Supporting Information

Naturally-occurring genetic variants in the oxytocin receptor alter receptor signaling profiles

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Table S1: Oxytocin response in Ca^{2+} assays for wild type (WT) and variant OXTRs

Log(EC50)

	Variant log(EC50) (95% CI)	WT log(EC50) (95% CI)	Significance (P)
V45L	-8.165 (-8.237 to -8.099)	-8.266 (-8.349 to -8.165)	0.0525
P108A	-8.195 (-8.355 to -8.195)	-8.307 (-8.432 to -8.185)	0.2499
V172A	-8.295 (-8.444 to -8.161)	-8.396 (-8.531 to -8.265)	0.2879
L206V	-8.432 (-8.657 to -8.212)	-8.352 (-8.492 to -8.208)	0.5264
A218T	-8.435 (-8.503 to -8.368)	-8.399 (-8.483 to -8.316)	0.4842
G221S	-8.465 (-8.655 to -8.277)	-8.533 (-8.638 to -8.427)	0.5237
A238T	-8.533 (-8.679 to -8.386)	-8.543 (-8.681 to -8.403)	0.9157
G252A	-8.734 (-8.932 to -8.516)	-8.659 (-8.769 to -8.545)	0.5224
V281M	-8.054 (-8.115 to -7.989)	-8.301 (-8.449 to -8.136)	**0.0028**
E339K	-8.167 (-8.282 to -8.055)	-8.445 (-8.572 to -8.314)	**0.0020**
R376G	-8.699 (-8.900 to -8.480)	-8.650 (-8.763 to -8.532)	0.6732

E_{max}

	Variant E_{max} (95% CI)	WT E_{max} (95% CI)	Significance (*P*)
V45L	93 (89 to 96)	97 (93 to 101)	0.0783
P108A	98 (91 to 107)	96 (91 to 102)	0.7520
V172A	91 (86 to 97)	98 (92 to 103)	0.0868
L206V	93 (85 to 102)	98 (92 to 104)	0.0783
A218T	91 (89 to 94)	98 (95 to 102)	**0.0019**
G221S	103 (95 to 111)	98 (94 to 102)	0.2751
A238T	100 (94 to 106)	99 (94 to 105)	0.8697
G252A	101 (93 to 109)	99 (95 to 103)	0.7182
V281M	74 (72 to 77)	97 (91 to 105)	**<0.0001**
E339K	76 (72 to 81)	98 (94 to 104)	**<0.0001**
R376G	98 (91 to 107)	98 (94 to 103)	0.9194

Results shown are point estimates and 95% confidence intervals from dose-response curves generated from three replicate experiments. Variant point estimates are shown next to point estimate from the WT control on the same plate. Statistically significant changes in log(EC50) or E_{max} are shown in bold [(extra sum of squares F test, $P<0.0045$ ($\alpha=0.05$ with Bonferroni correction for 11 comparisons)].

Table S2: Oxytocin-induced β-arrestin-1 recruitment for wild type (WT) and variant OXTRs

Log(EC50)

	Variant log(EC50) (95% CI)	WT log(EC50) (95% CI)	Significance (*P*)
V45L	-6.842 (-7.095 to -6.587)	-7.232 (-7.419 to -7.038)	0.0139
P108A	-6.793 (-6.990 to -6.593)	-7.363 (-7.474 to -7.249)	**<0.0001**
V172A	-7.083 (-7.334 to -6.829)	-7.232 (-7.419 to -7.038)	0.3240
L206V	-7.416 (-7.637 to -7.187)	-7.456 (-7.598 to -7.309)	0.7793
A218T	-7.411 (-7.665 to -7.148)	-7.363 (-7.474 to -7.249)	0.7072
G221S	-7.123 (-7.462 to -6.764)	-7.232 (-7.419 to -7.038)	0.5538
A238T	-7.392 (-7.710 to -7.053)	-7.232 (-7.419 to -7.038)	0.3924
G252A	-7.559 (-7.909 to -7.192)	-7.456 (-7.598 to -7.309)	0.5925
V281M	-7.579 (undefined)	-7.363 (-7.474 to -7.249)	0.6644
E339K	-6.750 (-7.443 to -5.984)	-7.363 (-7.474 to -7.249)	0.0478
R376G	-7.566 (-7.729 to -7.400)	-7.456 (-7.598 to -7.309)	0.3221

