

Single Channel Analysis of Isoflurane and Ethanol Enhancement of Taurine-activated Glycine Receptors

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Abbreviations: GlyR, glycine receptor; Tau, taurine; EtOH, ethanol; Iso, isoflurane;

P_{closed}, likelihood channel adopts a closed state; P_{flipped}, likelihood channel adopts a

flipped state; P_{open}, likelihood channel adopts an open state; τ , dwell-time; amp,

amplitude; SEM, standard error of the mean; tricine, N-[2-hydroxy-1,1-

bis(hydroxymethyl)ethyl]glycine

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Abstract

The amino acid taurine is an endogenous ligand acting on glycine receptors and is released by astrocytes in many brain regions, such as the nucleus accumbens and prefrontal cortex. Taurine is a partial agonist with an efficacy significantly lower than that of glycine. Allosteric modulators such as ethanol and isoflurane produce leftward shifts of glycine concentration-response curves but have no effects at saturating glycine concentrations. In contrast, in whole-cell electrophysiology studies these modulators increase the effects of saturating taurine concentrations. A number of possible mechanisms may explain these enhancing effects, including modulator effects on conductance, channel open times, or channel closed times. We used outside-out patch clamp single channel electrophysiology to investigate the mechanism of action of 200 mM ethanol and 0.55 mM isoflurane in enhancing the effects of a saturating concentration of taurine. Neither modulator enhanced taurine-mediated conductance. Isoflurane increased the probability of channel opening. Isoflurane also increased the lifetimes of the two shortest open dwell times while both agents decreased the likelihood of occurrence of the longest-lived intracluster channel-closing events. The mechanism of enhancement of GlyR functioning by these modulators is dependent on the efficacy of the agonist activating the receptor and the concentration of agonist tested.

Introduction

Although glycine receptors (GlyR) are responsible for most inhibitory neurotransmission in the brainstem and spinal cord, these receptors are also found in higher brain regions such as the hippocampus, nucleus accumbens, and prefrontal cortex (van den Pol and Gorcs, 1988; Malosio et al., 1991; Lynch, 2004; Waldvogel et al., 2007; Baer et al., 2009; Jonsson et al., 2009, 2012; Lu and Ye, 2011). GlyRs are members of the cys-loop ligand-gated ion channel superfamily, and each receptor is comprised of five subunits that coassemble around a central anion-conducting pore. Many different compounds modulate GlyR function, including zinc, alcohols and volatile anesthetics (Lynch, 2004; Yevenes and Zeilhofer, 2011). Some of the behavioral effects of these compounds are thought to be mediated by GlyR, as would be expected given GlyR subunit expression in the brain regions important for these effects (Yamashita et al., 2001; Molander et al., 2005, 2007; Badanich et al., 2013).

Alcohols and volatile anesthetics enhance GlyR-mediated currents in a concentration-dependent manner (Mascia et al., 1996a, 1996b), producing leftward shifts of glycine concentration-response curves, with no effects on currents generated by maximally-effective concentrations of glycine (Welsh et al., 2010; Kirson et al., 2012). At the single channel level, ethanol enhancement of currents produced by submaximal glycine concentrations is due to ethanol decreasing glycine unbinding rates, with no changes in open probability (P_{open}); i.e., ethanol increases burst durations (Eggers and Berger, 2004; Welsh et al., 2009).

The sulfonic acid taurine acts as a partial agonist of the GlyR, producing ~50% of the current produced by maximally-effective concentrations of glycine (Lape et al.,

2008). Taurine is the second most abundant amino acid in the brain, and taurine release leads to GlyR activation (Dahchour et al., 1996; Mori et al., 2002; Albrecht and Schousboe, 2005; Ericson et al., 2006; Choe et al., 2012). GlyRs have been implicated in mediating the effects of multiple drugs of abuse and, interestingly, taurine-mediated GlyR activation plays a role in the rewarding effects of ethanol (Molander et al., 2005, 2007; Ericson et al., 2011; Jonsson et al., 2014). Unlike glycine-activated GlyR currents, taurine-mediated currents measured on the whole-cell level are enhanced by many allosteric modulators when tested with maximally-effective concentrations of taurine (Kirson et al., 2012, 2013). We hypothesized that these modulators would increase P_{open} on taurine-activated GlyR, which could be due to increased mean channel open times and/or decreased mean channel closed times.

Here we report on studies conducted on human $\alpha 1$ homomeric GlyR expressed in *Xenopus* oocytes, investigating various single channel parameters for changes in taurine-mediated GlyR activation by ethanol and the volatile anesthetic isoflurane. We examined channel conductance, open and closed dwell times, cluster lengths, as well as determining the probabilities of channel opening in the absence and presence of these two modulators.

Materials and Methods

Reagents

Xenopus laevis were purchased from Nasco (Ft. Atkinson, WI) and housed at 19°C on a 12 hr light:dark cycle. Animals were housed, and surgeries performed, in accordance with AAALAC regulations. All reagents were purchased from Sigma-Aldrich

(St. Louis, MO) except isoflurane, which was obtained from Anaquest (New Providence, NJ). BioUltra taurine (Sigma product # 86329) was used in all experiments.

Single channel electrophysiology recording

Oocyte isolation, GlyR subunit cDNA nuclear injection, and oocyte storage are described in Kirson et al. (2012). Outside-out patches were pulled from the animal poles of *Xenopus* oocytes expressing wildtype $\alpha 1$ homomeric GlyRs. Before recording, each oocyte was placed in a high-osmolarity stripping solution [200 mM sodium methyl sulfate, 20 mM KCl, 10 mM HEPES, and 1 mM $MgCl_2$] to aid in manual removal of the vitelline membrane using forceps. Patch pipettes were pulled from thick-walled borosilicate glass (WPI, Sarasota, FL) using a P-97 Flaming/Brown Micropipette Puller (Sutter Instruments, Novato, CA). Pipettes were fire-polished with an MF-830 Microforge (Narishige, Tokyo, Japan) to obtain a smooth tip and those with resistances of 5 to 15 M Ω were used in recordings. Outside-out patches were held at -80 mV, and recordings were made according to standard methods (Hamill et al., 1981) using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Data were digitized at 100 kHz with a Digidata 1322A (Molecular Devices), low-pass filtered at 10 kHz, and recorded on a PC hard drive using pClamp 9 software (Molecular Devices). Taurine and drug solutions were prepared in external solution [100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 10 mM HEPES, 2 mM $CaCl_2$ at pH 7.4] + 2.5 mM tricine, to chelate free zinc, before being perfused over outside-out patches using an SF-77B Perfusion Fast Step apparatus (Warner Instruments, Hamden, CT). The pipette internal solution contained 102 mM CsCl, 10 mM HEPES, 2 mM $MgCl_2 \cdot 6H_2O$, 10 mM EGTA, and 1 mM $CaCl_2$ at pH 7.2.

