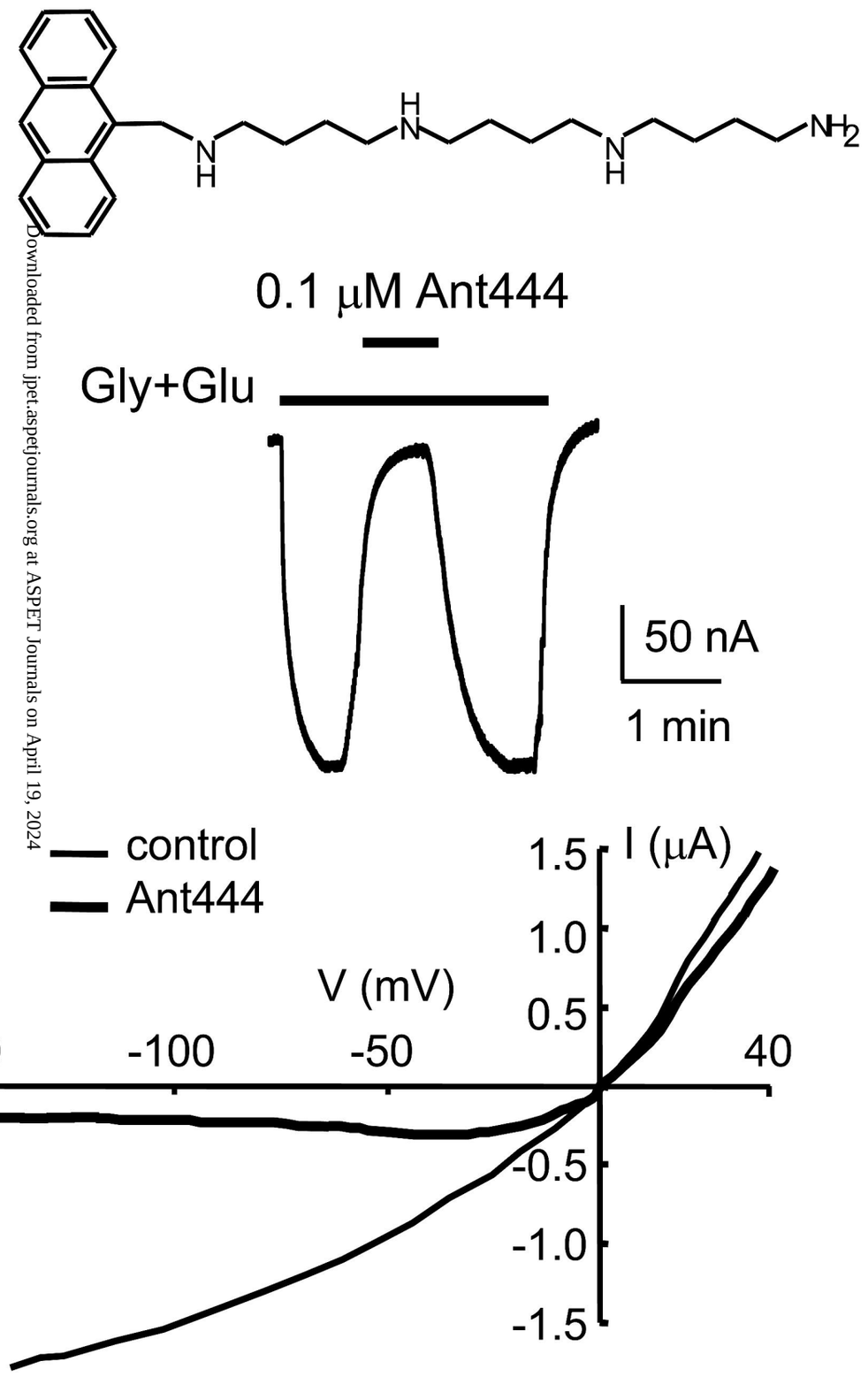


Fig. 2

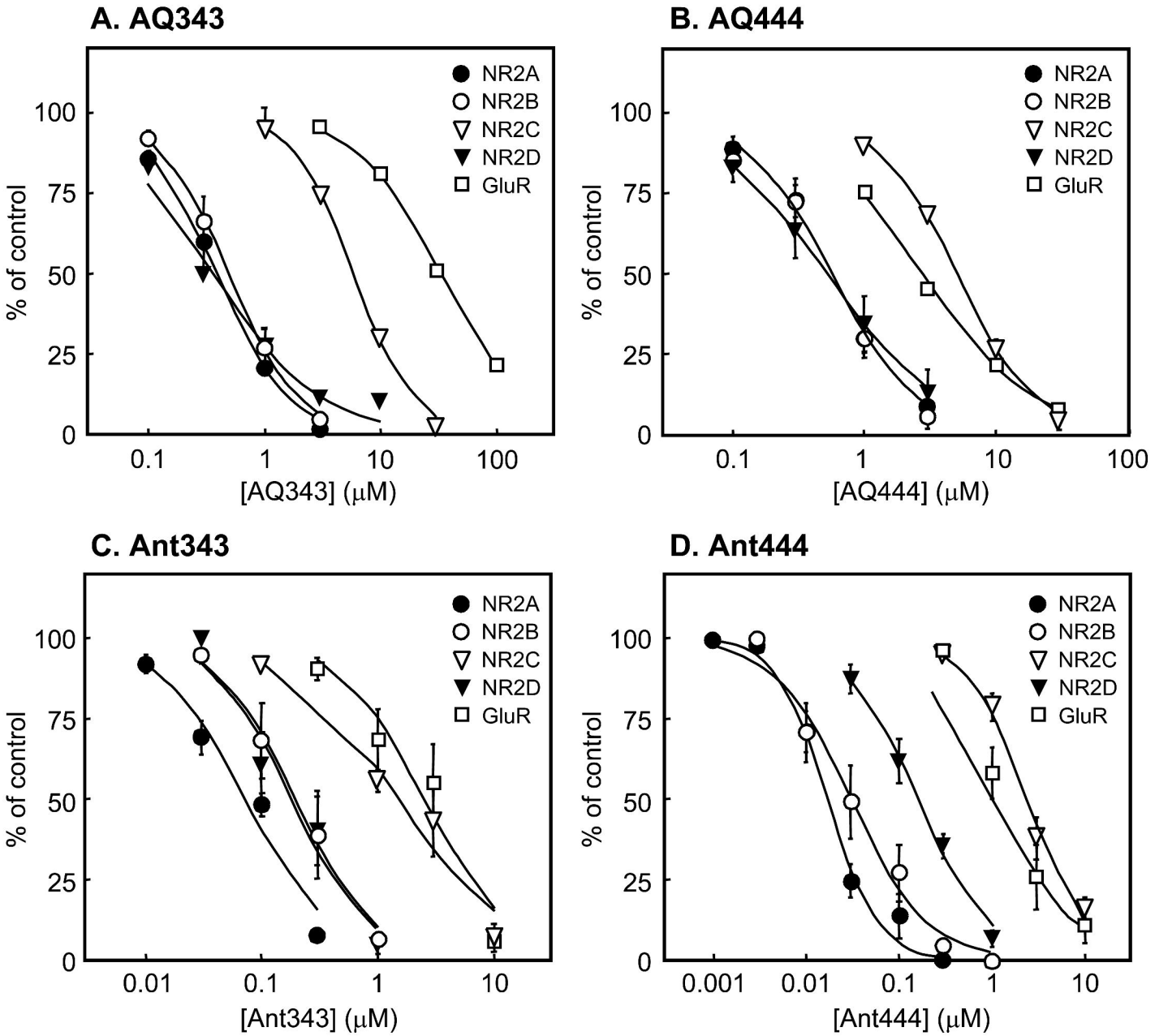


Fig. 3

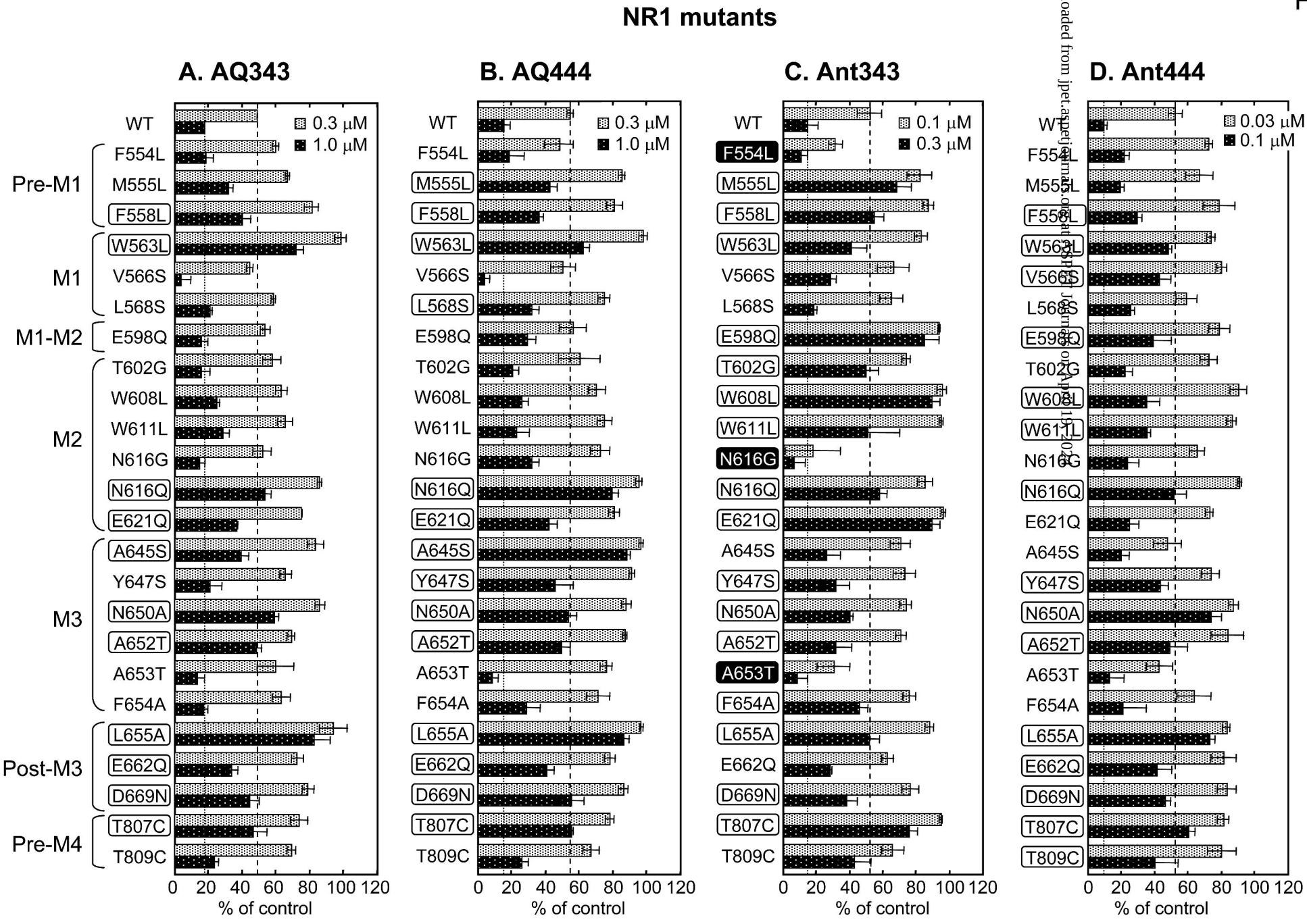