E_{max}

	Variant E_{max} (95% CI)	WT E_{max} (95% CI)	Significance (*P*)
V45L	73 (65 to 82)	99 (93 to 107)	**<0.0001**
P108A	101 (92 to 112)	102 (98 to 107)	0.8726
V172A	80 (73 to 89)	99 (93 to 107)	**0.0007**
L206V	134 (123 to 146)	102 (96 to 108)	**<0.0001**
A218T	79 (71 to 88)	102 (98 to 107)	**<0.0001**
G221S	88 (77 to 100)	99 (93 to 107)	0.0751
A238T	94 (83 to 107)	99 (93 to 107)	0.4363
G252A	112 (97 to 127)	102 (96 to 108)	0.2147
V281M	9 (4 to 18)	102 (98 to 107)	0.0174
E339K	26 (18 to 38)	102 (98 to 107)	**0.0006**
R376G	128 (121 to 137)	102 (96 to 108)	**<0.0001**

Results shown are point estimates and 95% confidence intervals from dose-response curves generated from three replicate experiments. Variant point estimates are shown next to point estimate from the WT control on the same plate. Statistically significant changes in log(EC50) or E_{max} are shown in bold [(extra sum of squares F test, $P<0.0045$ ($\alpha=0.05$ with Bonferroni correction for 11 comparisons)].

Table S3: Oxytocin-induced β-arrestin-2 recruitment for wild type (WT) and variant OXTRs

Log(EC50)

	Variant log(EC50) (95% CI)	WT log(EC50) (95% CI)	Significance (*P*)
V45L	-7.041 (-7.218 to -6.862)	-7.504 (-7.625 to -7.379)	**<0.0001**
P108A	-6.893 (-7.048 to -6.735)	-7.295 (-7.485 to -7.099)	**0.0017**
V172A	-7.206 (-7.378 to -7.030)	-7.504 (-7.625 to -7.379)	0.0050
L206V	-7.533 (-7.880 to -7.167)	-7.655 (-7.850 to -7.457)	0.5756
A218T	-7.349 (-7.538 to -7.153)	-7.295 (-7.485 to -7.099)	0.6795
G221S	-7.352 (-7.588 to -7.103)	-7.504 (-7.625 to -7.379)	0.2376
A238T	-7.392 (-7.603 to -7.169)	-7.504 (-7.625 to -7.379)	0.3463
G252A	-7.601 (-7.829 to -7.364)	-7.655 (-7.850 to -7.457)	0.7065
V281M	-7.287 (-7.612 to -6.940)	-7.295 (-7.485 to -7.099)	0.9767
E339K	-7.074 (-7.287 to -6.857)	-7.295 (-7.485 to -7.099)	0.1975
R376G	-7.570 (-7.740 to -7.395)	-7.655 (-7.850 to -7.457)	0.4945

E_{max}

	Variant E_{max} (95% CI)	WT E_{max} (95% CI)	Significance (*P*)
V45L	87 (81 to 94)	98 (94 to 103)	0.0056
P108A	120 (111 to 129)	103 (95 to 111)	**0.0039**
V172A	93 (87 to 99)	98 (94 to 103)	0.1178
L206V	149 (131 to 169)	103 (95 to 110)	**<0.0001**
A218T	94 (87 to 101)	103 (95 to 111)	0.0942
G221S	100 (91 to 109)	98 (94 to 103)	0.7994
A238T	105 (97 to 113)	98 (94 to 103)	0.1488
G252A	104 (95 to 113)	103 (95 to 110)	0.8088
V281M	26 (22 to 30)	103 (95 to 111)	**<0.0001**
E339K	51 (46 to 56)	103 (95 to 111)	**<0.0001**
R376G	113 (105 to 120)	103 (95 to 110)	0.0478

Results shown are point estimates and 95% confidence intervals from dose-response curves generated from three replicate experiments. Variant point estimates are shown next to point estimate from the WT control on the same plate. Statistically significant changes in log(EC50) or E_{max} are shown in bold [(extra sum of squares F test, $P<0.0045$ ($\alpha=0.05$ with Bonferroni correction for 11 comparisons)].

Table S4: Log(IC50)s for desensitization and internalization curves for wild type (WT) and variant OXTR.

