Supplementary Information

Table S1
Summary of results for agonist-activated ΔI and ΔF responses at unlabeled and MTSR-labeled α1-R19'C and α3-R19'C GlyRs. Electrophysiological and fluorescence data are shown in normal and bold type, respectively.

Agonist	Construct	EC_{50} (μM)	n_H	I_{max} (μA)	ΔF_{max} (%)	$\Delta F_{\text{half decay time}}/\Delta I_{\text{half decay time}}$	n
glycine	α3-WT unlabeled	74 ± 2	2.9 ± 0.2	5.4 ± 0.6	-	-	6
	α3-WT labeled	71 ± 2	2.9 ± 0.2	5.6 ± 0.5	-	-	6
	α3-WT labeled ΔF	**-**	**-**	**-**	**0**		**6**
	α3-19'C unlabeled	12700 ± 600a	1.4 ± 0.1a	1.7 ± 0.1a	-	-	7
	α3-19'C labeled	1740 ± 270ab	1.2 ± 0.1a	3.1 ± 0.3a	-	5.1 ± 1.0	13
	α3-19'C labeled ΔF	**1340 ± 210**	**1.1 ± 0.1**	**-**	**5.4 ± 0.5**		**13**
	α1-WT unlabeled	17.9 ± 1.5a	2.8 ± 0.1	4.8 ± 0.4	-	-	4
	α1-WT labeled	17.1 ± 0.6a	2.8 ± 0.2	4.9 ± 0.7	-	-	4
	α1-WT labeled ΔF	**-**	**-**	**-**	**0**		**4**
	α1-19'C unlabeled	4320 ± 220a	1.3 ± 0.1a	2.3 ± 0.4a	-	-	5
	α1-19'C labeled	499 ± 79ab	1.2 ± 0.2a	3.6 ± 0.6a	NA	1.0 ± 0.1	6
	α1-19'C labeled ΔF	**1110 ± 100c**	**1.0 ± 0.1**	**-**	**23 ± 4**		**6**
β-alanine	α3-19'C labeled	2610 ± 940a	1.5 ± 0.3a	0.4 ± 0.05a	NA	4.7 ± 0.7	5
	α3-19'C labeled ΔF	**22100 ± 2300c**	**1.1 ± 0.1**	**-**	**2.6 ± 0.5**		**5**
taurine	α3-19'C labeled	-	-	0.03 ± 0.01a	-	-	5
	α3-19'C labeled ΔF	**5990 ± 780**	**1.3 ± 0.4**	**-**	**1.3 ± 0.2**		**5**

a - significant difference to electrophysiological properties of glycine-activated unlabeled α3-WT GlyRs (Student's t-test, $p < 0.05$)
b - significant difference to electrophysiological properties before labeling in the same mutant GlyR (Student's t-test, $p < 0.05$)
c - significant difference of fluorescence properties to electrophysiological properties after labeling in the same mutant GlyR (Student's t-test, $p < 0.05$)
α1-WT data are reproduced from (36)

Table S2
Summary of results for glycine-activated ΔI and ΔF responses at chimeric and double mutant GlyRs incorporating MTSR labels at R19'C. Electrophysiological and fluorescence data are shown in normal and bold type, respectively.