Data analysis

Data were analyzed using the single channel analysis programs in QuB (Qin et al., 2000a, 2000b); version 2.0.0.9 was used for preprocessing, open/closed dwell time analysis and chopping of data into clusters. Tracings were first baseline-corrected to correct for drift and portions of the tracings that showed multiple channels activating simultaneously were excised. Portions of tracings clearly representing closed or open channel states were selected by eye and fit to Gaussian curves using the Amps modeling subroutine in QuB, to determine currents representing closed and open states in each patch. The conductance in each patch was determined by subtracting the mean closed from the mean open current and then dividing that number by the holding voltage (-80 mV). Channel opening and closing events were idealized using the segmental-k-means algorithm (Qin et al., 2000a, 2000b). Data were initially idealized with a simple two-state closed to open ($C \leftrightarrow O$) model. Multiple closed and open states were then added sequentially to form a star model (closed state as the center) using the maximum interval likelihood (MIL) fitting method, after imposing a deadtime resolution of 30 μ s. When this model was complete, as determined by lack of statistically-significant increases in log likelihood seen after adding additional closed and open states, the data were re-idealized and used for open and closed channel dwell time analyses. Durations of opening and closing events were sorted into 50 bins each, based on individual channel opening or closing durations. This allowed for the creation of dwell time distributions, constructed using log-time on the abscissa and the square-root of the number of events per bin divided by total events on the ordinate. These data were then fit with a mixture of exponentials components using the MIL function in QuB.

Clusters were defined as being separated by closed-time durations equal to or longer than the longest τ_{crit} value determined in each patch. Four closed time distributions were fit to the data. The longest and rarest (~0.12% prevalence) closed time was the most variable among patches in terms of lifetime when taurine was applied alone, with ethanol or with isoflurane and was consequently considered as occurring between clusters. The τ_{crit} values determined between the third- and fourth-longest closed states in each patch were used to chop data into individual clusters; these τ_{crit} values ranged from 6.2 ± 1.2 ms to 9.2 ± 1.1 ms among the three treatment groups. Once idealized, data were chopped into clusters, and the clusters were saved in a separate file for analysis using a kinetic model involving agonist-bound closed, flipped and open states (Lape et al., 2008).

Statistical analyses were performed using SigmaPlot version 11.0 (Systat Software, San Jose, CA) utilizing one-way ANOVAs and Bonferroni t-test post hoc comparisons.

Results

Ethanol and isoflurane effects on taurine-activated GlyR single channel conductance.

Single channel recording experiments were conducted on outside-out patches pulled from *Xenopus* oocytes expressing the human homomeric $\alpha 1$ GlyR. Patches were exposed to 200 mM ethanol or 0.55 mM isoflurane co-applied with 100 mM taurine, and data obtained compared to patches exposed to 100 mM taurine alone. This saturating concentration of taurine was chosen to maximize channel opening (Lape et al., 2008). Taurine applied alone, or in combination with one of the modulators, produced clearly-defined clusters of channel activity as shown in Fig. 1. There was a

significant effect of experimental condition on conductance [$F(2,18) = 5.24$, $p < 0.02$], with the conductance seen in the presence of ethanol slightly lower than that of the taurine-alone control (Fig. 2). Isoflurane had no effect on conductance.

Isoflurane increases taurine-activated GlyR mean open time.

When a saturating concentration of taurine was applied alone, GlyR exhibited a mean open time of 0.63 ± 0.04 ms ($n = 8$). The mean open time was determined by summing the products of the three open dwell times and likelihoods in each patch and was significantly increased by isoflurane but not ethanol [$F(2,18) = 8.45$, $p < 0.003$] (Fig. 3A). The closed mean time was calculated by summing the products of the three shortest closed dwell times and likelihoods in each patch. Both ethanol and isoflurane appeared to decrease the mean closed time, but not significantly (Fig. 3B).

Isoflurane increases the lifetimes of the first two open times.

Open dwell-time data were adequately described using three exponential components for all three experimental conditions, since the addition of extra open states did not significantly improve fits of data. In two of eight taurine control patches, as well as three of five taurine + isoflurane patches, the longest open time component (τ_3) could not be fit. Sample open dwell-time histograms from individual patches are shown in Fig. 4A for all three experimental conditions. The τ values correspond to the times at which peaks were observed for the three exponential functions fit in Fig. 4A. One-way ANOVAs revealed that isoflurane, but not ethanol, significantly increased the lifetimes of the briefest [$F(2,18) = 5.6$, $p < 0.015$] as well as the next shortest [$F(2,18) = 9.66$, $p < 0.002$] open times (τ_1 and τ_2 in Fig. 4B). Ethanol and isoflurane were also tested for their effects on the likelihoods of opening (amps) to these dwell times (Fig. 4C); this

refers to the areas under the curves of the three dwell times, as shown in the sample histograms in Fig. 4A. No effects of ethanol or isoflurane were seen on amps.

Ethanol and isoflurane have no effects on channel intracluster closed dwell-time components but do affect closed likelihoods.

Intracluster closed channel dwell-times were fit by three exponential components (τ s) for all three experimental conditions tested. A fourth, much longer and rare, closed time which varied considerably in time between patches, was considered to represent closings seen between clusters. Sample closed dwell-time histograms for all conditions can be seen in Fig. 5A. There were no significant effects of ethanol or isoflurane on the durations of τ_1 , τ_2 or τ_3 (Fig. 5B). Neither ethanol nor isoflurane affected the likelihoods of occurrence of the two shorter closed dwell-time components (Fig. 5C); however, both agents decreased the likelihood of observing the longest-lived intracluster dwell-time [$F(2,18) = 8.23$, $p < 0.003$] (Fig 5C).