Desensitization

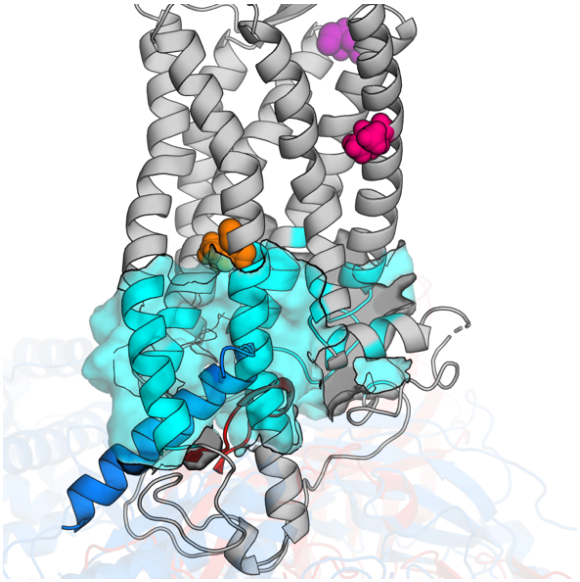
	Variant log(IC50) (95% CI)	WT log(IC50) (95% CI)	Significance (*P*)
V45L	-7.781 (-7.969 to -7.541)	-8.314 (-8.474 to -8.146)	**0.0001**
P108A	-7.840 (-8.054 to -7.530)	-8.414 (-8.490 to -8.336)	**<0.0001**
L206V	-8.414 (-8.609 to -8.200)	-8.427 (-8.541 to -8.310)	0.9040
V281M	-8.532 (-8.734 to -8.328)	-8.338 (-8.683 to -8.002)	0.3124
E339K	-7.828 (-7.976 to -7.654)	-8.355 (-8.518 to -8.180)	**<0.0001**

Internalization

	Variant log(IC50) (95% CI)	WT log(IC50) (95% CI)	Significance (*P*)
V45L	-8.016 (-8.285 to -7.746)	-8.436 (-8.606 to -8.269)	**0.0098**
P108A	-7.965 (-8.199 to -7.732)	-8.559 (-8.756 to -8.356)	**0.0003**
L206V	-8.556 (-8.670 to -8.441)	-8.657 (-8.909 to -8.384)	0.4626
V281M	-8.571 (-9.381 to -7.769)	-8.704 (-9.169 to -8.229)	0.7595
E339K	-8.350 (-8.687 to -8.026)	-8.585 (-8.796 to -8.369)	0.2135

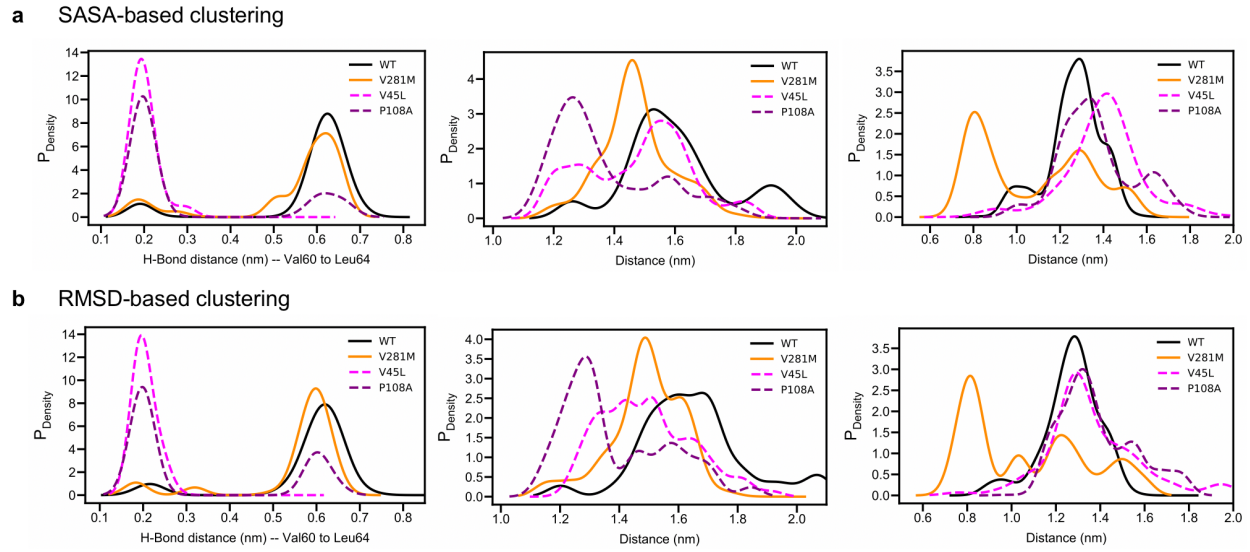
Results shown are point estimates and 95% confidence intervals (CI) from dose-response curves generated from three replicate experiments. Variant parameters are shown next to parameters from the WT control from the same experiment. *P* value shown from extra sum-of-squares F test comparing log(IC50) values between variant and WT.