Construct	EC_{50} (μM)	n_H	I_{max} (μA)	ΔF_{max} (%)	$\Delta F_{\text{half decay time}}/\Delta I_{\text{half decay time}}$	n
Chi1 unlabeled	2880 ± 390	3.2 ± 0.2	1.6 ± 0.5	-	-	5
Chi1 labeled	860 ± 490	1.1 ± 0.1	2.4 ± 0.5	-	3.2 ± 0.3b	5
Chi1 ΔF	**1450 ± 310**	**1.3 ± 0.2**	**-**	**2.3 ± 0.2b**		**5**
Chi2 unlabeled	2330 ±520	2.7 ± 0.1	3.2 ± 0.4	-	-	5
Chi2 labeled	530 ± 160	1.1 ± 0.01	2.6 ± 0.4	-	4.2 ± 0.8b	6
Chi2 ΔF	**970 ± 350**	**0.9 ± 0.3**	**-**	**4.8 ± 0.2b**		**6**
Chi3 unlabeled	5710 ± 1020	1.3 ± 0.1	1.4 ± 0.1	-	-	5
Chi3 labeled	610 ± 30	1.8 ± 0.2	3.0 ± 0.2	-	1.0 ± 0.1a	6
Chi3 ΔF	**1000 ± 190**	**1.3 ± 0.1**	**-**	**18.5 ± 3.3a**		**6**
Chi4 unlabeled	5300 ± 220	1.7 ± 0.1	1.9 ± 0.04	-	-	4
Chi4 labeled	770 ± 80	1.2 ± 0.03	2.8 ± 0.3	-	5.7 ± 1.6b	5
Chi4 ΔF	**640 ± 60**	**1.3 ± 0.05**	**-**	**4.4 ± 0.2b**		**5**
Chi5 unlabeled	3840 ± 340	1.6 ± 0.1	3.2 ± 0.2	-	-	5
Chi5 labeled	500 ± 40	2.0 ± 0.3	2.5 ± 0.3	-	1.2 ± 0.2a	5
Chi5 ΔF	**980 ± 210**	**1.3 ± 0.02**	**-**	**23.4 ± 3.7a**		**5**
Chi6 unlabeled	5640 ± 250	1.8 ± 0.2	3.0 ± 0.5	-	-	5
Chi6 labeled	630 ± 240	2.1 ± 0.4	2.4 ± 0.4	-	0.7 ± 0.01a	5
Chi6 ΔF	**1140 ± 190**	**1.0 ± 0.2**	**-**	**20.4 ± 3.2a**		**5**
ChiA unlabeled	10800 ± 610	1.1 ± 0.1	1.1 ± 0.3	-	-	5
ChiA labeled	780 ± 140	2.0 ± 0.3	0.9 ± 0.1	-	3.5 ± 0.4b	7
ChiA ΔF	**920 ± 240**	**0.8 ± 0.1**	**-**	**3.1 ± 0.5b**		**7**
ChiB unlabeled	3650 ± 920	1.8 ± 0.2	2.4 ± 0.6	-	-	6
ChiB labeled	410 ± 60	1.5 ± 0.2	2.6 ± 0.3	-	1.0 ± 0.1a	7
ChiB ΔF	**1150 ± 190**	**1.3 ± 0.2**	**-**	**21.9 ± 3.4a**		**7**
α3-R19'C,S346G unlabeled	6420 ± 370	1.5 ± 0.03	2.1 ± 0.2	-	-	5
α3-R19'C,S346G labeled	710 ± 40	1.4 ± 0.05	2.8 ± 0.3	-	1.8 ± 0.5a	5
α3-R19'C,S346G ΔF	**880 ± 210**	**1.3 ± 0.2**	**-**	**6.7 ± 0.5**		**5**
α3-R19'C,S346E unlabeled	8890 ± 280	1.8 ± 0.1	1.5 ± 0.1	-	-	5
α3-R19'C,S346E labeled	800 ± 70	1.4 ± 0.1	2.4 ± 0.1	-	3.7 ± 1.1b	5
α3-19'C,S346E ΔF	**1230 ± 350**	**1.1 ± 0.1**	**-**	**2.2 ± 0.1**		**5**

There was no significant difference among the EC_{50}, n_H or ΔI_{max} data using one way ANOVA followed by Dunnett's post hoc test. A statistical analysis of ΔF_{max} and $\Delta F/\Delta I$ half decay ratio data is presented in Fig. 3.

Table S3
Summary of results for agonist-activated ΔI and ΔF responses at MTS-TAMRA-labeled N203C mutant GlyRs. Electrophysiological and fluorescence data are shown in normal and bold type, respectively.

Construct	EC_{50} (μM)	n_H	I_{max} (μA)	ΔF_{max} (%)	n
α3-N203C ΔI glycine	57 ± 8	2.5 ± 0.2	4.9 ± 0.2	-	5
α3-N203C ΔF glycine	**732 ± 24**	**1.0 ± 0.1**	**-**	**4.0 ± 0.4**	**5**
α3-N203C ΔF strychnine	**-**	**-**	**-**	**5.5 ± 0.3**	**5**
α3-N203C-S346G ΔI glycine	43 ± 5	2.3 ± 0.2	4.7 ± 0.2	-	5
α3-N203C-S346G ΔF glycine	**588 ± 35**	**1.2 ± 0.2**	**-**	**7.1 ± 0.5**	**5**
α3-N203C-S346G ΔF strychnine	**-**	**-**	**-**	**8.4 ± 0.2**	**5**

Fig. S1. Amino acid sequence alignment of the coding region of the human α1 GlyR subunit (Uniprot accession number: P23415-2, isoform b) and the rat α3L GlyR subunit (Uniprot accession number: P24524). The alignment was generated using ClustalW. The residues that were mutated in this study are shown in blue. In addition to S346, the residues shown in red are also predicted to be serine phosphorylation sites according to the NetPhos 2.0 Server. Orange vertical lines denote the locations of subunit join sites used in creating α1–α3 chimeras.

