Nicotinic Cholinergic Mechanisms Causing Pathological Dopamine Release and Hyperactivity

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864.19/C1

Introduction

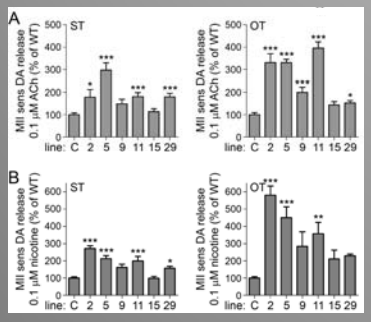
α6-Containing (α6*) nicotinic acetylcholine receptors (nAChRs) are expressed in dopaminergic neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA), adrenergic neurons in the locus coeruleus, and retinal ganglion cells. This limited expression pattern suggests that α6*-selective drugs could be potentially useful in modulating CNS dopamine (DA) release. To study the role of α6* nAChRs *in vivo*, we generated transgenic mouse lines expressing a mutant α6 subunit (α6L9'S) that dramatically increased the agonist sensitivity of the receptor, and measured nAChR-mediated synaptosomal DA release, nicotine- and novelty-induced locomotion, spontaneous ambulation, and spontaneous wheel-running. The results show that selective agonist activation of the mutant nAChRs substantially enhanced MII conotoxin-sensitive DA release from the SNc and VTA, and nicotine-induced ambulatory activity. Mice with six or more copies of the mutant gene displayed nocturnal hyperactivity, no intrasession locomotor habituation in response to a novel environment, and reduced spontaneous wheel running, consistent with increased endogenous dopamine release.

Mutant Gene Copy Numbers for the Transgenic α6L9'S Mouse Lines

Line #	# Mutant Gene Copies (mean ± SEM)	% Mutant Genes (assuming 2 WT α6 genes)	Group Classification
2	18 ± 1	90	High-copy number
11	16 ± 1	89	
5	5.5 ± 0.1	73	
9	2.3 ± 0.3	53	Low-copy number
15	2.0 ± 0.2	50	
29	2.0 ± 0.3	50	

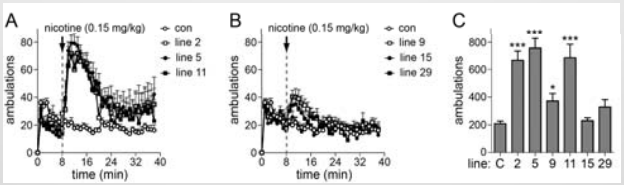
- We constructed six α6L9'S BAC transgenic mouse lines
- The number of mutant gene copies in each line was measured using real-time PCR
- Lines with 6 (5.5) or more copies were labeled "high-copy number" lines; those with less than 6 copies, "low-copy number" lines

α6L9'S Increased MII-Conotoxin-Sensitive, ACh- and Nicotine-Induced (100 nM) DA Release from Dorsal Striatal (ST) and Olfactory Tubercle (OT) Synaptosomes



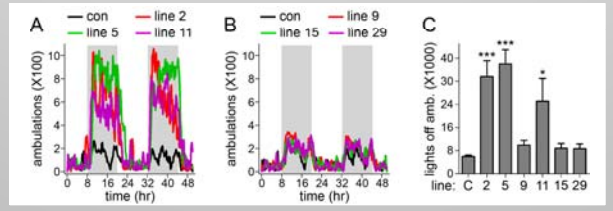
- Agonist-induced DA release from ST (A-B) and OT synaptosomes (C-D) was measured for WT controls (C) and six mutant lines (2,5,9,11,15,29)
- ST release mainly reflects release from SNc nerve terminals; OT release, release from VTA terminals
- Mutant values were normalized to control release (100%)
- Asterisks denote significant differences from control (*,**,*** P < 0.05, 0.01, 0.001)

Low-dose (0.15 mg/Kg) Nicotine Induced Ambulation in Mutant, but not Control, Mice



