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Differential Effects on Current Kinetics by Point Mutations in the *Lurcher* Motif of NR1/NR2A Receptors

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Running Title: The role of the *lurcher* motif in gating of NMDA receptors

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List of non-standard abbreviations:

NMDA, N-methyl-D-aspartate

SCAM, substituted cysteine accessibility method

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ABSTRACT

The extracellular proton site has emerged as the key site in regulating NMDA receptor function. However, the molecular determinants for the proton-sensitive gating of NMDA receptors are still not clearly delineated. The highly conserved *lurcher* motif plays an important role in determining the proton sensitivity of NMDA receptors. Mutations of several residues in the *lurcher* motif of either NR1 or NR2A significantly reduce proton sensitivity of recombinant NR1/NR2A receptors. It remains uncertain how these residues play a role in proton inhibition of NMDA receptors. Mutations of these residues could directly reduce the proton affinity. Alternatively, they could alter the proton IC_{50} indirectly by increasing channel open probability. In the present study, we recorded the macroscopic NMDA currents in HEK 293 cells with a piezo-based rapid solution exchange system. We show that zinc slows the deactivation of NR1a(A653T)/NR2A receptors and NR1a/NR2A(A651T) receptors. However, NR1a(T648C)/NR2A, NR1a/NR2A(T646C), NR1a(A649C)/NR2A, NR1a/NR2A(A647C) and NR1a(A653T)/NR2A exhibit significantly slower rise time and deactivation time constants under nominally zinc-free conditions. Our data suggest that the channel open probability for these mutant receptors may be significantly increased. The reduction in proton sensitivity by these mutations could be accounted for, at least partially, by the increased channel open probability. In contrast, NR1a/NR2A(A651T) exhibits normal macroscopic currents, suggesting that the reduction of proton sensitivity by this mutation cannot be attributed to any significant change of

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open probability. Further experiments are needed to determine the exact role of this residue in proton-sensitive gating of NMDA receptors.

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INTRODUCTION

NMDA receptors play a critical role in learning and memory, and in the regulation of neuronal development (Dingledine et al., 1999). Overactivation of NMDA receptors, causing calcium overload in neurons, are implicated in a variety of pathological conditions, such as stroke (Dirnagl et al., 1999; Lee et al;, 1999), limbic seizures (Meldrum et al., 1999), head trauma (Obrenovitch and Urenjak, 1997) and degenerative neurological diseases (Olney et al., 2001). Hypoactivity of NMDA receptors has been suggested to play a role in psychosis (Olney et al., 1997). The involvement of NMDA receptors in such diverse physiological and pathological processes has lead to considerable interest in developing a detailed understanding of cellular and molecular mechanisms involved in the regulation of NMDA receptor function.

The NMDA receptor current is modulated by a number of extracellular allosteric modulators, including proton, zinc and polyamines. The extracellular proton site has emerged as the most critical site because the effects of several allosteric regulators of NMDA receptor are mediated by the proton site. The high affinity Zn^{2+} inhibition of NR2A-containing receptors is due to enhancement of tonic proton inhibition (Choi and Lipton, 1999; Low et al., 2000). A change in tonic proton inhibition is also the underlying mechanism for polyamine potentiation of NR2B-containing receptors (Traynelis et al., 1995), and for ifenprodil inhibition of NR2B-containing receptors (Park and Williams, 1997; Mott et al., 1998). To fully understand the gating of NMDA receptors, detailed knowledge of the molecular determinants of the proton-sensitive

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gating of NMDA receptors are essential.

Drawing analogy to pH-sensitive gating of potassium channels (Schulte and Fakler, 2000; Yang et al., 2000), we hypothesized that the molecular determinants for the proton site is localized near the extracellular end of the M3 transmembrane domain of NMDA receptors that contains the *lurcher* motif (SYTANLAAF). The *lurcher* motif is conserved in all glutamate receptors and is thought play a critical role in gating (Kohda et al., 2000; Taverna et al., 2000; Jones et al., 2002). We have identified residues in the *lurcher* motif that can shift the proton IC_{50} by more than 200 fold in combination (Low et al., 2003). However, a residue with an ionizable side chain is the most likely candidate to participate directly in proton-sensitive gating. All residues identified in the *lurcher* motif so far lack ionizable side chains. It remains unclear how these residues alter proton sensitivity of NMDA receptors.

One possibility is that the effects of all these mutants in the *lurcher* motif on proton sensitivity are indirect. As in the case of apparent agonist affinity (Colquhoun, 1998), the apparent proton sensitivity of a mutant NMDA receptor can reduced by a drastic increase in the open probability, since more receptors would be shifted into the open state. Such change in open probability would also result in significantly slower deactivation time constant (Colquhoun, 1998). A previous study has reported changes in the deactivation time constants by the *lurcher* mutation in NMDA receptors, suggesting that the *lurcher* mutations may change channel open probability (Kohda et al., 2000). However, the slower deactivation could also be explained by

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the presence of ambient zinc. We have shown previously that an extracellular zinc site in the amino terminal domain of NR2A is allosterically coupled to the glutamate binding site in the S1/S2 domain of NR2A (Zheng et al, 2001), and binding of zinc to that site inhibits NMDA channels by enhancing the proton sensitivity of NMDA receptors (Low et al., 2000; Zheng et al., 2001). When the proton sensitivity is significantly reduced by a point mutation, zinc-bound but unprotonated receptors can transit into the open state. The deactivation of these zinc-bound receptors will be slower since they exhibit higher affinity for glutamate due to the positive allosteric interaction between the glutamate binding site and the zinc binding site. In the present study, we attempted to determine whether mutations with reduced proton sensitivity in the *lurcher* motif exhibit changes in the gating properties. Our data suggests that, although ambient zinc contributes to the slower deactivation time constants of the *lurcher* mutants, most of the mutations with reduced proton sensitivity exhibit significantly slower rise time and deactivation time constant. Our data suggest that the change in proton sensitivity caused by these mutants may be due to the change of pH-independent gating properties of the NMDA receptors, at least partially. However, NR2A(A653T) exhibits normal macroscopic currents, suggesting that the shift of proton sensitivity caused by this point mutation could reflect a direct alteration of the proton binding.