Mechanistic fits to intra-cluster data

Lape et al. (2008) proposed that partial agonism at glycine and nicotinic acetylcholine receptors was not due to a decreased efficacy (E) of partial agonists compared to higher efficacy agonists, as had long been thought. E is defined as the rates of transition from closed to open states (β) divided by transition rates from open to closed (α). They showed that the E values of glycine and taurine were quite similar. Instead agonists varied in their abilities to transition the receptor from an agonist-bound closed state to another pre-open closed state they termed flipped (Lape et al., 2008). The transition rate from closed to flipped states (δ) divided by the converse (γ) was defined as F, and this differed markedly between glycine and taurine. We reasoned

that, at a concentration of 100 mM taurine, receptors would be found in the triply-liganded state within clusters and that the intracluster behaviour could be modeled as transitions among closed (A_3R), flipped (A_3F) and open (A_3R^*) states. Fig. 6A shows the transition rates calculated in the absence and presence of allosteric modulators using the kinetic model depicted. Isoflurane, but not ethanol, increased the δ [$F(2,18) = 4.62$, $p < 0.03$] and β [$F(2,18) = 9.1$, $p < 0.002$] transition rates, decreased α [$F(2,18) = 8.42$, $p < 0.003$] and had no effect on γ (Fig. 6A) compared to taurine alone. This led to isoflurane-induced increases in both F [$F(2,18) = 5.62$, $p < 0.014$] (Fig. 6B) and E [$F(2,18) = 10.42$, $p < 0.001$] (Fig. 6C). In a mechanistic model of channel activation involving a second closed state (flipped), channel opening involves transitions between closed and flipped, as well as flipped and open states, as shown in Fig. 6A. Thus the traditional method of defining efficacy as β/α is inadequate at GlyR since the δ and γ transition rates between closed and flipped states, yielding $F = \delta/\gamma$, must also be taken into account. Lape et al. (2008) defined effective efficacy (E_{eff}) to combine F and E such that $E_{\text{eff}} = EF/(1 + F)$. This E_{eff} value was more than doubled by isoflurane but not affected significantly by ethanol [$F(2,18) = 14.81$, $p < 0.001$] (Fig. 6D). The likelihoods of channels being found in the closed, flipped or open states were also calculated from the transition rates. Isoflurane increased P_{open} [$F(2,18) = 8.89$, $p < 0.003$] (Fig. 7A), decreased P_{closed} [$F(2,18) = 6.56$, $p < 0.008$] (Fig. 7B) but had no effect on P_{flipped} (Fig. 7C). The average open (Fig. 7D), closed (Fig. 7E) and flipped (Fig. 7F) state lifetimes were also determined. Ethanol had no effect on any state lifetime but isoflurane did increase the open state lifetime [$F(2,18) = 10.25$, $p < 0.001$].

Discussion

In the present report we examined the effects of anesthetically-relevant concentrations of ethanol and isoflurane for their effects on taurine-activated GlyR. The concentration of isoflurane used (0.55 mM) is slightly less than twice the EC₅₀ of isoflurane in mammals (0.32 mM) and would be expected to anesthetize essentially all subjects (Franks and Lieb, 1994). A concentration of 200 mM ethanol is equivalent to a blood alcohol level of 920 mg%, which would be expected to be either anesthetizing or lethal depending on a subject's level of functional tolerance. We chose these concentrations because larger modulator effects would make it easier to detect more subtle drug responses.

We previously showed that these allosteric modulators studied on the whole-cell level enhance the effects of saturating concentrations of taurine, but not glycine, on $\alpha 1$ GlyR function (Kirson et al., 2012, 2013). However, in these studies we were not able to distinguish among the possible mechanisms of action of these compounds. One, admittedly unlikely, possibility is that modulators could enhance taurine-mediated but not glycine-mediated GlyR conductance, explaining why they enhance the effects of saturating concentrations of the former but not the latter. The data shown in Fig. 2 disprove that hypothesis; if anything ethanol slightly decreased conductance. Another possibility is that modulators could affect taurine affinity, but this would not be expected to be the case when saturating concentrations of taurine are used. Under saturating agonist conditions, the unbinding of an agonist molecule would almost immediately be followed by another agonist binding event, preventing modulators from exerting their effects via changes in agonist affinity. A third possible explanation is that modulators

increase the channel P_{open} , after activation by taurine but not glycine, since the latter produces a P_{open} near 0.95 at saturating concentrations. This increase in taurine's P_{open} could be accomplished in four different ways, by: (1) increasing channel open lifetimes (τ_s); (2) decreasing channel closed lifetimes; (3) increasing the likelihoods (amps) of longer-lived open states or; (4) decreasing the likelihoods of longer-lived closed states.

In the present report we show that significant increases in the two shorter time constants of opening events were found after application of isoflurane (Fig 4B) but not ethanol. In addition, both ethanol and isoflurane decreased the likelihood of occurrence of the longest-lived closed state (amp3 in Fig. 5C). Both of these findings would be consistent with an increase in P_{open} in the presence of a modulator, so these phenomena both likely contribute to the overall increase in P_{open} that we observed in the presence of isoflurane (Fig. 7A).