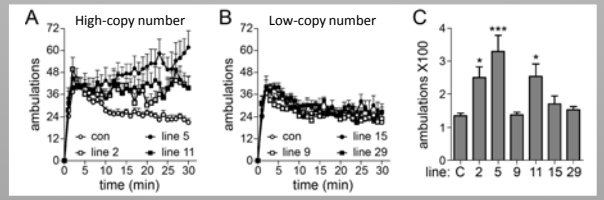
- Ambulation was measured as consecutive beam breaks for control (con) and mutant mice (lines 2,5,9,11,15,29) after injecting 0.15 mg/Kg nicotine (i.p.)
- Nicotine-induced locomotor responses were greater in the high-copy mutants than in the control (A) and low-copy mutants (B)
- Nicotine-induced ambulatory activity between 9 and 20 min (C) was significantly greater for the high-copy mutants (2, 5,11) and the line 9 low-copy mutant than the controls

High-Copy Mutants were Hyperactive during Lights Off



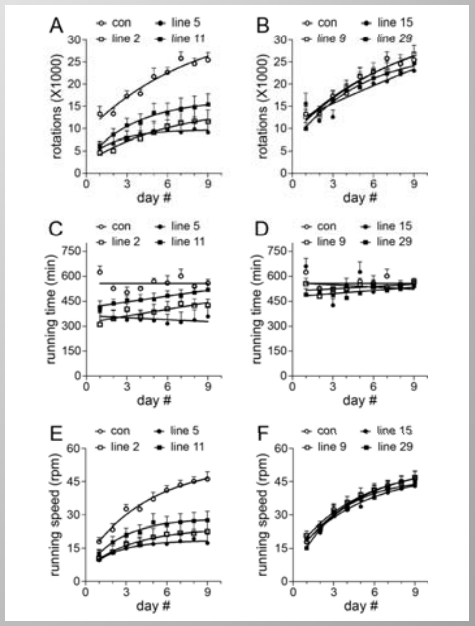
- We measured home-cage ambulation of the control (con), high-copy (A), and low-copy (B) mutants over a 48 h period (12:12 L:D cycle)
- Grey shading (A-B) denotes lights off
- High-copy mutants (lines 2, 5, 11) displayed significantly more total ambulation during lights off (C) than the low-copy mutants (lines 9,15,29) and control

High-Copy Mutants Fail to Habituate after Exposure to a Novel Environment



- Intrasession locomotor habituation was measured for control (con), high-copy (A), and low-copy mutant mice (B) after placement in a new cage (at min 0)
- High-copy mutants did not display locomotor habituation within the 30 min session (A)
- Ambulatory activity was summed during the last 5 min of the test (C) and was significantly greater for the high-copy mutants than the control

High-Copy Strains Display less Spontaneous Wheel Running than the Control



- Daily wheel running (rotations per 24 h) was measured continuously for control (con), high-copy (A), and low-copy (B) mice for a 9 d period (12:12 L:D cycle)
- Daily wheel running increased in a negative exponential fashion during the 9 d experiment
- However, high-copy mutants (A) ran significantly less (P < 0.01) than the low-copy mutants (B) and controls (con)
- All mice ran almost exclusively during lights off .
- The reduction in lights-off running for the high-copy mutants was due to both a reduction in the time spent running (C-D) and the running speed (E-F)

Conclusions

- The α6L9'S mutation enhances ACh- and nicotine-induced DA release from SNc and VTA nerve terminals and nicotine-induced ambulation, suggesting that that drugs which target α6* nAChRs will be able to modulate midbrain DA release *in vivo*
- Mice with six or more mutant gene copies display nocturnal hyperactivity and no intrasession locomotor habituation in response to a novel environment, consistent with increased α6* nAChR-mediated DA release
- The reduction in wheel running in the high-copy mice suggests that they lack motivation to expend the additional effort required to obtain DA release from wheel-running because baseline extracellular DA is already high