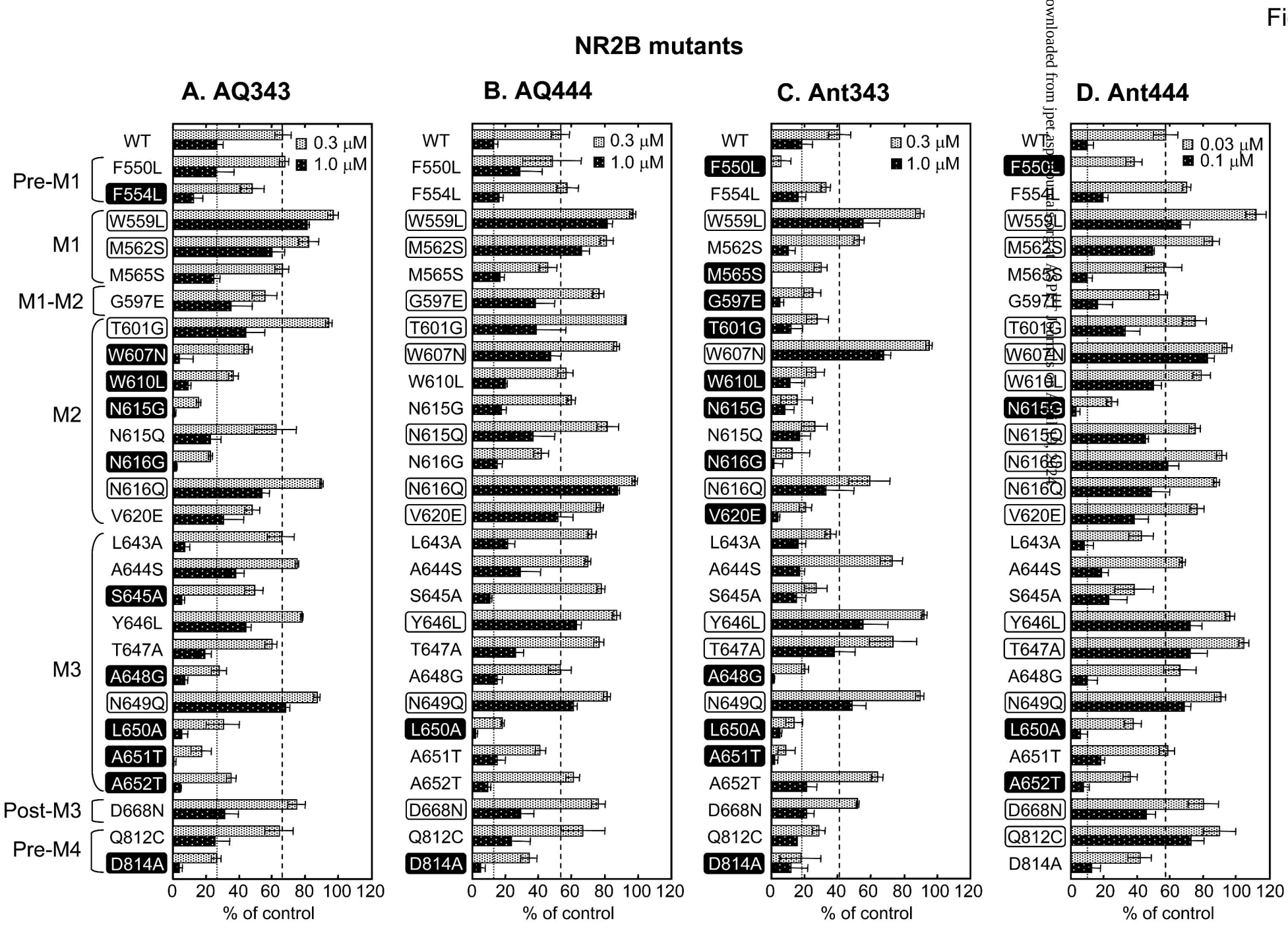


Fig. 4



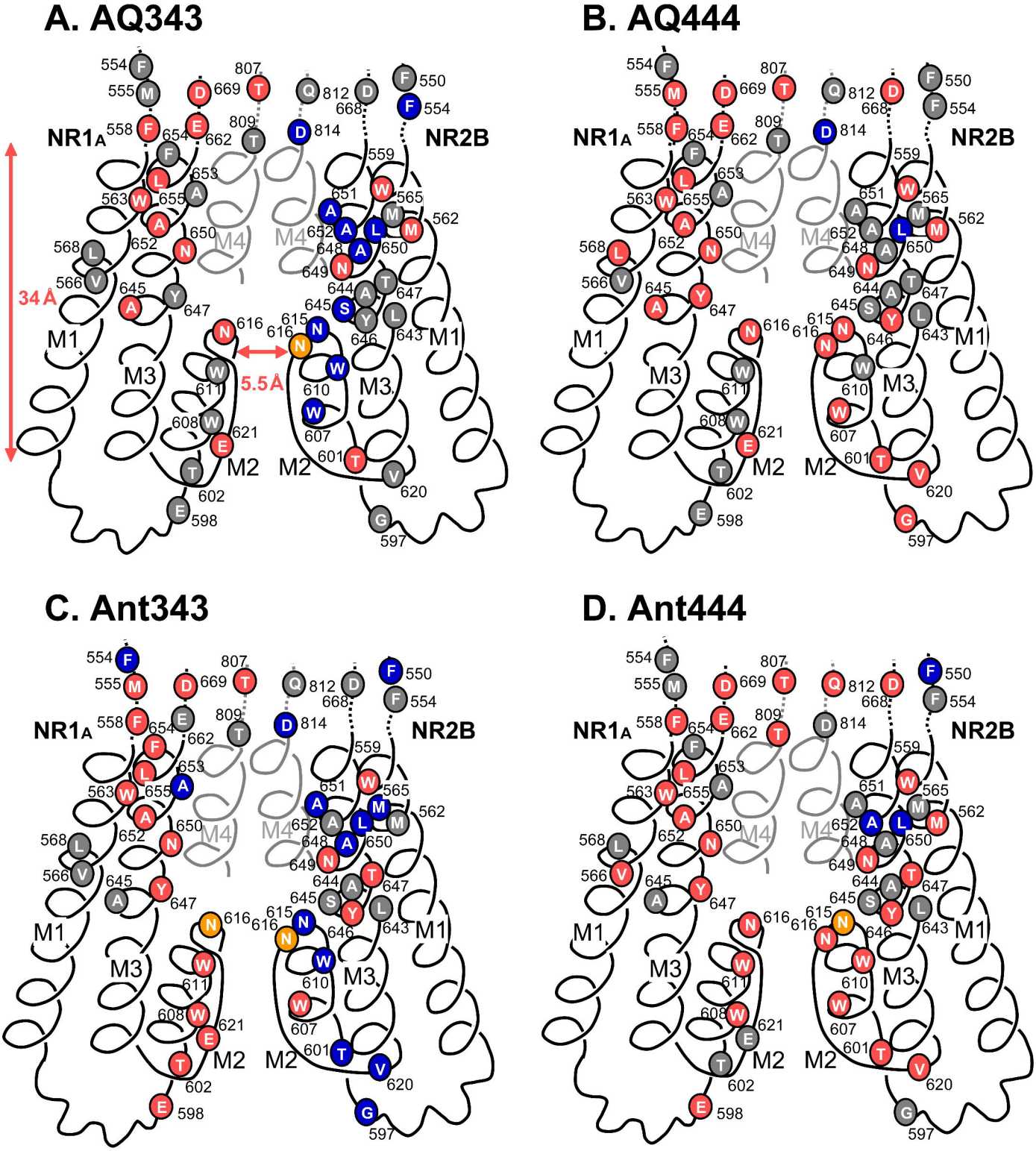

A. AQ343
B. AQ444