METHODS

Plasmid constructions and Site-directed mutagenesis. All plasmid encoding wild type

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NR1/NR2A receptors and mutants used in this study have been described previously (Low et al., 2003).

Transfection of HEK cells. HEK 293tsa cells (Dr. Rick Horn, Tuft University, Philadelphia, PA) were maintained at 37° and 5% CO_2 in DMEM supplemented with L-glutamine (200 μM), sodium pyruvate (100 μM), penicillin/streptomycin (100 units/ml), and 10% fetal bovine serum (Invitrogen, Calsbad, CA). Low confluency cells were transfected by the calcium phosphate precipitation method (Chen and Okayama, 1987). Cells were cotransfected with a mixture containing NR1, NR2, and GFP plasmids (1, 2, and 0.3 μg per 12 mm diameter coverslip, respectively). After transfection, D-AP5 (100-200 μM) and 7-Cl-kynurenic acid (50-100 μM) was added to the culture medium. For some mutants, MgCl2 (2 mM) was also needed to reduce excitotoxicity. NMDA antagonists were purchased from either Sigma (St. Louis, MO) or Tocris Cookson (Ballwin, MO).

Whole cell patch-clamp recordings and outside-out patch recording from HEK 293 cells. Patch clamp recording in the whole cell configuration and outside-out patch recording were made as described previously with Axopatch 200B amplifier (Axon Instruments, Union City, CA). Recording electrodes (5-12 MΩ) were filled with (in mM): 140 Cs-gluconate, 5 HEPES, 4 NaCl, 2 $MgCl_2$, 0.5 $CaCl_2$, 1 ATP, 0.3 GTP and 5 BAPTA (pH 7.4). The recording chamber was continually perfused with recording solution comprised of (in mM): 150 NaCl, 10 HEPES,

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1.0 $CaCl_2$, 3 KCl, and 20 mM mannitol. Glutamate (50-100 μM) and glycine (30-60 μM) was applied using a multibarrel pipette driven by a piezo-based fast solution jumping system (Burleigh LSS 3100, EXFO Burleigh, Victor, NY). The rise time of the junction potential change produced by this system is 0.66 ms. The tricine-buffered Zn^{2+} solutions were prepared according to the empirically established binding constant 10^{-5} M (Paoletti et al., 1997) by adding into 10 mM tricine 1.23 μM Zn^{2+} to obtain 100 nM free Zn^{2+} at pH7.4.

Data Analysis and Statistics. Five current traces were collected at a sampling rate of 5-20 kHz with pClamp 9 (Axon Instruments) and averaged with Clampfit 9 (Axon Instruments). The averaged current traces were then analyzed with ChannelLab (S.T. Traynelis, Emory University, Altanta, GA). The rise time was determined by linear regression of the rising phase of the current responses. The deactivation time constant was determined by fitting the decay of the current responses with a single exponential component. All pooled data are expressed as mean±SEM. One way ANOVA and Tukey post-hoc test were used to determine significance ($p<0.05$) unless stated otherwise.

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RESULTS

In a previous study (Low et al., 2003), we have identified 3 residues in the *lurcher* motif of NR1 subunits that greatly reduce proton sensitivity of NMDA receptors when it is mutated (Fig.1). Point mutations of the corresponding residues in the NR2A subunit have similar effects (Fig.1). To determine whether these point mutations alter the kinetic properties of NMDA receptors, we measured the rise time and the deactivation time constant of these mutants expressed in HEK 293 cells. To eliminate any potential effects of extracellular free zinc on the deactivation time constants, the whole-cell NMDA current responses were recorded in the presence of metal chelators (10 μM EDTA or 10 mM tricine). The free zinc concentration was reduced to nominally zero (less than 10 pM) in the presence of either one of the chelators used in our experiments.

The first pair of point mutations tested was the *lurcher* point mutation of NR1a and NR2A that reduce proton sensitivity of NR1/NR2A receptors by approximately 5 fold. The *lurcher* mutation of NR2A (NR2A(A651T)) showed no detectable changes in the macroscopic current (Fig.2A,B and Table 1). On the other hand, the rise time and deactivation time constant for the NR1a (A653T) mutant was significantly slower than the wild type (Fig. 2C, D and Table 1). However, the deactivation time constant was only 0.4 sec, not as high as the value reported in a previous report (Khoda et al., 2000). The difference is likely due to the presence of ambient zinc in that study as indicated by the presence of the fast desensitization caused by zinc.

We next determined the effects of the point mutations of NR1a(T648C) or

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NR2A(T646C) on the macroscopic currents. In our previous study (Low et al., 2003), these two mutants caused comparable reductions in proton sensitivity to either NR1a(A653T) or NR2A(A651T) mutations. Like NR1a(A653T), these two mutants exhibited a slower rise time and a greater deactivation time constant (Fig.3, Table 1). The substitution of the neighboring residue to cysteine caused even greater change in macroscopic currents (Fig.4). The deactivation of NR1a(A649C)/NR2A required tens of seconds that we were unable to quantify it by regular curve fitting routine (n=4) and the exact deactivation time constant remains undetermined. The deactivation of NR1a/NR2A(A647C) was equally slow (n=4). The slow deactivation time course caused failure in the curve fitting routine so the deactivation time constant was not determined. We have also recorded NMDA receptor currents in HEK293 cells expressing other cysteine substitutions in the *lurcher* motif of NR2A (i.e. NR2A(Y645C), NR2A(N648C), NR2A(L649C), NR2A(A650C), NR2A(A651C) and NR2A(F652C). The deactivation of these mutants appears normal (data not shown, n=3-5). We have also recorded NMDA receptors currents for two additional NR1 cysteine substitutions in the *lurcher* motif (NR1a(A652C) and NR1a(F654C)). The deactivation of these mutants also appears normal (data not shown, n=3-5). In summary, we observed slower rise time and deactivation time constants for most point mutations in the *lurcher* motif of either NR1 or NR2A that reduce proton sensitivity. The only exception is NR2A(A651T), which exhibits normal kinetics for whole cell currents. A change in the deactivation time constant could be caused by a change in the affinity of the agonist binding site or a change in the channel open probability. Since the *lurcher* motif is outside the glutamate