After chopping our data into clusters we fit the intra-cluster opening and closing events to a mechanistic model equivalent to the fully-liganded flipped model described by Lape et al. (2008), in which three taurine molecules are bound; we believe this to be a reasonable model since the receptor was exposed to a saturating concentration of 100 mM taurine. The rate constants we determined for α , β , γ and δ (Fig. 6A) were generally quite similar to those found for taurine by Lape et al. (2008). Their values for the GlyR that had bound three taurine molecules were 14,500 (α), 133,000 (β), 5,170 (γ) and 740 (δ); only in δ did our numbers differ markedly. In addition we obtained similar numbers when calculating F (0.29 vs. 0.15) and E (8.8 vs. 9.2) values. The calculated values for P_{open} , P_{closed} , P_{flipped} , and their respective lifetimes, were also generally close to those determined by Lape et al. (2008). Ethanol and isoflurane were then tested for

their effects on these different single channel properties. Isoflurane had several effects that would all tend to increase GlyR function: an increased open channel lifetime (Fig. 7D), which is a reflection of decreased α (Fig. 6A), and a decreased likelihood that the channel would be found in the closed state (Fig. 7B), since isoflurane increased δ (Fig. 6A). These effects would be expected to contribute to the increase seen in channel P_{open} (Fig. 7A). Isoflurane enhanced transitions from closed to the flipped as well as open states (Figs. 7B & 7C) and both of these contributed to the increase in the effective efficacy of taurine (Fig. 7D). Although ethanol at times trended towards having an effect, particularly on closed times, it generally was without effect. In previous whole-cell studies (Kirson et al., 2012, 2013) we found that isoflurane and other volatile anesthetics produce greater enhancement of the effects of maximally-effective concentrations of taurine than does 200 mM ethanol and this likely explains why we saw more of an effect with isoflurane on the single channel level. In several instances ethanol appeared to be having effects similar to those of isoflurane (egs. Figs. 3B & 6B), but not great enough to be statistically different from the taurine alone control.

In our previous single channel investigations into the effects of ethanol on the glycine-activated GlyR, only subsaturating glycine concentrations were examined and no significant changes in P_{open} or open times were seen, although there was a trend towards an increase in mean open time in the presence of 200 mM EtOH. Under those conditions ethanol instead exerted its potentiating effects by decreasing the rate of glycine unbinding (Eggers and Berger, 2004; Welsh et al., 2009). Thus, at the single channel level, ethanol and isoflurane enhancement of GlyR functioning is dependent on which agonist activates the receptor and at what concentration that agonist is tested.

In summary, we investigated at the single channel level the effects of ethanol and isoflurane on saturating concentrations of taurine applied to the GlyR in isolated outside-out patches. Isoflurane increased the average lifetimes of the two shortest open dwell-times while both compounds decreased the likelihood that channels will adopt the longest-lived intra-cluster channel closed state. These effects seen with saturating concentrations of taurine are not seen when the modulators are coapplied with a saturating concentration of the more efficacious agonist glycine. As evidence accumulates for the physiological role of the taurine-activated GlyR, it is important to note that the nature of allosteric modulation of these receptors can differ depending on the agonist activating the receptors.

Authorship Contributions

Participated in research design: Kirson, Todorovic and Mihic

Conducted experiments: Kirson and Todorovic

Performed data analysis: Kirson, Todorovic and Mihic

Wrote or contributed to the writing of the manuscript: Kirson, Todorovic and Mihic

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Footnotes

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The first two authors contributed equally to this research.

Figure Legends

Figure 1. Sample tracings of taurine-activated GlyR alone and with co-applied ethanol or isoflurane. Representative tracings show ~2-3 s of single channel homomeric $\alpha 1$ GlyR responses to 100 mM taurine (A), 100 mM taurine + 200 mM EtOH (B), or 100 mM taurine + 0.55 mM isoflurane (C). The horizontal red line above a portion of each top trace denotes the ~150 ms expanded view seen in the trace below.

Figure 2. Ethanol and isoflurane effects on taurine-activated GlyR conductance.

The conductance (pS) is graphed for each experimental condition. Taurine-activated GlyR conductance was 88.7 ± 1.4 pS ($n = 8$ patches; 368,347 total events) as seen in the white bar. Ethanol (grey bar) slightly decreased conductance to 81.1 ± 1.9 pS ($n = 6$ patches; 1,075,247 total events) while conductance in the presence of isoflurane was 87.5 ± 2.4 pS respectively ($n = 5$ patches; 222,663 total events). Data are shown as mean + SEM of 5–8 patches. *, $p < 0.05$.

Figure 3. Isoflurane significantly increases mean open time. A) Summary graph showing the effects of ethanol (grey bar) and isoflurane (black bar) on mean open times of the taurine-activated GlyR. Only isoflurane significantly increased mean open time of channels. B) Summary graph showing the effects of ethanol (grey bar) and isoflurane (black bar) on mean closed times of the taurine-activated GlyR. Neither isoflurane nor ethanol significantly decreased mean closed time of channels. Data are shown as mean + SEM of 5–8 patches. *, $p < 0.05$.

Figure 4. Ethanol and isoflurane effects on lifetimes and likelihoods of channel opening events. A) Representative histograms demonstrating the fits for the open dwell-times. Each condition was fit using three open time components (τ_s). In each graph, the solid lines describe the individual dwell-time exponential functions and the dashed line is a fit of all the data. The vertical lines denote the 1 ms time point of open times to better illustrate differences of fit among experimental groups. B) Only isoflurane significantly increased the average durations of the two shortest open dwell-times. C) Neither ethanol nor isoflurane affected the likelihoods of observing specific open times. Data are shown as mean + SEM of 2–6 patches; the longest open times could be fit in 6 of 8 taurine-alone and 2 of 5 taurine + isoflurane patches. *, $p < 0.05$.

Figure 5. Ethanol and isoflurane effects on lifetimes and likelihoods of channel closing events. A) Representative histograms demonstrating the fits for the closed dwell-times. Each condition was fit using three closed time components (τ_s). In each graph, the solid lines describe the individual dwell-time exponential functions and the dashed line is a fit of all the data. The vertical lines denote the 1 ms time point of open times to better allow for comparisons among experimental groups. B) Neither modulator affected average durations of closed dwell-times. C) Ethanol and isoflurane both decreased the likelihood of occurrence of the longest-lived closed time component. Data are shown as mean + SEM of 5–8 patches.

Figure 6. Application of the flipped kinetic model to ethanol and isoflurane effects on taurine-activated GlyR. (A) Clusters of single channel recordings were isolated and fit

to a kinetic scheme involving two closed states (A_3R and A_3F) as well as a single open state (A_3R^*). The numbers over and under arrows represent the mean \pm S.E.M. of rate constants describing transitions among states. The top scheme represents 100 mM taurine alone and the Greek symbols identify the relevant transition rates. (B) The F ratio is a unitless number describing transitions between closed and flipped states ($F = \delta/\gamma$) and was significantly increased by isoflurane. F values were determined for each patch and averaged. (C) The E ratio is also unitless and describes transitions between flipped and open states ($E = \beta/\alpha$), and was significantly increased by isoflurane. E values were determined for each patch and averaged. (D) The effective efficacy (E_{eff}) is a function of E and F as described in Results and was significantly enhanced by isoflurane but not ethanol. Data are shown as mean + SEM of 5-8 patches. *, $p < 0.05$.

