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Supplementary Data

Corticotropin-Releasing Factor Receptor 1 Activation During Exposure to Novelty Stress Protects Against Alzheimer's Disease-Like Cognitive Decline in AβPP/PS1 Mice

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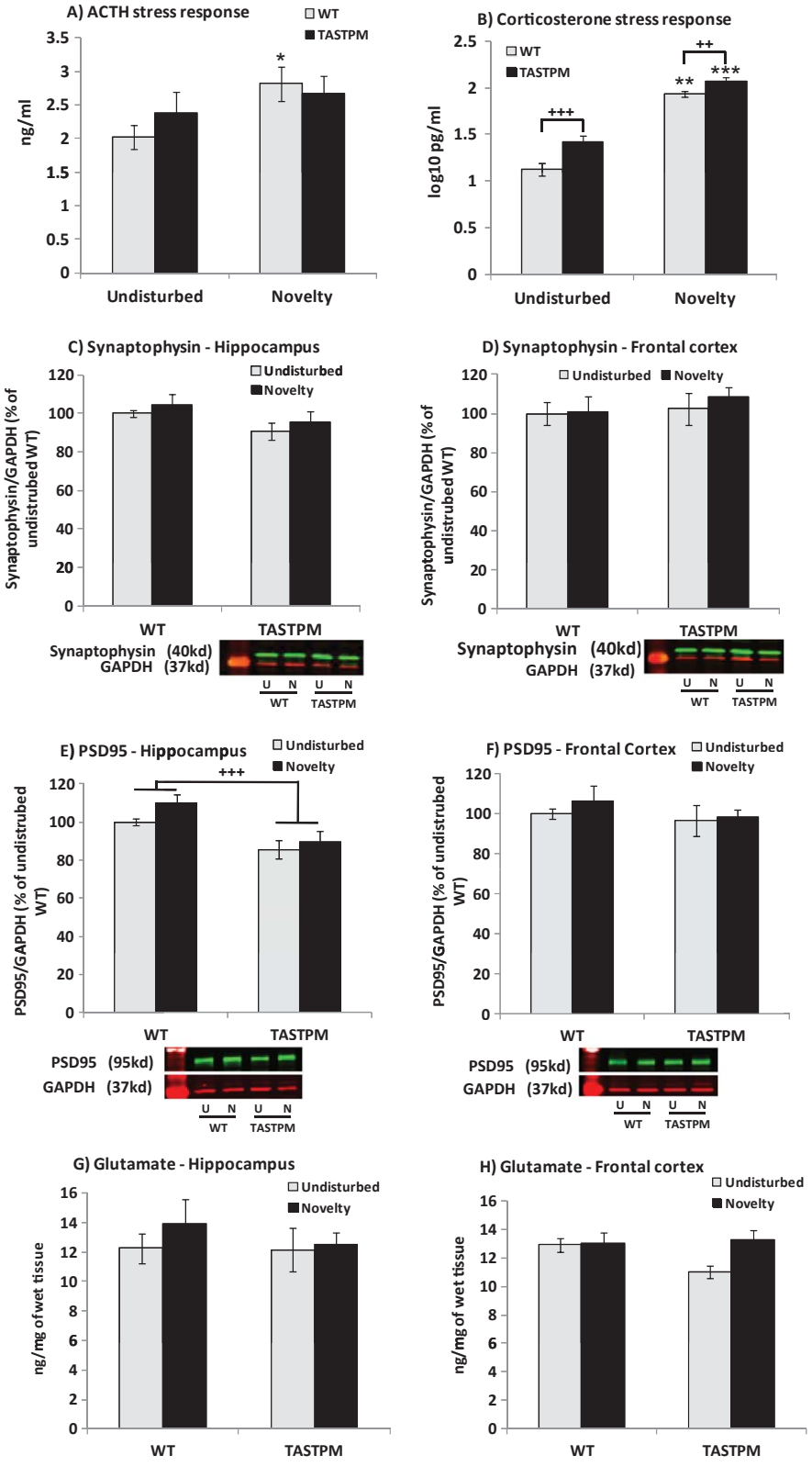


Supplementary Figure 1.