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binding domain of NR2 or the glycine binding domain of NR1 (Armstrong et al., 1998; Furukawa and Gouaux, 2003), it is unlikely that these mutants in the *lurcher* motif could directly alter the agonist binding site. Therefore, our data suggest that the channel open probability may be altered by NR1a(T648C), NR1a(A649C), NR1a(A653T), NR2A(A646C) and NR2A(A647C).

In a final set of experiments, we investigated the effects of zinc on the time constant for the deactivation of *lurcher* mutants. As outlined earlier, our working hypothesis regarding zinc regulation of NMDA receptor activity predicts that zinc should slow the deactivation of any mutant receptors with significantly reduced affinity for proton binding. Since we had shown earlier that zinc can not modulate gating directly and its effects is mediated by the proton site (Low et al., 2000), we assume that zinc-bound, unprotonated receptors can transit into open state. For wild type receptors, most zinc-bound receptors will be protonated at physiological pHs so the zinc-bound, unprotonated receptors make little contribution to the macroscopic currents. For a mutant receptor with greatly reduced affinity for the proton binding site, the proportion of zinc-bound, unprotonated receptors will increase significantly and will make greater contribution to the macroscopic currents. Since glutamate affinity is higher for zinc-bound receptors, the deactivation for these receptors should be slower. On the other hand, zinc should have no significant effects on the deactivation time constants for mutants that reduce proton sensitivity indirectly by increasing channel open probability. This is due to the notion that the proportion of zinc-bound, unprotonated receptors will not change and the contribution to the

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macroscopic currents by these receptors will remain the same. The effect of zinc on the deactivation of NR1a(A653T)/NR2A could explain the discrepancy between our results and a previous report (Khoda et al., 2000). We measured the deactivation time constants in the presence and absence of extracellular free zinc from the same HEK 293 cells for NR1a(A653T)/NR2A receptors and NR1a/NR2A(A651T) receptors (Fig. 5). The deactivation time constant of NR1a/NR2A(A651T) was doubled in the presence of zinc, which is consistent with the hypothesis that NR2A(A651T) alters proton dissociation constant directly. The deactivation time constant for NR1a(A653T)/NR2A was also significantly greater in the presence of zinc, which suggests that NR1a(A653T) may reduce proton sensitivity by two separate mechanisms, i.e. by reducing the channel closing rate and by reducing the dissociation constant for proton. Zinc has no detectable effects on the deactivation time constant of NR1a(T648C)/NR2A or NR1a/NR2A(T646C). The deactivation time constants for NR1a(T648C)/NR2A or NR1a/NR2A(T646C) were 667±132ms (n=3) and 337±25 ms (n=8), respectively. These values are not significantly different from the deactivation time constant for these mutants under zinc free conditions (Table 1; $p>0.05$, t-test). The effects of zinc on deactivation for NR1a(A649C) and NR2A(A647C) were not determined since the deactivation is too slow to be reliably fitted.

DISCUSSION

Our data show that several mutations in the *lurcher* motif alter the macroscopic current

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kinetics of recombinant NR1/NR2A receptors. In addition to NR1a(A653T), mutations of two consecutive residues in the *lurcher* motif of either NR1 (T648 and A649) or NR2A (T646 and A647) increase the deactivation time constants by more than 4 folds. Our data provide further support for the view that the highly conserved *lurcher* motif is critical for the gating of all glutamate receptors (Khoda et al., 2000; Taverna et al., 2000; Jones et al., 2002). The changes in current kinetics caused by these mutants in the *lurcher* motif also have clear implications on data interpretation for some earlier works (Beck et al., 1999; Sobolevsky et al., 2002a, b; Jones et al., 2002; Low et al., 2003). The role of the lurcher motif in gating has been investigated before using substituted cysteine accessibility method (SCAM). There is contradicting evidence as to whether Ala649 is aqueous accessible (Beck et al., 1999; Jones et al., 2002). The modification rate of substituted cysteine residues will be influenced by the gating rates of NMDA receptors. If the gating rates are altered by cysteine substitution as suggested by our present data, the data from most SCAM studies (Beck et al., 1999; Sobolevsky et al., 2002a, b; Jones et al., 2002) will need to be reevaluated. The conclusions in our earlier publication (Low et al. 2003) need to be reevaluated in light of our new data since we have not considered the potential effects on proton sensitivity caused by changes in gating rates produced by mutations in the *lurcher* motif.

Figure 6A shows the simplified kinetic scheme for proton-sensitive gating of NMDA receptors. The key presumption of this scheme is that protonated receptors can not proceed to open state and receptors in open state can not be protonated. Single channel analysis of proton inhibition of NR1/NR2B receptors appears to support this presumption since proton can not

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facilitate closure and there is little changes in the shut time distribution (Banke et al., 2004). Since the *lurcher* motif is located outside the binding domain for glutamate (Armstrong et al., 1998), mutations in the *lurcher* motif could not directly alter glutamate binding. Therefore, the apparent proton sensitivity could be altered in two distinct ways. The first way is to alter the proton dissociation constant directly by altering either the k_{on} or k_{off} for the proton-binding site. The second way is to drastically alter the channel open probability by changing the channel open rate or the close rate. Such changes in open probability could also result in changes in apparent proton sensitivity since it would alter the distribution of receptors in various states. Therefore, a point mutation could reduce the proton sensitivity of NMDA receptors by: (a) directly reducing the dissociation constant for proton; or (b) increasing the open probability and subsequently reducing the number of protonated receptors; or (c) a combination of both (a) and (b). The mutants in the *lurcher* motif that reduce proton sensitivity could fall into these three categories.