Figure 7. Ethanol and isoflurane effects on open and closed likelihoods and lifetimes determined from the flipped kinetic model. (A-C) The probabilities of channels being found in open (A), closed (B) and flipped (C) states were determined in the absence and presence of allosteric modulators. In each patch P_{open} was determined as $(\beta/\alpha * \delta/\gamma) / (1 + \beta/\alpha + \beta/\alpha * \delta/\gamma) * 100$, while P_{closed} was defined as $(\alpha/\beta * \gamma/\delta) / (1 + \alpha/\beta + \alpha/\beta * \gamma/\delta) * 100$ and $P_{\text{flipped}} = 100 - P_{\text{open}} - P_{\text{closed}}$. Isoflurane enhanced P_{open} and decreased P_{closed} . No effects on P_{flipped} were noted. (D-F) The lifetimes of open, closed and flipped states were determined in the absence and presence of allosteric modulators. In each patch the open lifetime (D) was determined as $1/\alpha$, the closed lifetime (E) as $1/\delta$ while the flipped lifetime (F) was $1 / (\beta + \gamma)$. Isoflurane increased the open lifetime compared to

the taurine alone condition. Data are shown as mean + SEM of 5-8 patches. *, $p < 0.05$.

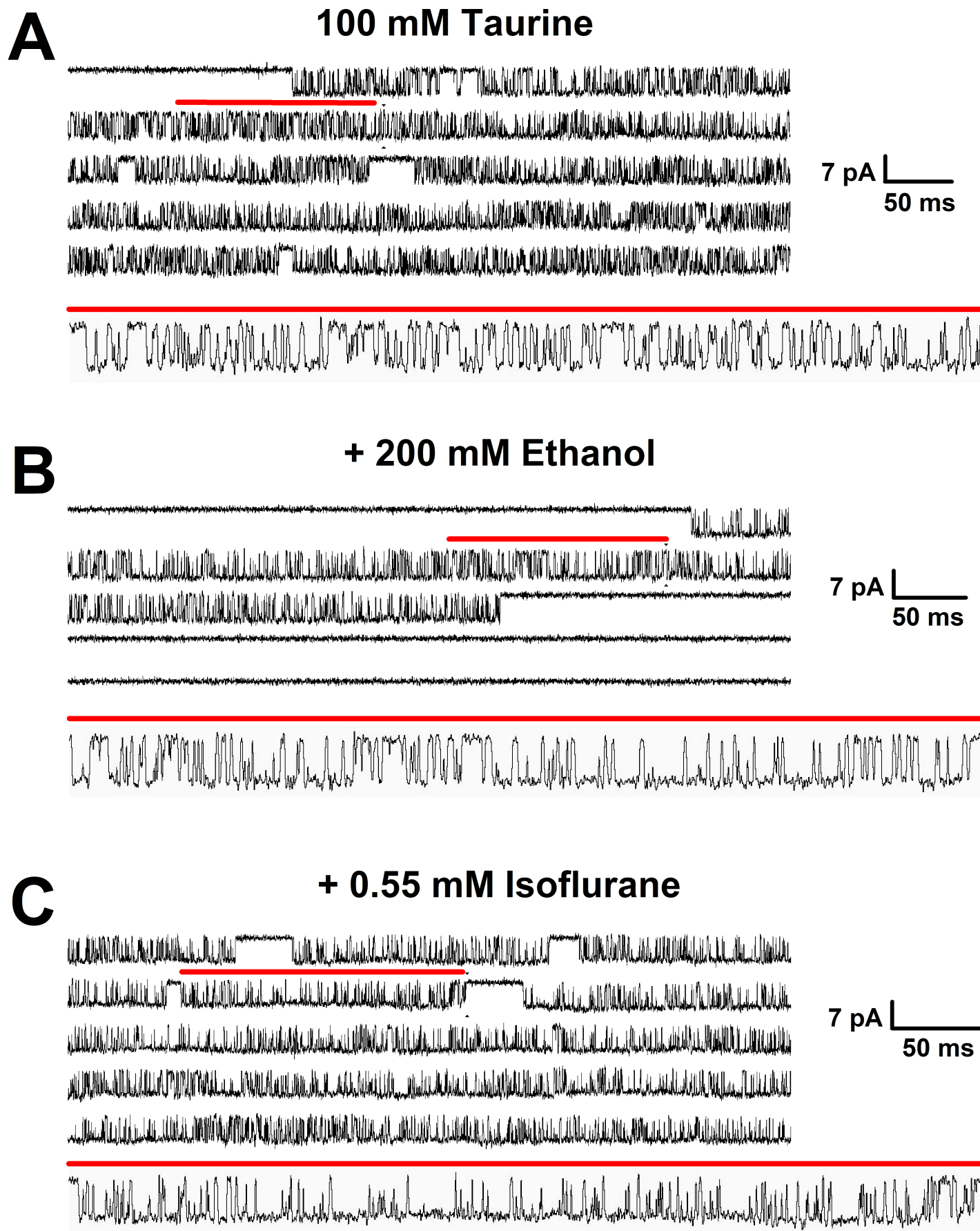
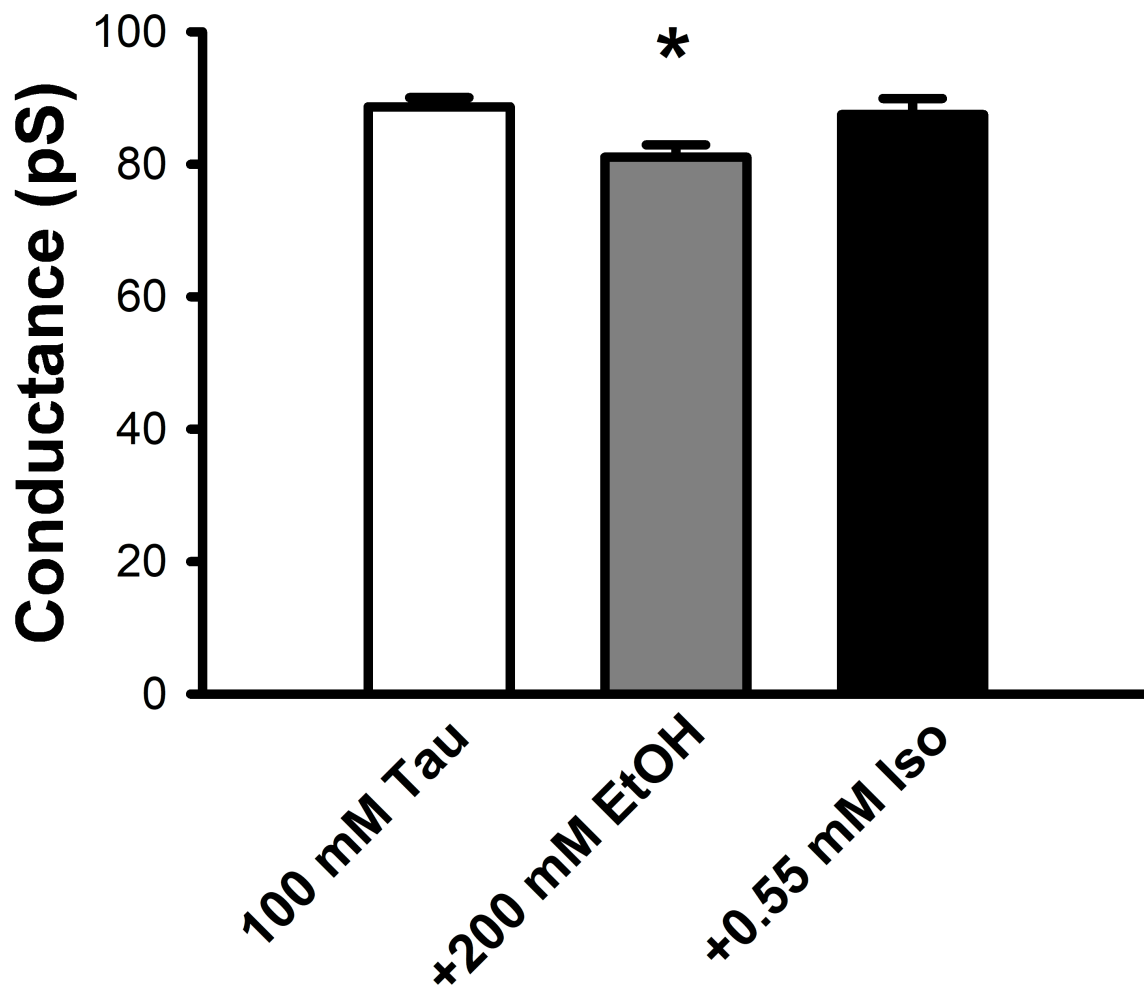