Figure S1: Atoms included in DiffNets analysis.



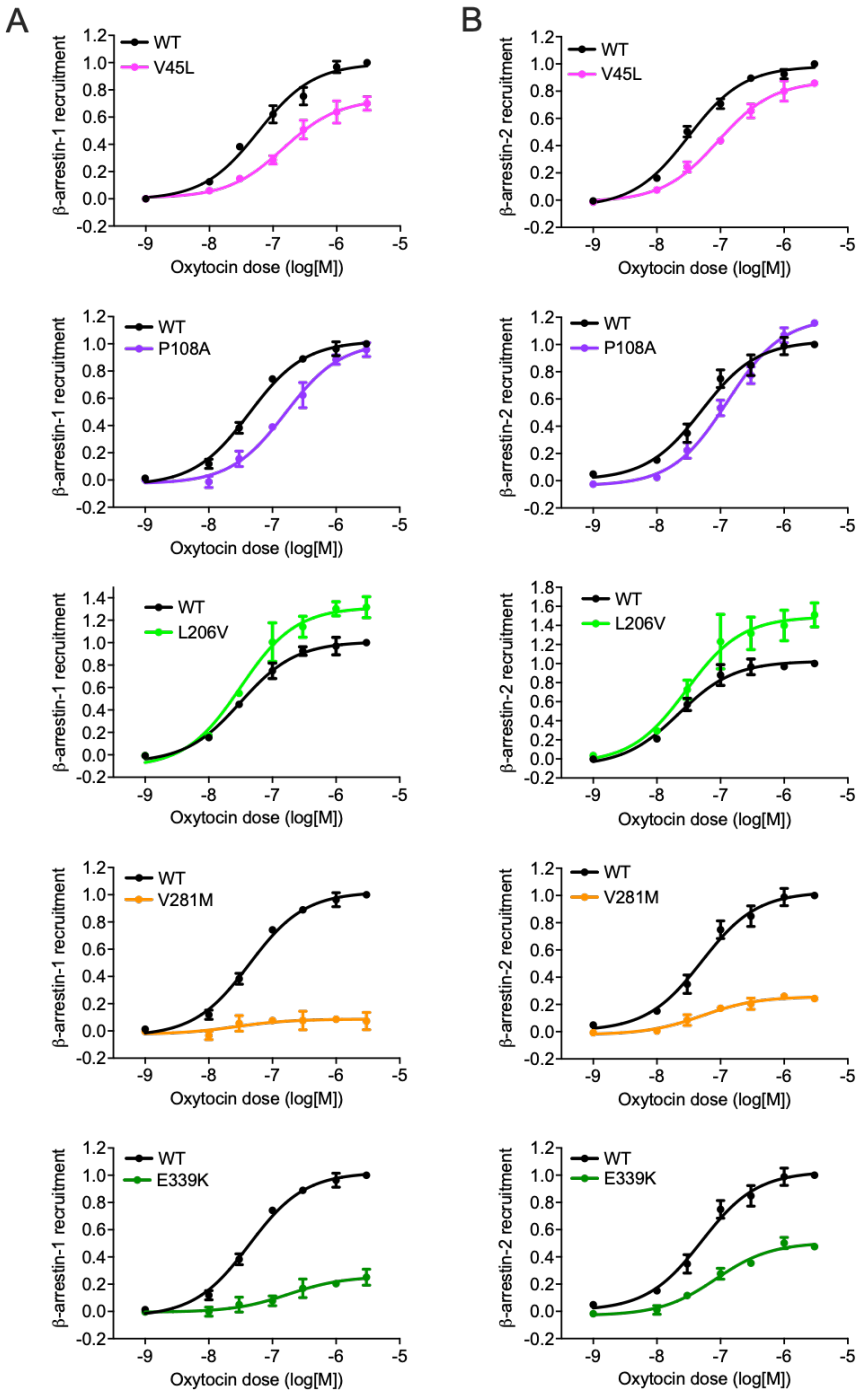
Atoms included in DiffNets analysis (cyan). OXTR homology model (grey) showing variants V45L (magenta), P108A (purple), and V281M (orange). Superimposed structures show β-arrestin-1 (red) and G protein (blue).

Figure S2: Comparison of equilibrium properties calculated from simulations using different clustering methods.