Supplementary Table 1

At the end of the 5 weeks of novelty sessions, baseline corticosterone levels were no longer elevated in TASTPM mice (genotype: $F_{(1,28)} = 0.18$; $p = 0.67$) and baseline adrenocorticotropic hormone (ACTH) levels did not differ (genotype: $F_{(1,20)} = 0.59$; $p = 0.45$) irrespective of the injection status (ACTH: $F_{(1,20)} = 0.11$; $p = 0.74$; corticosterone: $F_{(1,28)} = 2.81$; $p = 0.10$). Activity levels in the open-field were not significantly altered by the genotype ($F_{(1,32)} = 2.25$; $p = 0.14$) or the repeated injections ($F_{(1,32)} = 1.23$; $p = 0.27$) in unexposed mice. Hippocampal synaptophysin levels were reduced in TASTPM mice (genotype: $F_{(1,22)} = 1.99$; $p = 0.17$; injections : $F_{(1,22)} = 4.12$; $p = 0.06$). Synaptophysin levels in the frontal cortex were unaltered by the genotype ($F_{(1,32)} = 2.12$; $p = 0.15$) or repeated injections ($F_{(1,32)} = 0.39$; $p = 0.54$). Hippocampal PSD95 levels were reduced in TASTPM mice and by repeated injections in wild-type (WT) mice, but increased in injected TASTPM mice (genotype × injection: $F_{(1,32)} = 20.29$; $p < 0.001$), while those levels in the frontal cortex were unaltered by any of the experimental conditions (genotype: $F_{(1,22)} = 0.05$; $p = 0.83$; injections : $F_{(1,22)} = 0.00$; $p = 0.96$)

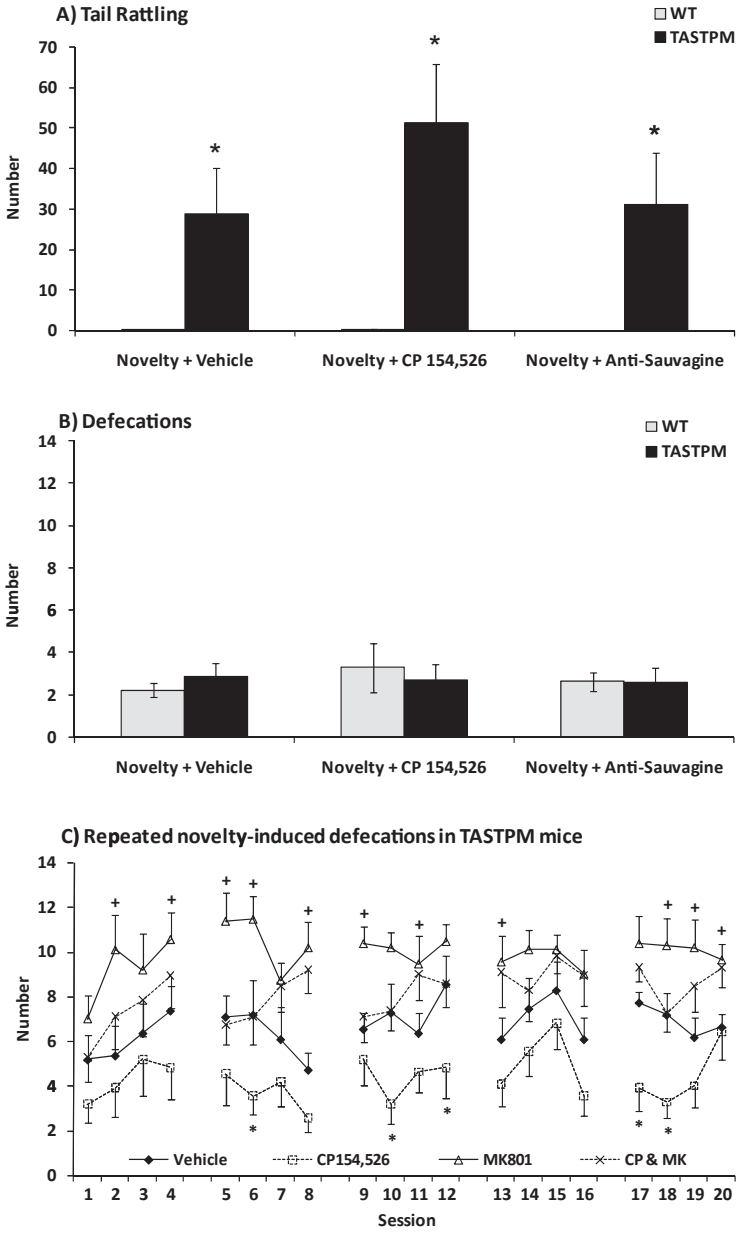
	WT		TASTPM	
	Non-injected	Injected	Non-injected	Injected
ACTH (ng/ml)	2.51 ± 0.21	3.51 ± 0.62	3.79 ± 0.33	2.97 ± 0.40
Corticosterone (log10 pg/ml)	1.12 ± 0.10	1.18 ± 0.12	1.04 ± 0.12	1.37 ± 0.15
Distance moved in open-field (m)	63.11 ± 2.16	54.56 ± 3.67	53.88 ± 1.97	54.73 ± 3.69
Synaptophysin (% of undisturbed WT)				
Hippocampus	100.00 ± 3.42	97.29 ± 5.72	93.92 ± 3.58	91.56 ± 3.40
Frontal Cortex	100.00 ± 3.59	93.96 ± 5.06	98.57 ± 2.67	90.56 ± 2.82
PSD95 (% of undisturbed WT)				
Hippocampus	100.00 ± 4.43	82.33 ± 3.97	78.85 ± 4.53	95.76 ± 2.00
Frontal Cortex	100.00 ± 4.35	107.17 ± 3.63	105.46 ± 3.90	100.09 ± 2.64