We simulated the NMDA receptor currents based on the kinetic scheme in Fig.6A. As shown in Fig.6B, reducing the close rate by 10 fold is sufficient to reduce apparent proton sensitivity by 4 fold. Such changes in gating also result in changes in the rise time and deactivation time constant as shown in our experimental data for several mutants in the *lurcher* motif (Fig.6B). In contrast, direct changes in the proton dissociation constant will not result in detectable kinetic changes in the macroscopic current (Fig.6C). Thus, the simple kinetic scheme

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is sufficient to account for our experimental findings.

Could the changes in gating rates that would result in the observed deactivation time course account for the shift in proton dose response curve reported previously? The mutants in the *lurcher* motif reported in Low et al. (2003) appear to be a heterogenous group. At one end of the broad spectrum are the cysteine substitutions of NR1a A649 and NR2A A647. These two mutants exhibit very slow deactivation time constants that would require the channel closing rates to be reduced by approximately 100 fold. Consistent with the greatest change in gating as suggested by the slow deactivation, NR1a(A649C) produces the greatest shift in proton sensitivity by any single point mutation. NR2A(A647C) also produces the greatest shift in proton sensitivity by a single point mutation (IC_{50}, 1178 nM, personal communication, Le and Traynelis). The reported proton IC_{50} for NR1a/NR2A(A647C) in a previous study (Low et al., 2003) is incorrect. It is possible that the effects of these two mutants on proton sensitivity could be entirely due to the suggested changes in gating rates and this residue may not play any direct role in proton-sensitive gating of NMDA receptors.

At the other end of the spectrum is the *lurcher* mutation of NR2A (NR2A(A651T)). NR2A(A651T) exhibits normal rise time and deactivation time constant while it produces a similar reduction in proton sensitivity as other point mutations in the *lurcher* motif. Our data suggest that the change in proton sensitivity caused by NR2A(A651T) can not be accounted for by changes in gating rates. Other lines of evidence also support our hypothesis that

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NR2A(A651T) could alter proton sensitivity directly. Zinc slows the deactivation of this mutant as predicted for a mutant that reduces proton affinity. Finally, the effects of NR2A(A651T) is additive to the effects of NR1a(A649C) that may be due to change of open probability (Low et al., 2003). If both mutants cause reduction of proton sensitivity by the same mechanism, i.e. changing channel open probability, the effects could not be independent as suggested by the coupling coefficient (Low et al., 2003).

The *lurcher* mutant of NR1a (NR1a(A653T)) may be in the middle of the spectrum. We observed a significant change in the current kinetics that suggests significant changes in the channel gating rates. The changes in gating clearly would contribute to the reduction of proton sensitivity by this mutation. However, NR1a(A653T) may also have some direct effects on proton affinity. Zinc slowed the deactivation of this mutant just as it slowed the deactivation of NR2A(A651T). The change of gating rate suggested by deactivation time constant for this mutant appears also to be insufficient to produce the observed shift in proton IC_{50}.

One clear implication of our present data is the need of abundant caution in interpretating mutagenesis data. Our present data clearly demonstrated that the reduction of proton sensitivity by some mutants in the *lurcher* motif is caused by changes of gating rates, at least partially. It is also possible that reduction in proton sensitivity produced by some mutants in other regions of NR1 could also be accounted for by changes in channel gating rates. Those mutations with great effects on proton sensitivity will need to be reevaluated just as the mutants in the *lurcher* motif.

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The gating properties of these mutants as well as mutants in the *lurcher* motif need to carefully be investigated with single channel analysis or non-stationary variance analysis. A kinetic model on proton-inhibition of NMDA receptors, validated by experimental data, is also critical. The simple model presented in Fig.6A has not been rigorously tested with experimental data. However, it shares the many features proposed in a recent study that has been tested more rigorously (Banke et al., 2004). We only intended to use it as a starting point in our attempt to evaluate the true impact of mutants in the *lurcher* motif on proton-sensitive gating. Detailed analyses of gating guided by kinetic modeling are needed to determine whether any of the mutants reported earlier (Low et al., 2003) alter proton sensitivity directly.

Our data also suggest that the *lurcher* mutant of NR2A is distinct from other *lurcher* mutants that reduce proton sensitivity since it is the only one with normal kinetics for macroscopic currents. Since Ala and Thr both lack ionizable side chains, it seems unlikely that Ala651 of NR2A acts directly as the proton sensor. However, Ala651 of NR2A could modulate the proton binding site by participating in hydrogen bond with the proton sensor through the peptide backbone. Alternatively, the oxygen in the side chain for threonine could be interacting with a protonated side chain through hydrogen bond. Systematic substitution of side chains of this residue may yield additional information that could improve our understanding about the molecular determinants of pH-sensitive gating of NMDA receptors.

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Footnotes:

a. This work was supported by the NINDS NS39418 (FZ).

b. Address reprint requests, correspondence, and requests for materials to Dr. Fang Zheng, Dept. of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, AK 72205-7199, USA.

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Figure Legends:

Fig. 1. The *lurcher* motif of glutamate receptors. The *lurcher* motif is marked by the box. Residues that are critical for proton sensitivity of NMDA receptors are shaded.

Fig. 2. The effects of the *lurcher* mutation on the deactivation of NR1/NR2A receptors. NMDA receptor currents were recorded in HEK 293 cells expressing wild type or mutant receptors (V_h=-40 mV or -50 mV). A rapid perfusion system (Burleigh LSS3100) applied 100 μM glutamate for 500 ms. Glycine (30-60 μM) was present all the time. Free zinc in the recording solution was considered to be 0 nM by adding 10 μM EDTA or 10 mM tricine. Representative current responses for NR1a/NR2A(A651T) receptors and NR1a(A653T)/NR2A receptors were scaled and superposed on the current trace of wild type receptors to show the rising phase (A, C) and deactivation (B, D).

