

Supplementary Material

Activation of protein kinase G after repeated cocaine administration is necessary for the phosphorylation of α-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid receptor GluA1 at serine 831 in the rat nucleus accumbens

Ju Hwan Yang^{+,1}, Su Yeon Seo^{+,1,2}, Jeong Hwan Oh^{1,3}, In Soo Ryu^{1,4}, Jieun Kim¹, Dong Kun Lee⁵, Yeonhee Ryu², Eun Sang Choe^{1,*}

¹Department of Biological Sciences, Pusan National University, South Korea

²Korea Institute of Oriental Medicine, South Korea

³Institute of Fisheries Sciences, Pukyong National University, South Korea

⁴Substance Abuse Pharmacology Group, Korea Institute of Toxicology, South Korea

⁵Department of Physiology, School of Medicine and Institution of Health Sciences, Gyeongsang National University, South Korea

⁺Equal contributors

* Correspondence: Eun Sang Choe, eschoe@pusan.ac.kr

Supplementary Table S1. Rates of changes in the immunoreactivity of pGluA1-S831 in the NAc after repeated systemic injections of saline or cocaine at different time points

	Repeated saline	Repeated cocaine
Time (min)	M	ean ± SEM
5	100 ± 15.412	127.33 ± 39.443
10	100 ± 12.428	129.873 ± 6.131
30	100 ± 16.493	$422.652 \pm 52.047^{*}$
60	100 ± 23.862	$264.855 \pm 24.21^{*}$
120	100 ± 4.517	$164.846 \pm 15.488^{\#}$
240	100 ± 5.722	$121.777 \pm 3.903^{\#}$

*p < 0.05 vs. repeated saline group at the same time point; p < 0.05 vs. repeated cocaine group at the 30 min time point.

		Repeated saline	Repeated cocaine
Treatment		Mean ±	SEM
WB	Vehicle	100 ± 14.02	$260.8 \pm 22.053^*$
	KT5823	114.225 ± 3.383	$123.575 \pm 14.446^{\#}$
	Rp-cGMP	113.2 ± 2.587	88.3 ± 14.849 [#]
IHC	Vehicle	100 ± 3.095	$193.1 \pm 11.656^{*}$
	KT5823	111.525 ± 4.509	$111.025 \pm 8.742^{\#}$
	Rp-cGMP	107.025 ± 6.716	$113.225 \pm 7.035^{\#}$

Supplementary Table S2. Rates of changes in the immunoreactivity of pGluA1-S831 in the NAc after intra-NAc infusion of KT5823 or Rp-cGMP prior to the final injection of saline or cocaine

*p < 0.05 vs. repeated saline vehicle group; # p < 0.05 vs. repeated cocaine vehicle group; WB, western immunoblotting; IHC, immunohistochemistry.

Supplementary Table S3. Rates of changes in number of co-localization of pGluA1-S831 with NeuN in the NAc after the intra-NAc infusion of KT5823 or Rp-cGMP prior to the final injection of saline or cocaine

	Repeated saline	Repeated cocaine
Treatment	Ν	Aean ± SEM
Vehicle	100 ± 2.68	184.895 ± 3.815*
KT5823	99.248 ± 4.4	$95.873 \pm 4.09^{\#}$
Rp-cGMP	106.62 ± 5.262	$88.951 \pm 4.784^{\#}$

	Repeated saline	Repeated cocaine
Treatment		Mean ± SEM
PKG	100 ± 5.289	$228.4 \pm 13.16^{*}$
pGluA1 ^{S831}	100 ± 11.53	$202 \pm 9.809^{*}$
GluA1	100 ± 8.264	106.7 ± 8.209

Supplementary Table S4. Rates of changes in the immunoreactivity of PKG, pGluA1-S831 or GluA1 in the GluA1 precipitate of the NAc after the repeated systemic injections of saline or cocaine

*p < 0.05 vs. repeated saline group.

Supplementary Table S5. Rates of changes in the immunoreactivity of pGluA1-S831 after intra-NAc infusion of Tat-peptides prior to the final injection of saline or cocaine

	Repeated saline	Repeated cocaine
Treatment	Mean :	± SEM
Vehicle	100 ± 2.634	$200.74 \pm 2.837^{*}$
Tat-GluA1-c (0.2 nmol)	98.36 ± 2.837	$181.64 \pm 11.163^*$
Tat-GluA1-c (2.0 nmol)	95.48 ± 0.856	$176.46 \pm 10.206^{*}$
Tat-GluA1-i (0.2 nmol)	106.96 ± 4.599	$175.62 \pm 3.267^{*}$
Tat-GluA1-i (2.0 nmol)	82.3 ± 3.578	$99.82 \pm 6.098^{\#}$

		Repeated saline	Repeated cocaine
Treatment		Mean ± SEM	
	Vehicle	100 ± 4.293	214.933 ± 7.031*
PKG	Tat-GluA1-c (2.0 nmol)	107.667 ± 8.019	$169.267 \pm 13.762^{\ast}$
	Tat-GluA1-i (2.0 nmol)	112.933 ± 12.203	$93.833 \pm 6.912^{\#}$
	Vehicle	100 ± 1.02	97 ± 0.603
GluA1	Tat-GluA1-c (2.0 nmol)	98.1 ± 2.854	95.233 ± 6.306
	Tat-GluA1-i (2.0 nmol)	100.8 ± 0.874	95.1 ± 4.912

Supplementary Table S6. Rates of changes in the immunoreactivity of PKG or GluA1 in the GluA1 precipitate after the intra-NAc infusion of Tat-peptides prior to the final injection of saline or cocaine



Supplementary Table S7. Changes in total distance travelled for 60 min after the intra-NAc infusion of Tat-peptides prior to the final injection of saline or cocaine

	Repeated saline	Repeated cocaine
Treatment	М	ean ± SEM
Vehicle	1068.9 ± 144.882	$17617.2 \pm 1574.275^{*}$
Tat-GluA1-c	1093.5 ± 83.314	$15075.6 \pm 1531.533^{*}$
Tat-GluA1-i	863.6 ± 126.171	$5649.4 \pm 379.234^{\#}$