Supplementary information for

Guanine Base Modifications in Antisense Oligonucleotides Mitigate Acute CNS Toxicity

Maho Katsuyama [a, c-e], Taiki Matsubayashi [a-e], Yang Ying [a], Su Su Lei Mon [a,c,d], Takayuki Kuroda [a,c,d], Kie Yoshida-Tanaka [a,c,d], Rintaro Iwata Hara [a,c,d], Takeshi Yamada [a,c,d], Kumiko Ui-Tei [c,d], Kotaro Yoshioka [a,c,d*]

AFFILIATIONS

[a] Department of Neurology and Neurological Science, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, 1-5-45 Yushima, Bunkyo-Ku, Tokyo, 113-8519, Japan

[b] Department of Neurology, National Hospital Organization Disaster Medical Center, 3256 Midori-chou Tachikawa-shi, Tokyo 190-0014, Japan

[c] Center for Brain Integration Research, Institute of Science Tokyo, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8519 Tokyo, Japan

[d] NucleoTIDE and PepTIDE Drug Discovery Center, Institute of Science Tokyo, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8519 Tokyo, Japan

[e] These authors contributed equally

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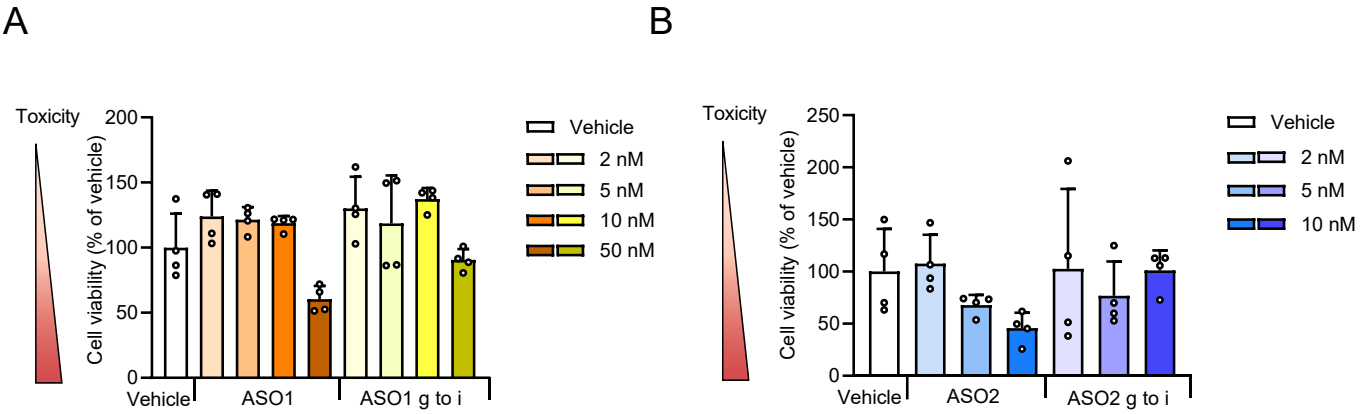
		Score 1	Score 2	Score 3	Score 4
Category 1 Consciousness	Decreased exploration or voluntary movement	Decreased exploration	Decreased exploration	No exploration	No exploration
	Decreased responsiveness	Normal	Slightly (e.g., reduced response to touch or handling)	Moderately (e.g., reduced response to lift, no blink)	Marked (e.g., reduced response to a tail pinch)
Category 2 Motor function	Ataxia	(-)	Mild (e.g., slow righting response, swaying)	Moderate (e.g., staggering, impaired walking)	Severe (e.g., crawling)
	Strength	Decreased strength of lower limbs	No ability to climb a slope	No ability to walk	No ability to right
Category 3 Appearance	Abnormal posture	Slight	Mild (e.g., hunched, extended, low posture, straub tail)	Moderate (e.g., ventral recumbency)	Severe (e.g., lateral recumbency)
	Abnormal breathing	Normal	Normal	Shallow	Labored
Category 4 Hyperactivity	Increased home-cage exploration	Slightly increased (e.g., increased exploration)	Increased (e.g., digging, burying)	Moderately increased (e.g., scratching limbs)	Marked increased
	Stereotype	(-)	Slight (e.g., increased grooming)	Moderate (e.g., circling, repetitive behavior)	Marked
Category 5 Involuntary movement	Tremors	Detectable	Marked	N.A.	N.A.
	Seizure (e.g., running, bouncing, clonic and/or tonic seizures)	(-)	(-)	Few or partial	Repeated or continuous (e.g., >1 min)
Death	Score 22				

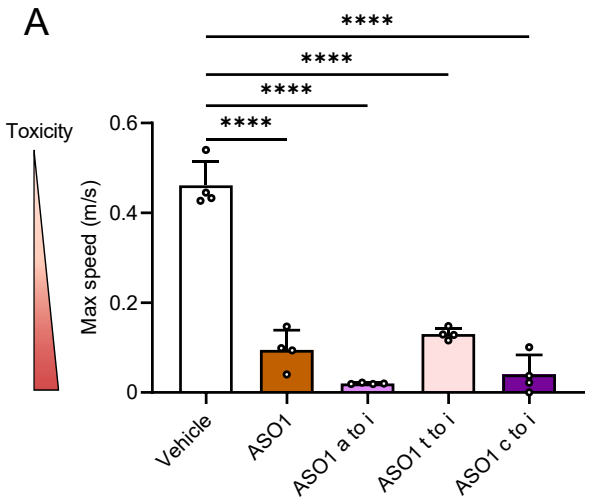
Supplementary Table S1. Acute tolerability scoring system