Fig. 3. Macroscopic currents of NR1a(T648C)/NR2A and NR1a/NR2A(T646C). NMDA receptor currents were recorded in HEK 293 cells expressing wild type or mutant receptors (V_h=-40 mV or -50 mV). A rapid perfusion system (Burleigh LSS3100) applied 100 μM glutamate for 500 ms. Glycine (30-60 μM) was present all the time. Free zinc in the recording solution was considered to be 0 nM by adding 10 μM EDTA or 10 mM tricine. Representative current responses for NR1a/NR2A(T646C) receptors and NR1a(T648C)/NR2A receptors were scaled

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and superposed on the current trace of wild type receptors to show the rising phase (A, C) and deactivation (B, D).

Fig. 4. The deactivation time course of NR1a(A649C)/NR2A receptors (A) and NR1a/NR2A(A647C) receptors (B). NMDA receptor currents were recorded in HEK 293 cells expressing wild type or mutant receptors (V_h=40 mV or 50 mV). A rapid perfusion system (Burleigh LSS3100) applied 100 μM glutamate for 1 or 2 sec. Glycine (30-60 μM) was present all the time. Free zinc in the recording solution was considered to be 0 nM by adding 10 μM EDTA or 10 mM tricine.

Fig. 5. Zinc increases the deactivation time constants of the *lurcher* mutants. NMDA receptor currents were recorded in HEK 293 cells (V_h=-40 mV or -50 mV). A rapid perfusion applied 100 μM glutamate for 500 ms. Glycine (30-60 μM) was present all the time. EDTA (10 mM) or tricine were used to obtain zinc-free conditions. The free zinc concentration ranged from 100-270 nM . Typical current traces in the presence and absence of zinc from the same HEK cell are shown for NR1a/NR2A(A651T) (A) and NR1a(A653T)/NR2A (B). Pooled data from the same cells are shown in C and D. (**: $p<0.01$, paired t-test).

Fig. 6. A kinetic model for proton-sensitive gating of NMDA receptors. This model shown in

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panel A is built on the model first proposed by Lester and Jahr (1993). We assume that there is a single proton-binding site. We further assume that protonated receptors cannot transit into open state directly. For simplicity, the desensitization states are not considered. The rates used for simulating macroscopic currents of wild type NR1/NR2A receptors were as follows: k1, $1x10^{7}M^{-1}s^{-1}$; k2, 8 s^{-1}; k3, $1x10^{9}M^{-1}s^{-1}$; k4, 8 $s^{\uparrow 1}$; α, 100 s^{-1}; β, 250 s^{-1}. Macroscopic currents were simulated with Channel Lab (S. T. Traynelis). Changes in the open probability (a reduction of β by 10 fold) will shift the proton dose-response (B). However, the deactivation and rise time are also significantly slower (inset in panel B). On the other hand, direct changes of the proton affinity (an increase of k4 by 5 fold) will shift the proton dose-response curve (C) without detectable changes of the macroscopic currents (inset in panel C).

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Table 1. Effects of mutations in the *lurcher* motif of NMDA receptors on activation and deactivation time constants of macroscopic currents

Receptors	10-90% Rise time (ms)	Deactivation time constant (ms)
Wild Type (n=28)	14.31±0.62	85.88±6.11
NR1a(A653T)/NR2A (n=14)	21.27±1.26[a]	415.6±22.2[b]
NR1a/NR2A(A651T) (n=5)	13.63±0.43	96.53±11.62
NR1a(T648C)/NR2A (n=5)	22.33±0.80[a]	542.9±34.1[b]
NR1a/NR2A(T646C) (n=8)	26.80±2.89[a]	365.7±39.0[b]

a, b: significantly different from wild type receptors (ANOVA and Tukey post-hoc tests, $p<0.01$).