C. Ant343
D. Ant444
NR1A
NR2B
M1
M2
M3
M4
34 Å
5.5 Å
Mutations reduce block
Mutations enhance block
Mutations affect block differently depending on the size of mutated amino acid
Mutations have little or no effect

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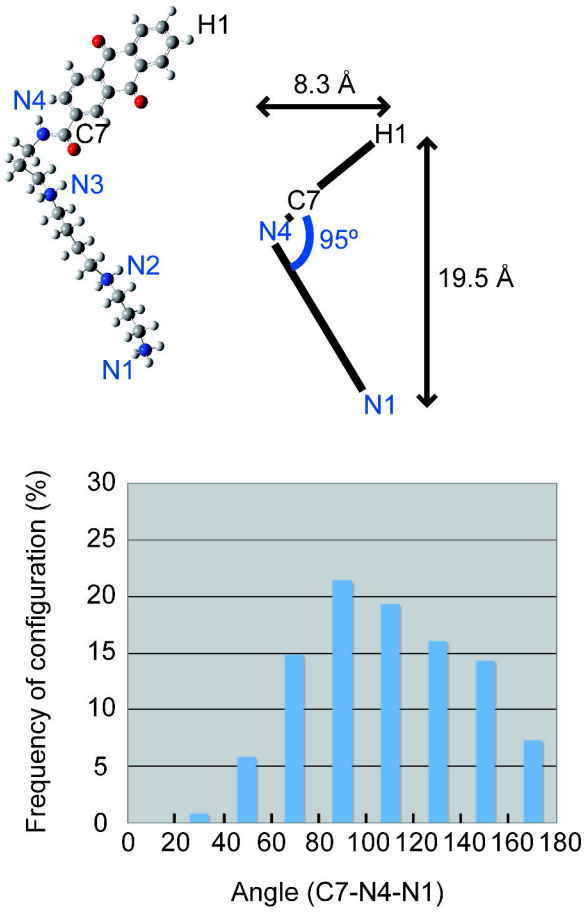
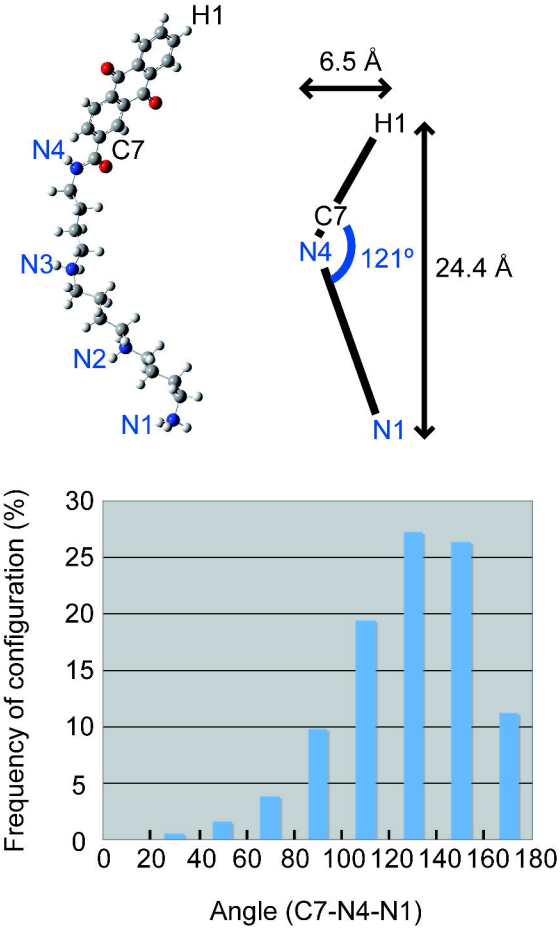
Fig. 6

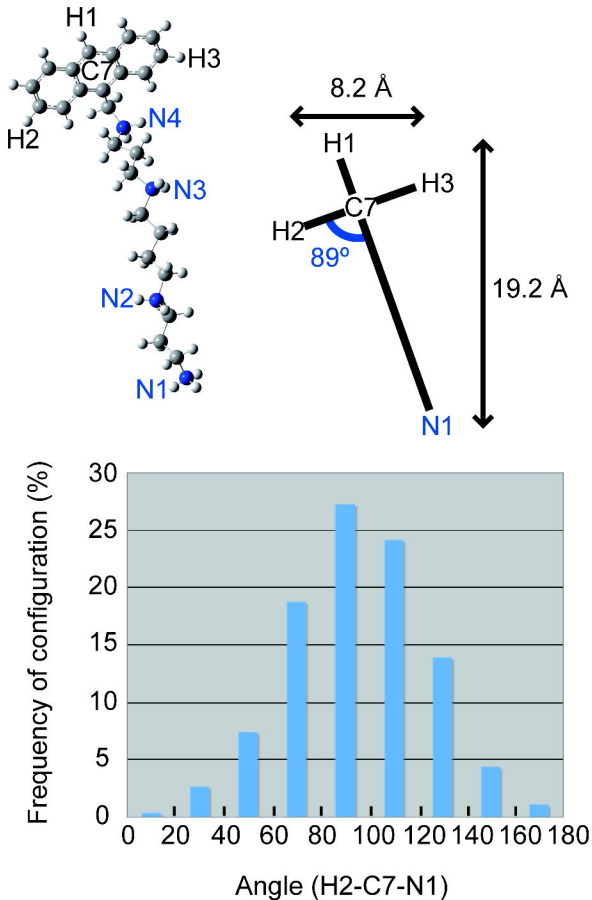