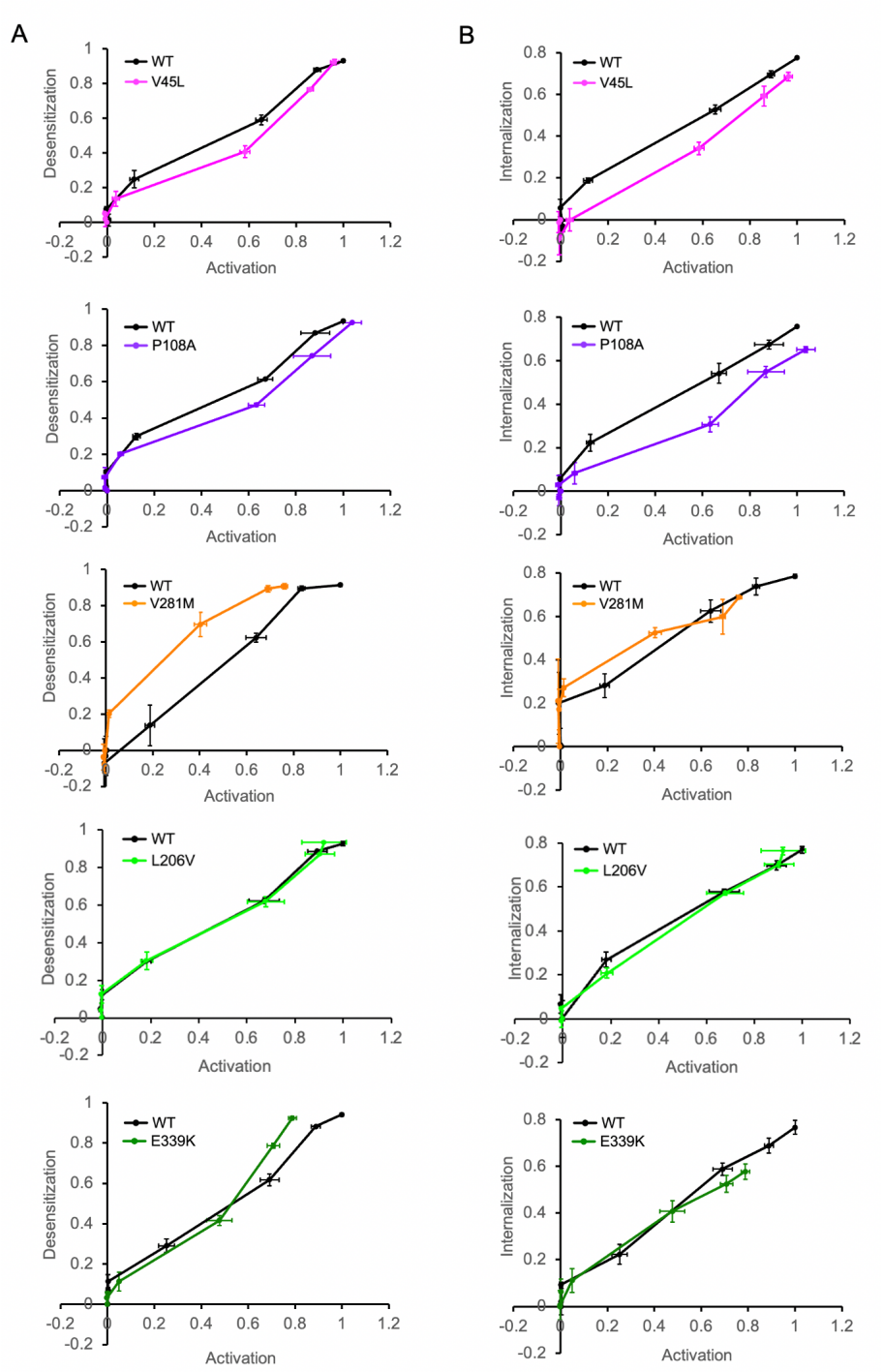
The three distance distributions from Figure 6A, 6C, and 7A are replotted here from left to right using SASA-based clustering (a) and RMSD-based clustering (b). (a) and (b) are highly consistent suggesting that the choice of clustering used prior to MSM construction does not strongly affect the computed equilibrium properties of the system.

Figure S3: Oxytocin-induced ß-arrestin recruitment to wild type (WT) and variant OXTRs.



Dose response curves for β-arrestin-1 (A) and β-arrestin-2 (B) recruitment are shown for WT and variant OXTR. Error bars show standard error from *N*=3 independent experiments.

Figure S4: Bias plots for wild type (WT) and variant OXTRs



Each point represents activation and desensitization (A) or internalization (B) for one oxytocin dose (10^{-12}-10^{-6} M). Error bars are SEM from *N=3* independent experiments.