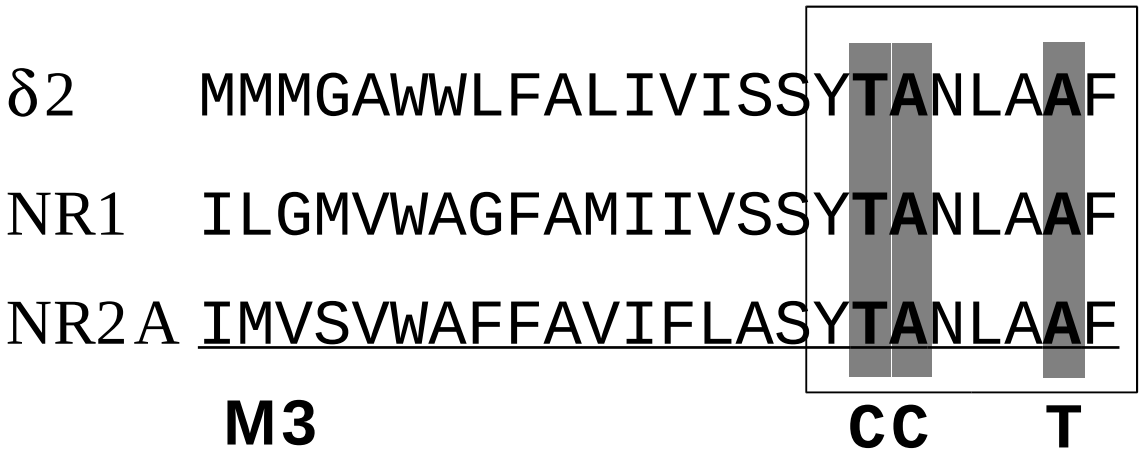
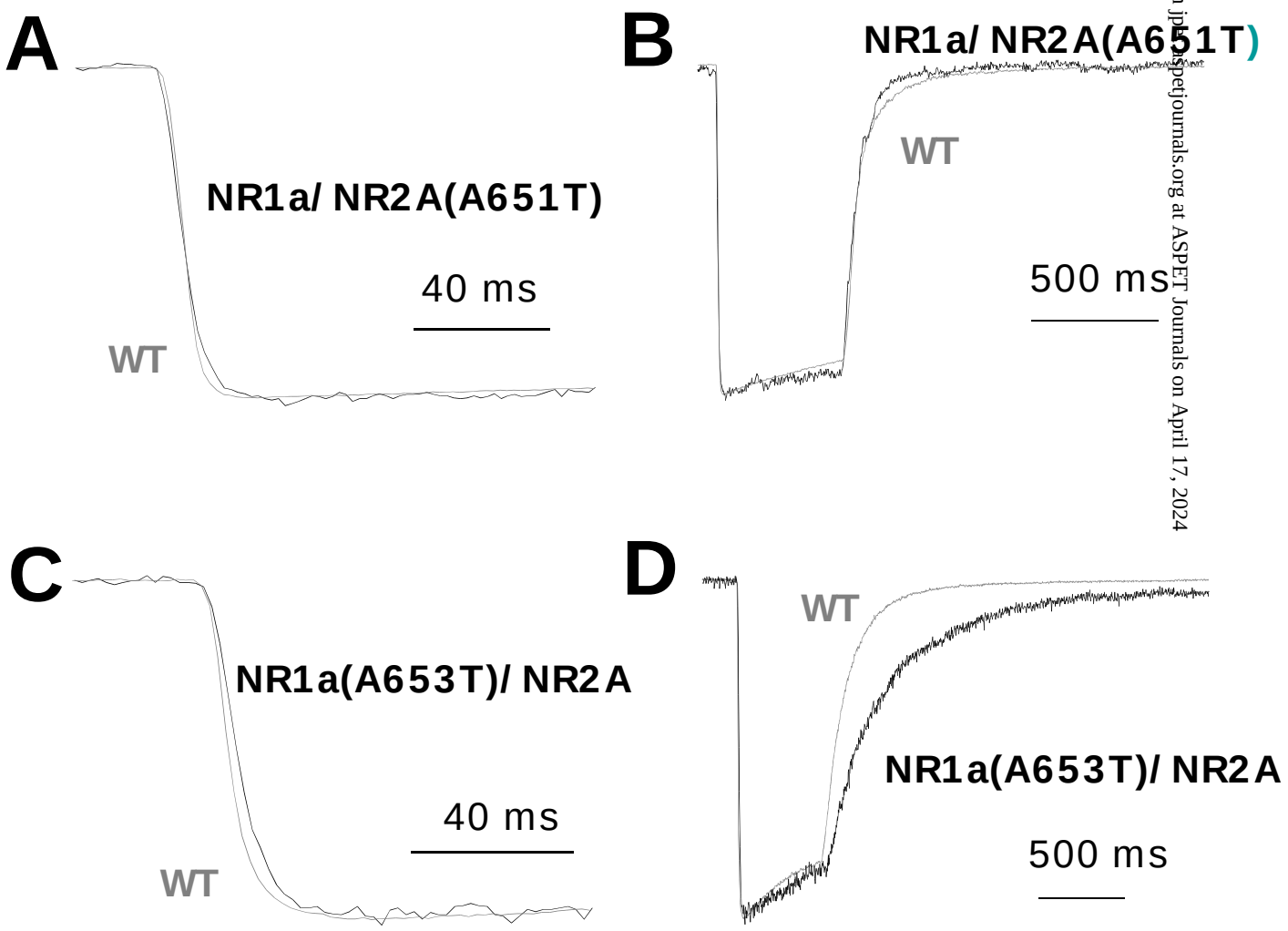


Figure 1. JPET77388



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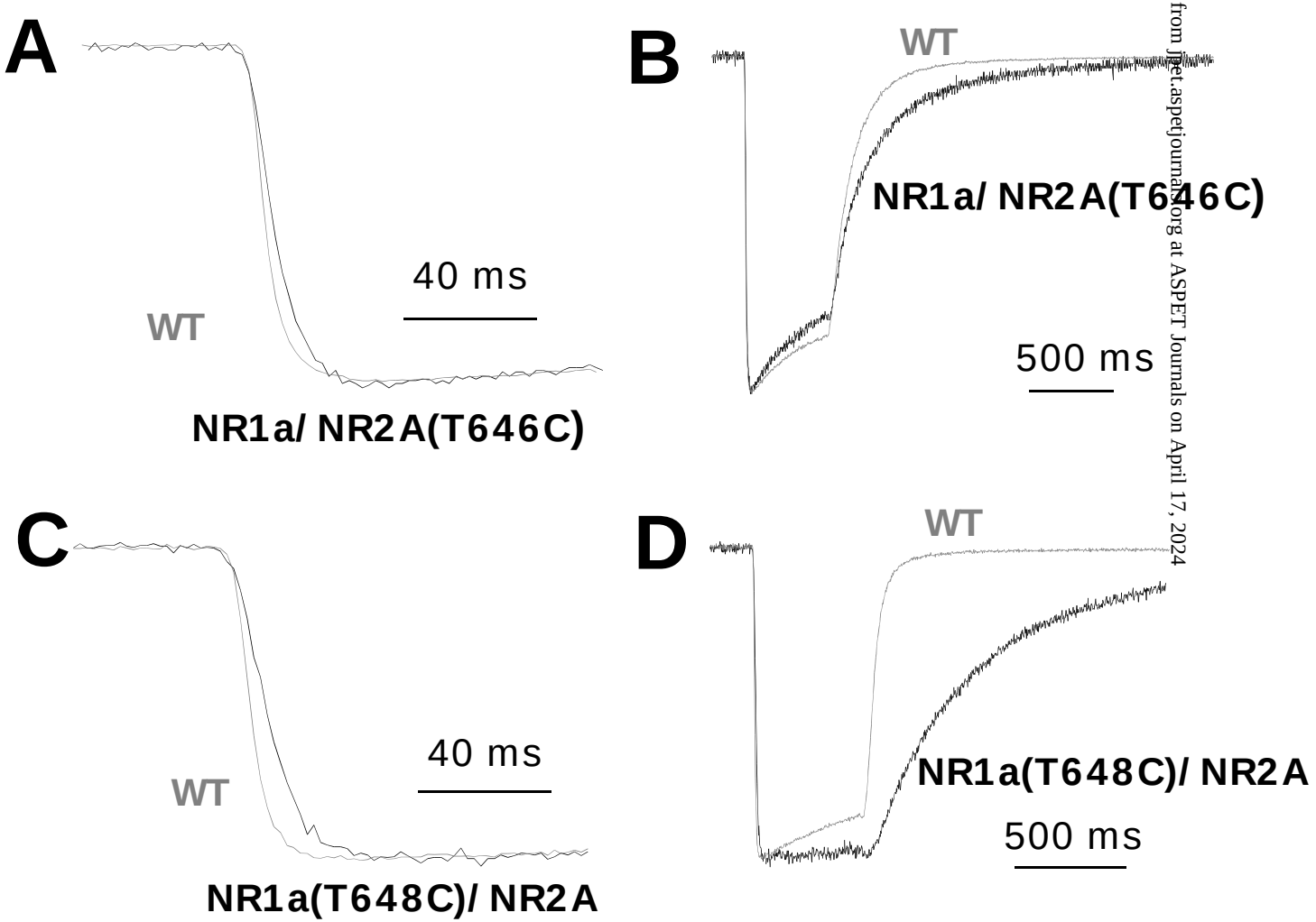