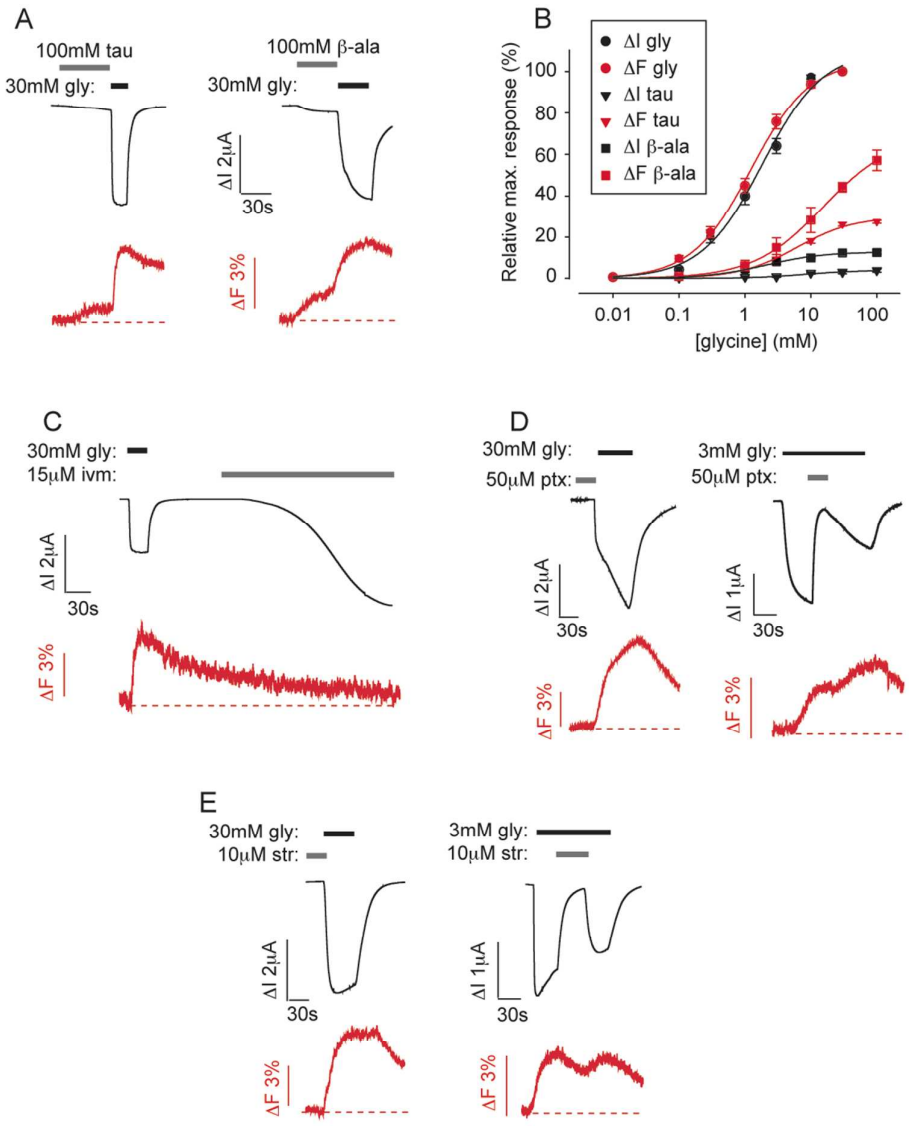

Fig. S2. Pharmacological characterization of MTSR-labeled α3-R19'C GlyRs. A. Sample ΔI_{max} and ΔF_{max} recordings in response to consecutive applications of saturating taurine (tau) and glycine (gly, left panel) or saturating β-alanine (β-ala) and glycine (right panel). Gray bars indicate duration of applications of taurine and β-alanine. B. Averaged ΔI and ΔF dose-response relationships for glycine, taurine and β-alanine. All dose-response curves are normalised to those of glycine as measured in the same oocyte. Mean parameters of best fit to all dose-response curves are presented in Table S1. C. Sample ΔI_{max} and ΔF_{max} recordings in response to saturating applications of glycine (black bar) and ivermectin (ivm, gray bar). This recording is representative of 5 others in which no detectable ivermectin-induced ΔF was observed. D. Sample ΔI_{max} and ΔF_{max} recordings in response to the application of picrotoxin (ptx) alone (left) or co-application of EC_{50} glycine with saturating picrotoxin (right). E. Sample ΔI_{max} and ΔF_{max} recordings in response to application of strychnine (str) alone (left) or co-application of EC_{50} glycine with saturating strychnine (right). Averaged values for experiments in panels D and E are given in the main text.