A. AQ343



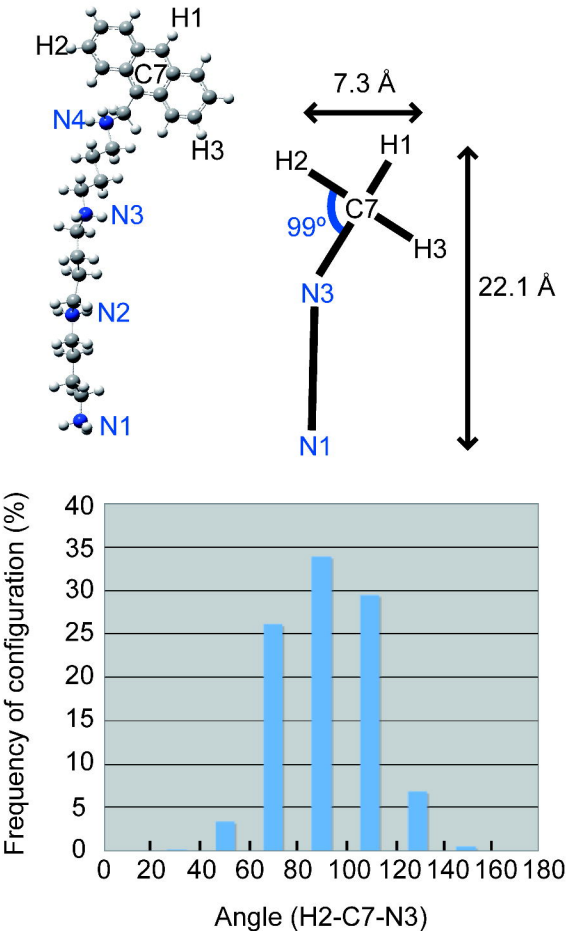
B. AQ444

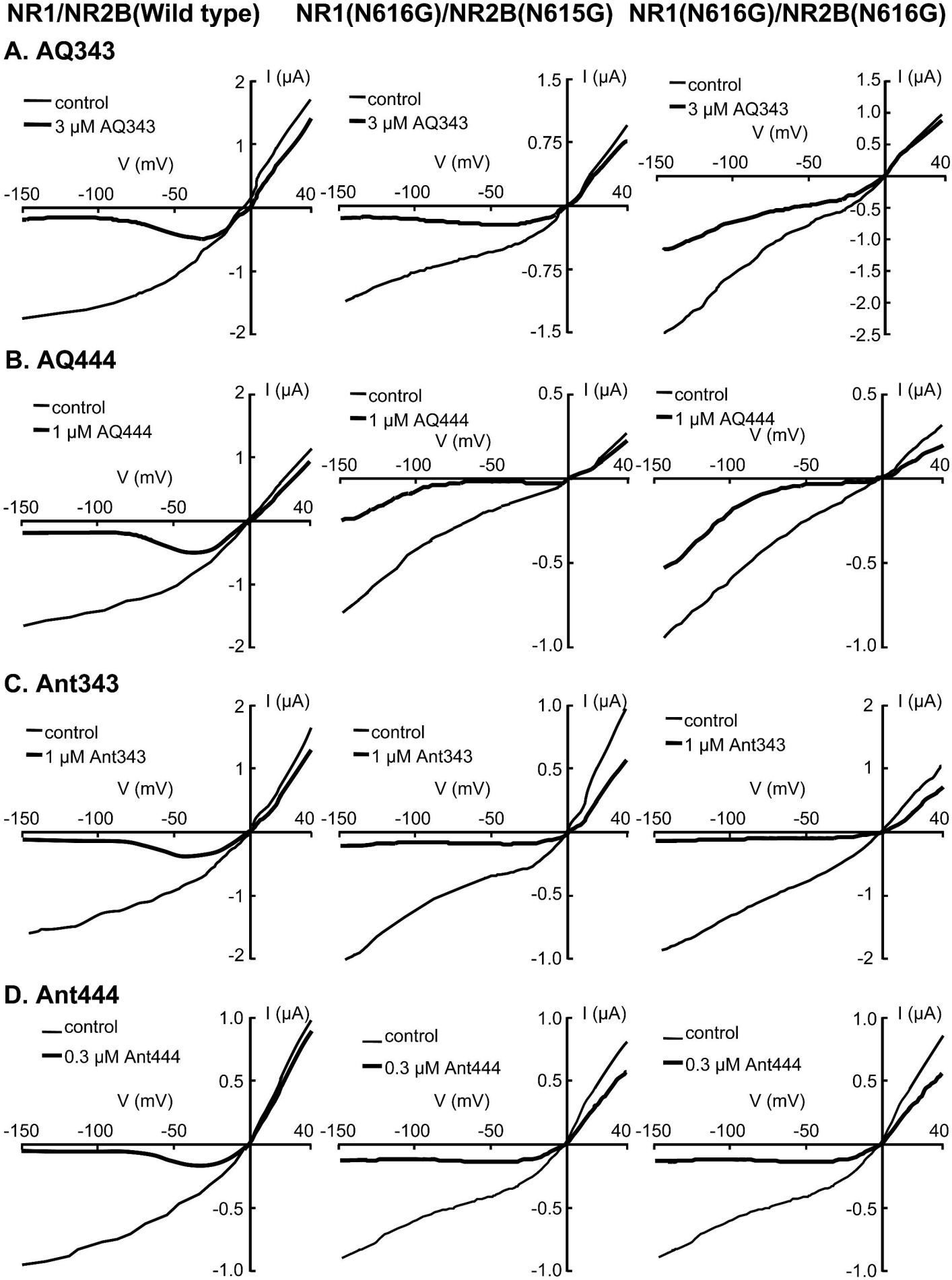


C. Ant343



D. Ant444




NR1/NR2B(Wild type)
NR1(N616G)/NR2B(N615G)
NR1(N616G)/NR2B(N616G)
A. AQ343
control
3 μM AQ343
I (μA)
V (mV)
B. AQ444
control
1 μM AQ444
I (μA)
V (mV)
C. Ant343
control
1 μM Ant343
I (μA)
V (mV)
D. Ant444
control
0.3 μM Ant444
I (μA)
V (mV)