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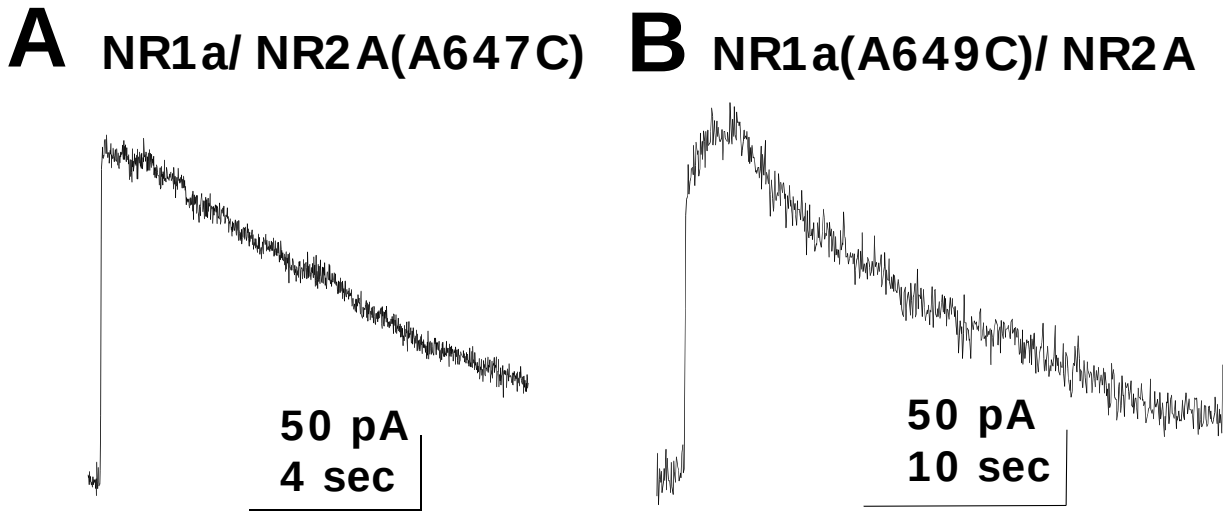