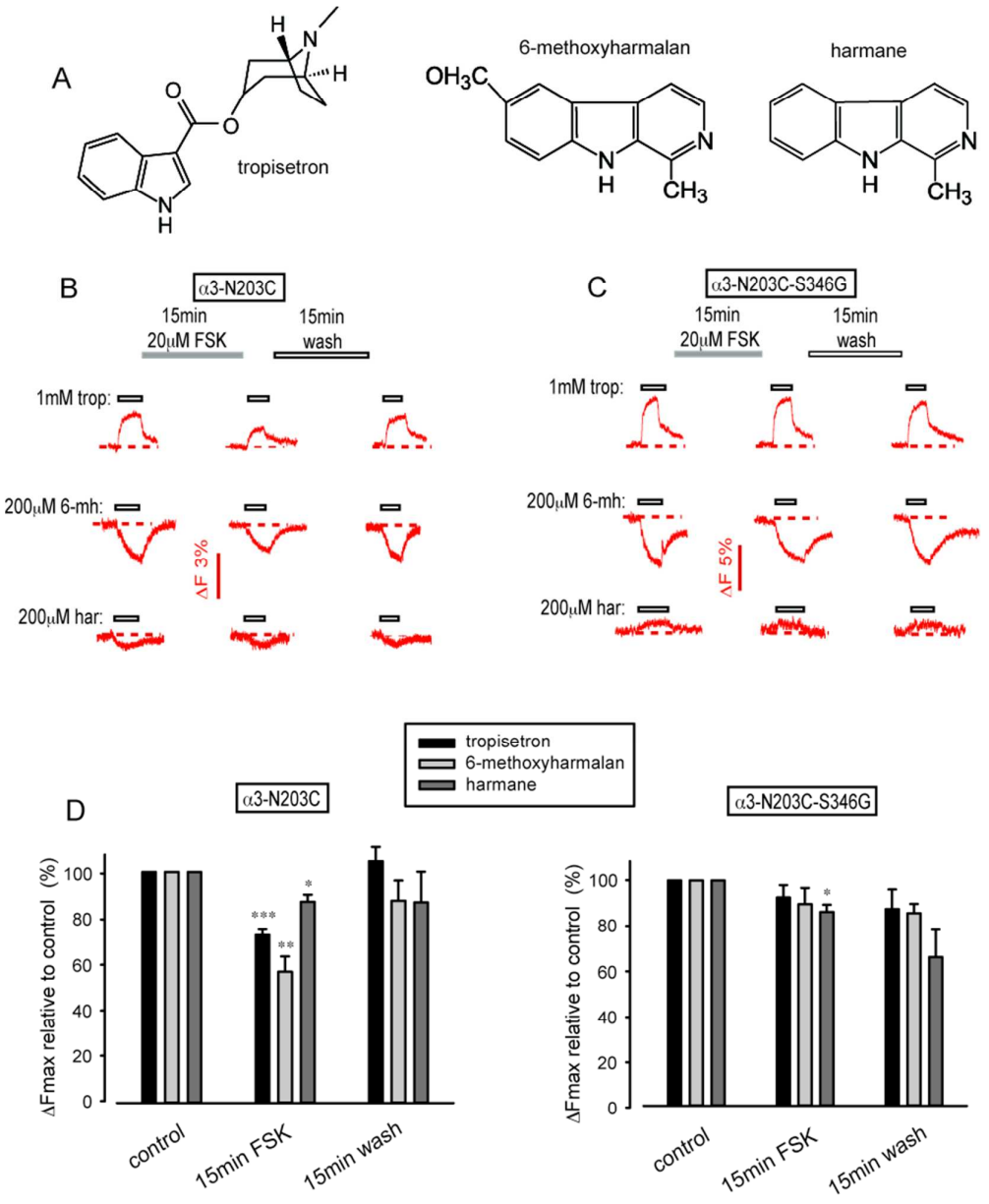

Fig. S3. Effects of phosphorylation on ΔF_{max} responses induced by tropisetron, 6-methoxyharmalan and harmane in MTS-TAMRA-labeled α3-N203C GlyRs. A. Structures of the three molecules. B. Examples of ΔF_{max} responses induced by 1 mM tropisetron (trop), 200 μM 6-methoxyharmalan (6-mh) and 200 μM harmane (har) on MTS-TAMRA-labeled α3-N203C GlyRs before and after a 15 min forskolin (FSK) treatment and a 15 min wash. C. Corresponding experiments on MTS-TAMRA-labeled α3-N203C-S346G GlyRs. D. Averaged data for the experiments shown in B and C (all n = 6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to control using paired t-test.