Figure 2



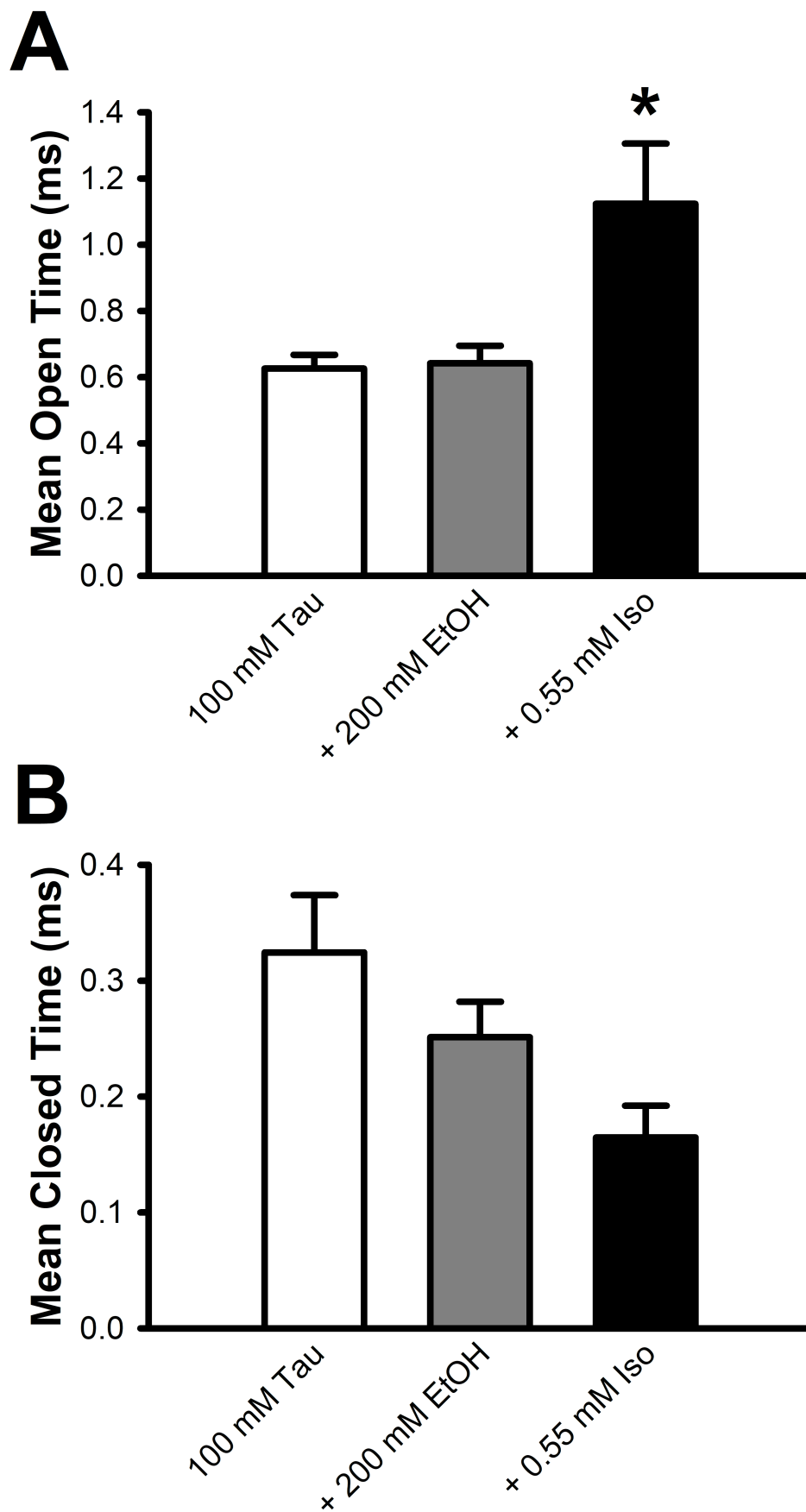


Figure 4