Acute toxicity scoring system used for mouse subjects. The scoring system consists of five neurobehavioral categories: (1) Consciousness, (2) Motor function, (3) Appearance, (4) Hyperactivity, and (5) Involuntary movement. Mice that died during or after i.c.v. administration were assigned a score of 22. N.A., not applicable.



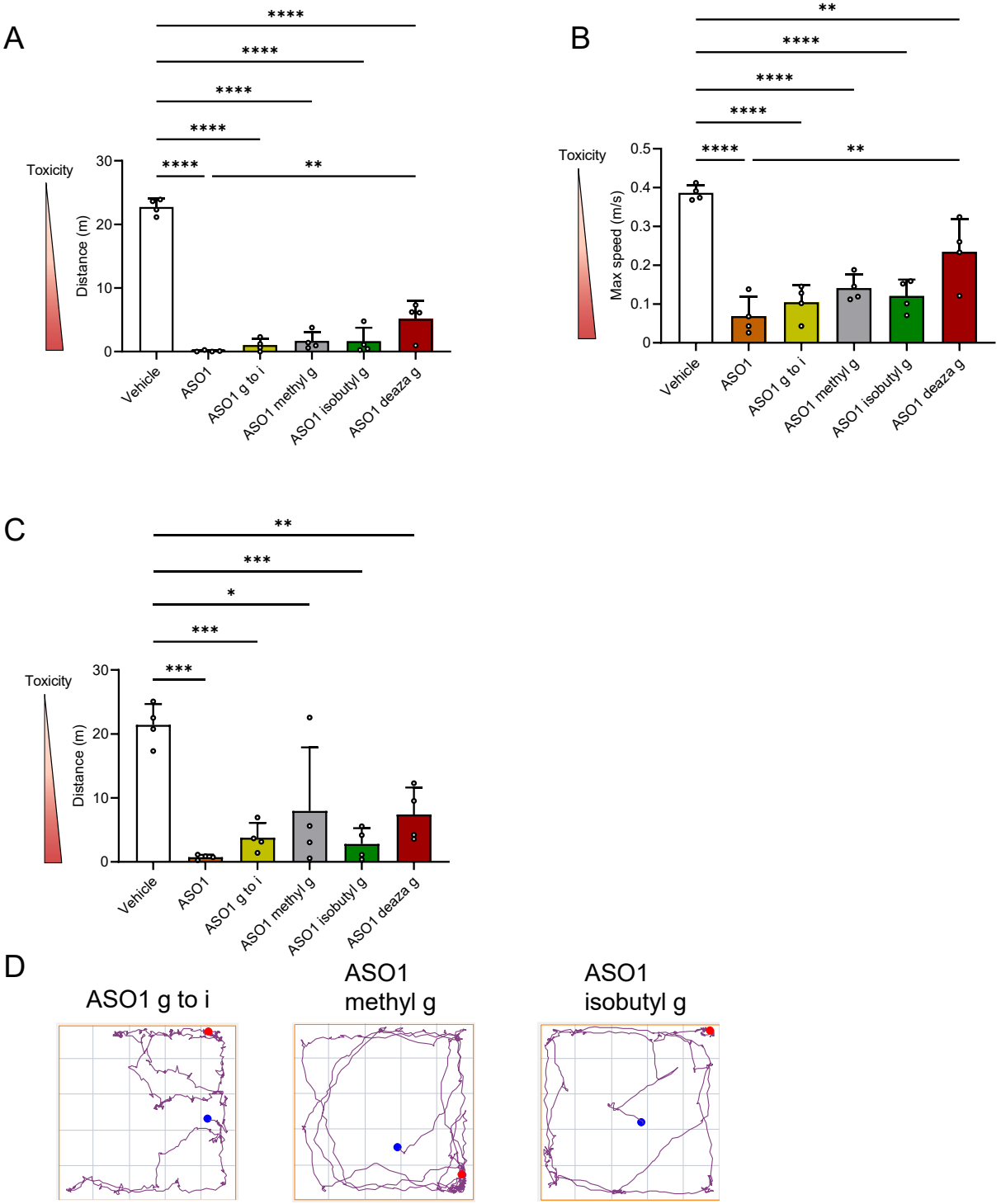
Supplementary Figure S1. Effects of guanine-to-hypoxanthine substitution on ASO cytotoxicity *in vitro*

(A) Cell viability in Neuro-2a cells 48 h after transfection with 2, 5, 10, or 50 nM ASO1, expressed relative to vehicle. (B) Cell viability in Neuro-2a cells 48 h after transfection with 2, 5, or 10 nM ASO2, expressed relative to vehicle. Data are shown as mean ± SEM (n = 4). Statistical differences were examined using a one-way ANOVA followed by Tukey's post hoc test. We observed no significant differences in group means ($p > 0.05$).