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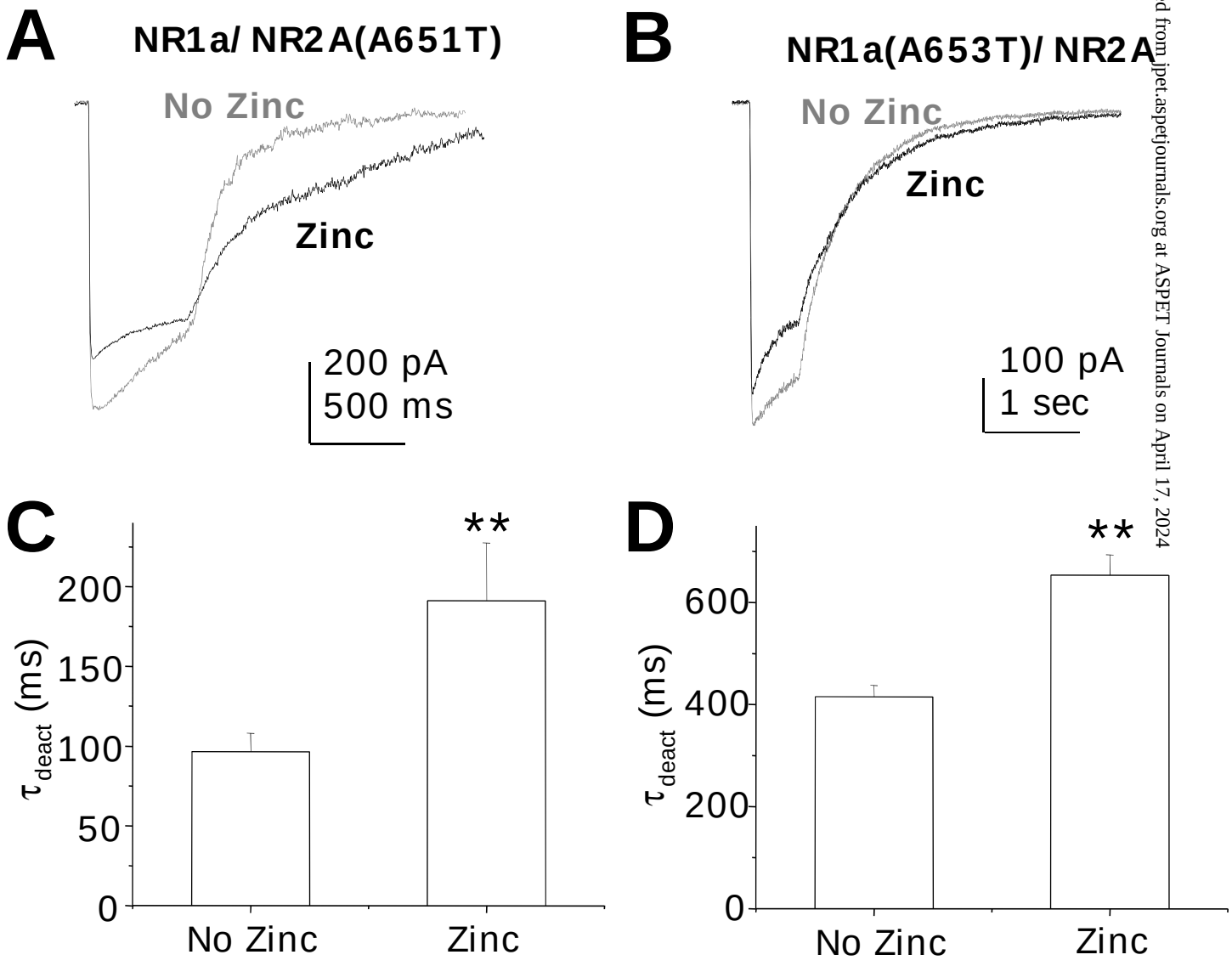

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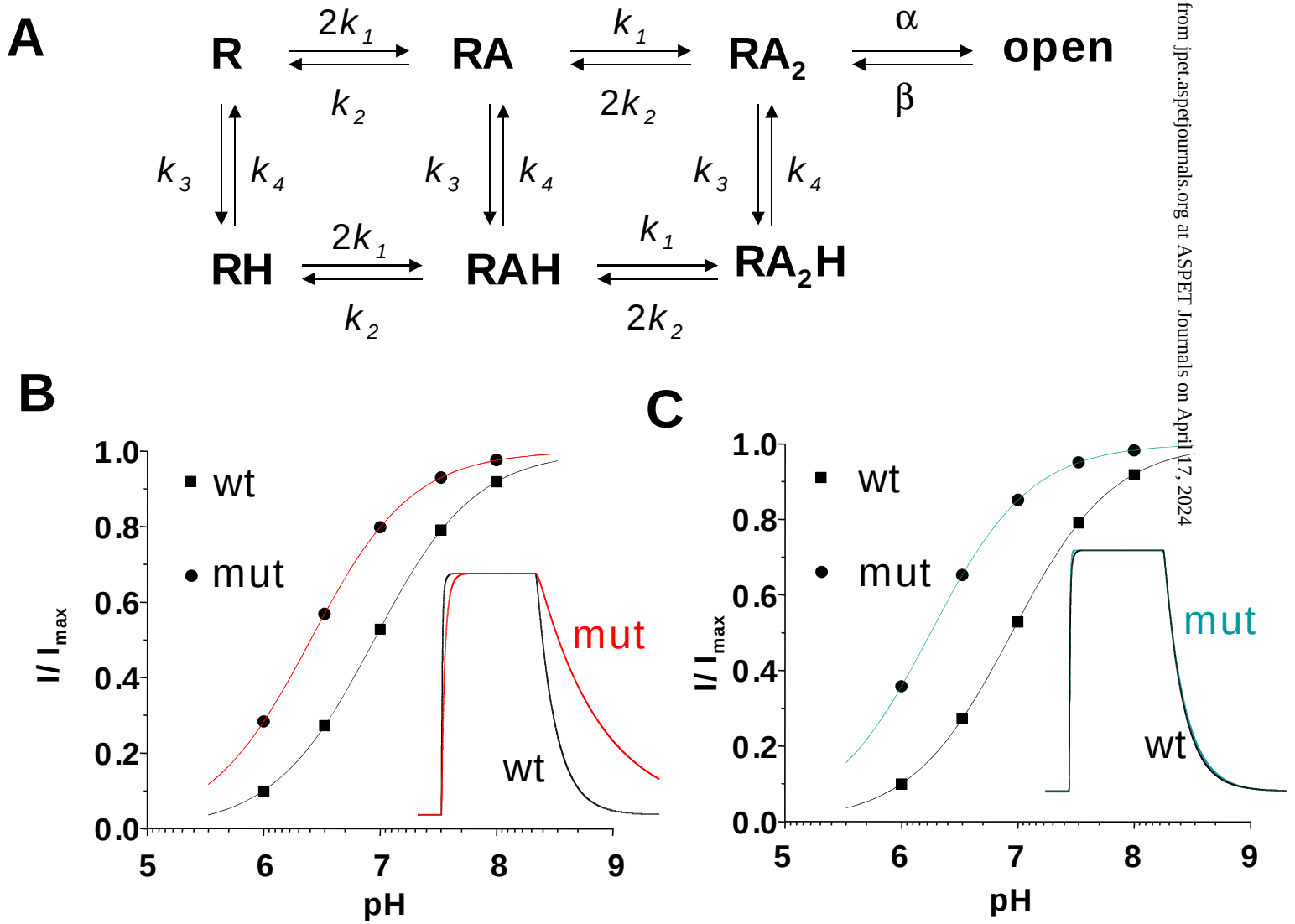

Figure 6. JPET77388