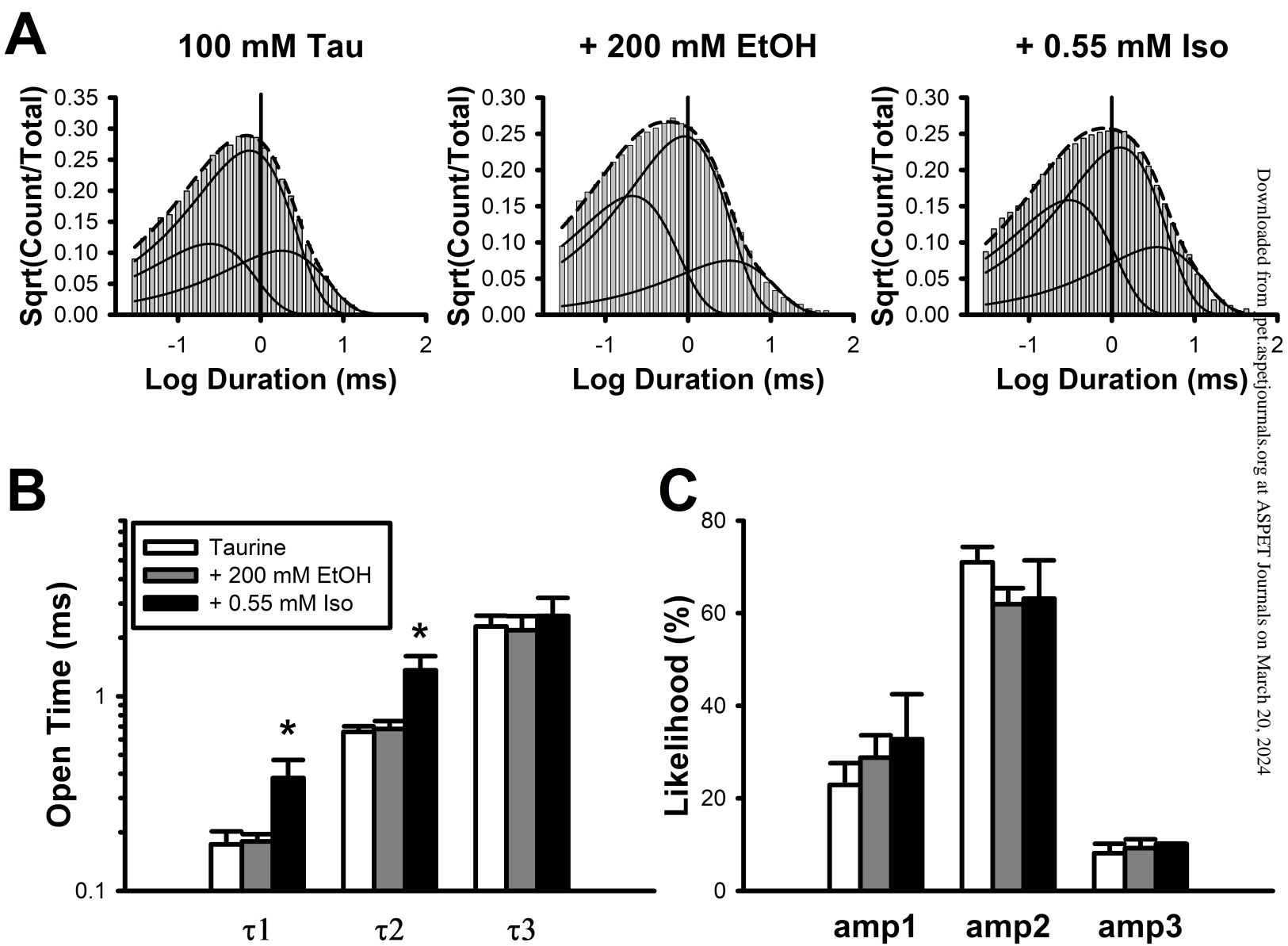


Figure 5

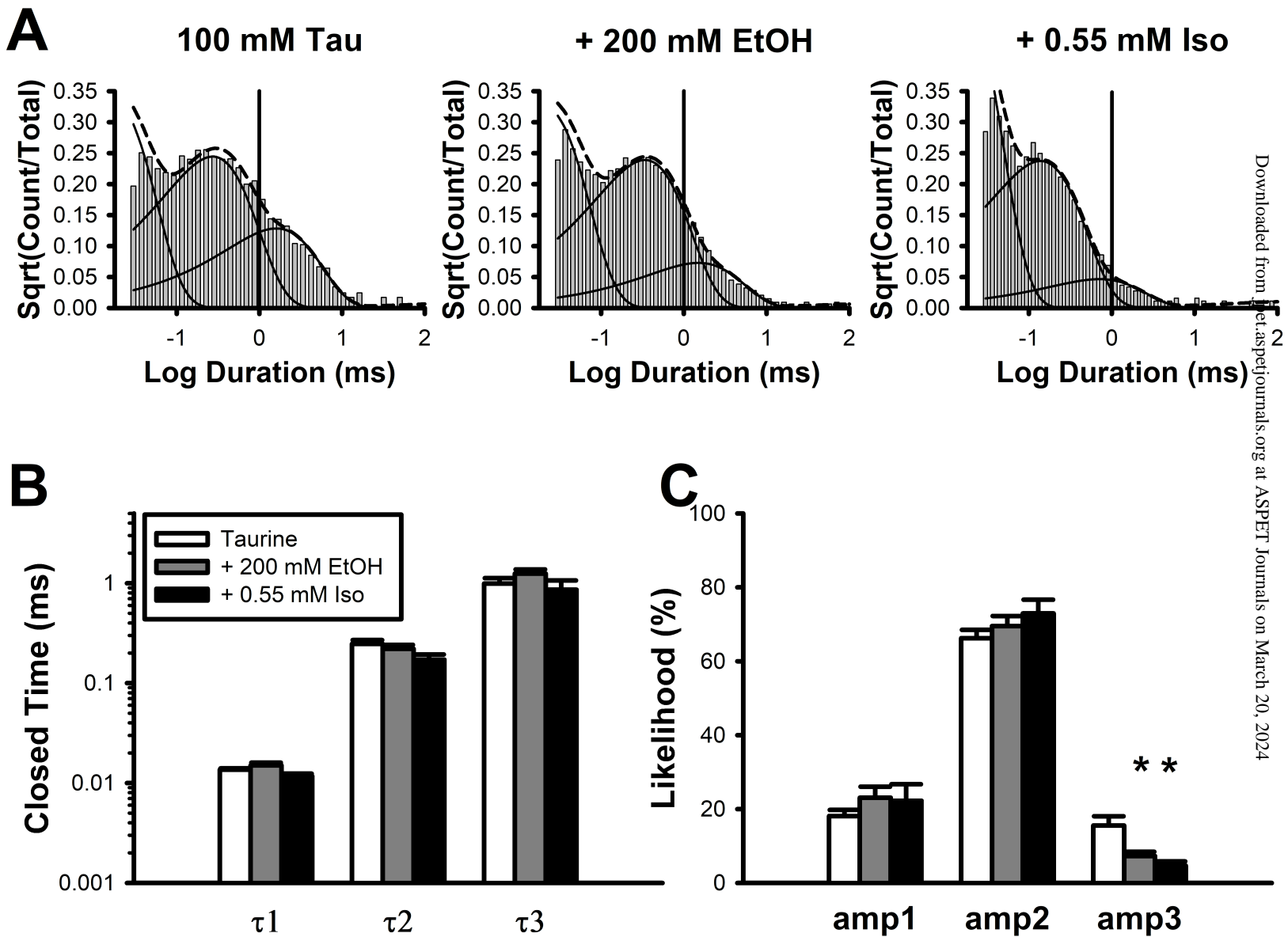