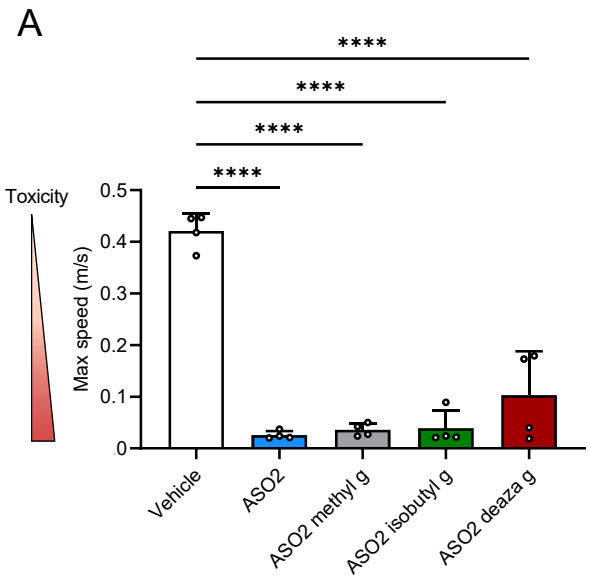
Supplementary Figure S2. Locomotor activity of mice following i.c.v. injection of hypoxanthin-substitution ASOs assessed by open-field test

(A) Locomotor activity of mice assessed after i.c.v. injection of ASO1, ASO1 a to i, t to i, and c to i (9.4 nmol, 50 μg/mouse). Maximum speed at 1 h post-injection in open field tests. Data are shown as mean ± SEM (n = 4). Statistical differences were examined using a one-way ANOVA followed by a Tukey's post hoc test (vs. Vehicle or ASO1; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). We observed no significant differences in group means ($p > 0.05$).



Supplementary Figure S3. Locomotor activity of mice following i.c.v. injection of guanine-modified ASO1s assessed by open-field test

(A–C) Locomotor activity of mice assessed after i.c.v. injection of ASO1, ASO1 g to i, ASO1 methyl g, ASO1 isobutyl g, and ASO1 deaza g (9.4 nmol, 50 μg/mouse). Locomotor activity parameter (A) Represents distance traveled at 1 h post-injection in open-field tests. (B) Maximum speed at 1 h post-injection in open field tests. (C) Distance traveled at 3 h post-injection in open field tests. (D) Representative track plots from open-field tests conducted 3 h post-injection. Data are shown as mean ± SEM (n = 4). Statistical differences were examined using a one-way ANOVA followed by a Tukey's post hoc test (vs. Vehicle or ASO1; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). we observed no significant differences in group means ($p > 0.05$).



Supplementary Figure S4. Locomotor activity of mice following i.c.v. injection of guanine-modified ASO2s assessed by open-field test

(A) Locomotor activity of mice assessed after i.c.v. injection of ASO2, ASO2 methyl g, ASO2 isobutyl g, and ASO2 deaza g (11 nmol, 50 μg/mouse). Maximum speed at 1 h post-injection in open-field tests. Data are shown as mean ± SEM (n = 4). Statistical differences were examined using a one-way ANOVA followed by Tukey's post hoc test (vs. vehicle or ASO1; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). We observed no significant differences in group means ($p > 0.05$).

Supplementary Video S1. Videos demonstrating mitigation of CNS toxicity in mice following i.c.v. injection of ASO

(A) Mouse recorded 1 h after i.c.v. injection of vehicle. (B) Mouse recorded 1 h after i.c.v. injection of 11 nmol ASO1. (C) Mouse recorded 1 h after i.c.v. injection of 11 nmol ASO1 g to i from the experiment shown in Figure 2.

Supplementary Video S2. Videos of mice exhibiting CNS toxicity after i.c.v. injection of ASOs

(A) Mouse recorded 3 h after i.c.v. injection of vehicle. (B) Mouse recorded 3 h after i.c.v. injection of 11 nmol ASO1. (C) Mouse recorded 3 h after i.c.v. injection of 11 nmol ASO1 a to i. (D) Mouse recorded 3 h after i.c.v. injection of 11 nmol ASO1 t to i. (E) Mouse recorded 3 h after i.c.v. injection of 11 nmol ASO1 c to i from the experiment shown in Figure 3.

Supplementary Video S3. Videos showing mitigation of CNS toxicity in mice following i.c.v. injection of guanine-modified ASOs

(A) Mouse recorded 3 h after i.c.v injection of 9.4 nmol ASO1. (B) Mouse recorded 3 h after i.c.v injection of 9.4 nmol ASO1 g to i. (C) Mouse recorded 3 h after i.c.v. injection of 9.4 nmol ASO1 methyl g. (D) Mouse recorded 3 h after i.c.v. injection of 11 nmol ASO1 isobutyl g. (E) Mouse recorded 3 h after i.c.v. injection of 11 nmol ASO1 deaza g from the experiment shown in Figure 4.