Figure 6

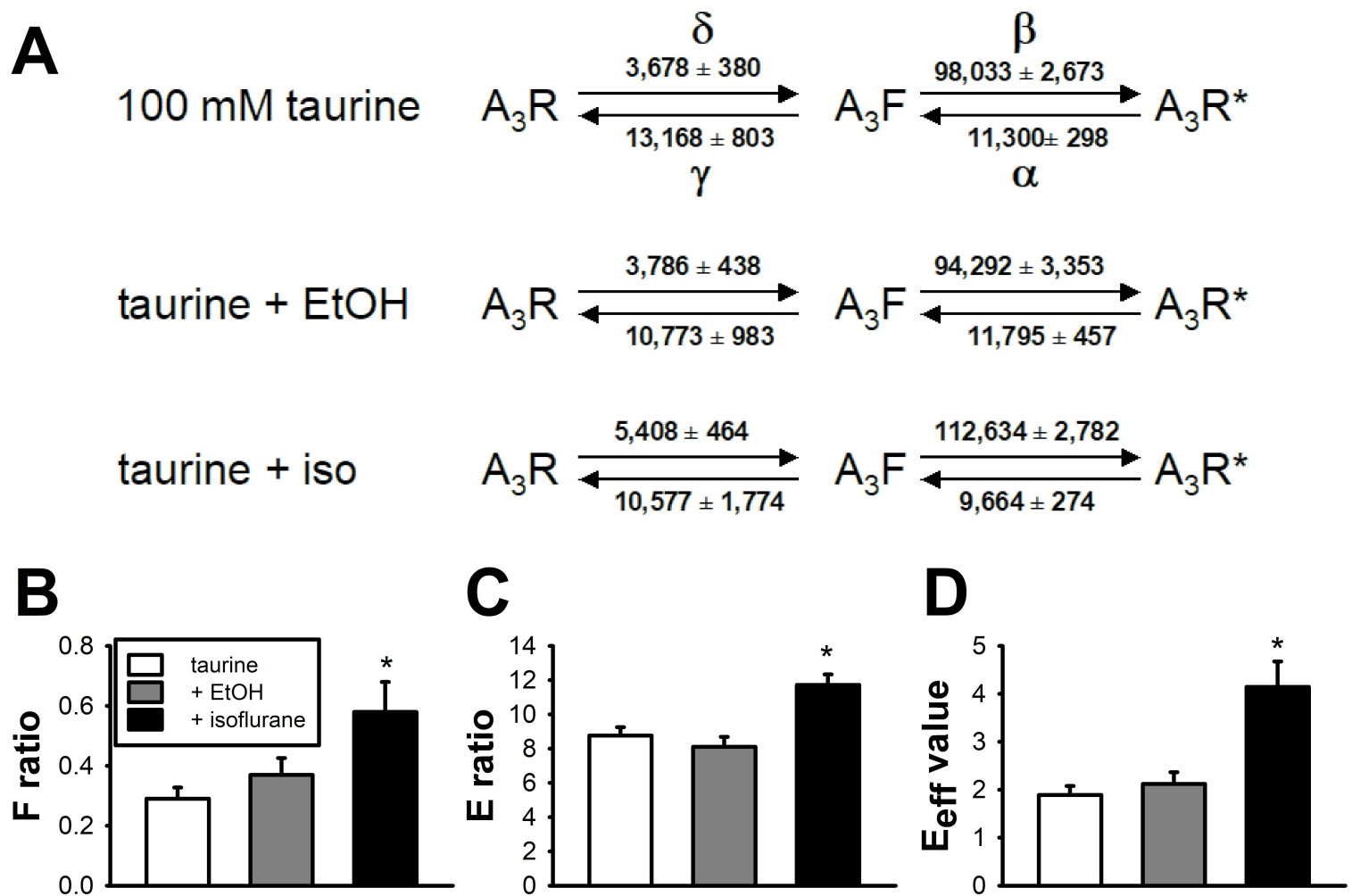


Figure 7