Supplementary Table 2

None of the experimental conditions altered plasma adrenocorticotropic hormone (ACTH) ($F_{(4,34)} = 1.25$; $p = 0.31$) or corticosterone levels ($F_{(4,40)} = 1.25$; $p = 0.49$), body weight ($F_{(3,36)} = 0.03$; $p = 0.99$) or the distance travelled in the open-field ($F_{(4,47)} = 1.1$; $p = 0.37$) in TASTPM mice

	Vehicle	Novelty + vehicle	Novelty + CP154,526	Novelty + MK801	Novelty + CP & MK
ACTH (ng/ml)	2.97 ± 0.39	3.53 ± 0.64	3.16 ± 0.38	4.02 ± 0.42	4.10 ± 0.47
Corticosterone (log10 pg/ml)	1.28 ± 0.14	1.05 ± 0.17	1.06 ± 0.11	1.32 ± 0.17	1.31 ± 0.13
Body weight (g)					
Baseline	26.50 ± 0.71	26.13 ± 0.60	26.60 ± 0.70	26.72 ± 0.53	26.54 ± 0.42
After repeated novelty	27.33 ± 0.89	26.25 ± 0.57	26.97 ± 0.42	26.74 ± 0.65	27.04 ± 0.71
Distance moved in open-field (m)	54.73 ± 3.70	51.44 ± 4.80	53.43 ± 4.07	44.15 ± 3.21	54.73 ± 5.51

Supplementary Figure 1. TASTPM mice exhibit altered hypothalamic-pituitary-adrenal responses to stress at a prepathological age. Wild-type (WT) and TASTPM mice aged 4 months were exposed to an acute 1-hour novelty session at the end of which trunk blood, hippocampi and frontal cortex were collected. A) This challenge significantly increased adrenocorticotropic hormone (ACTH) levels in WT ($p = 0.04$) but not TASTPM mice ($p = 0.45$) but baseline levels were unaltered (novelty: $F_{(1,47)} = 3.68$; $p = 0.048$). B) Corticosterone release was also induced in both WT ($p < 0.001$) and TASTPM ($p < 0.001$) mice (novelty: $F_{(1,50)} = 171.92$; $p < 0.001$). Although, TASTPM mice exhibited higher basal ($p < 0.001$) but not novelty-induced ($p = 0.11$) circulating levels of corticosterone (genotype: $F_{(1,50)} = 14.83$; $p < 0.001$), but both genotypes showed a similar rise in plasma corticosterone levels (+306.32 ± 48.50% for WT and +311.89 ± 44.95% for TASTPM mice). Synaptophysin levels of WT and TASTPM mice did not differ significantly in both the hippocampus ($F_{(1,28)} = 3.82$; $p = 0.06$, C) and frontal cortex ($F_{(1,28)} = 0.42$; $p = 0.47$, D) and were unaltered by a single exposure to novelty (C, hippocampus: $F_{(1,28)} = 0.99$; $p = 0.33$ & D, frontal cortex: $F_{(1,28)} = 0.25$; $p = 0.62$). In contrast, PSD95 levels of TASTPM mice were selectively reduced in the hippocampus ($F_{(1,28)} = 13.85$; $p < 0.001$, E) but not frontal cortex ($F_{(1,28)} = 0.87$; $p = 0.36$, F), and unaltered by a single exposure to novelty (E, hippocampus: $F_{(1,28)} = 2.36$; $p = 0.14$; F, frontal cortex: $F_{(1,28)} = 0.42$; $p = 0.52$). Glutamate levels in the hippocampus (G) and frontal cortex (H) were unaltered in TASTPM mice (Genotype: $F_{(1,22)} = 0.25$; $p = 0.62$ and $F_{(1,28)} = 1.99$; $p = 0.17$, respectively) or by acute exposure to novelty ($F_{(1,22)} = 0.58$; $p = 0.46$ and $F_{(1,28)} = 4.12$; $p = 0.06$, respectively). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus undisturbed (same genotype). $^{++}p < 0.01$, $^{+++}p < 0.001$.



Supplementary Figure 2. Behavioral and physiological stress responses of TASTPM mice. 4-month-old wild-type (WT) and TASTPM mice were subjected to a 1-hour novelty session 30 minutes after receiving an i.c.v. injection of saline, CRFR1 or CRFR2 antagonists (7 nmol CP154,526 and 100 pmol anti-sauvagine, respectively). A) TASTPM mice exhibited higher frequency of tail rattling than WT mice ($F_{(1,39)} = 27.16$; $p < 0.001$), and this behavior was not altered by CRFR1 or CRFR2 antagonism (Treatment: $F_{(1,39)} = 0.94$; $p = 0.40$). $^{*}p < 0.05$ compared to WT mice (same treatment). B) Defecations induced by the acute exposure to novelty were not significantly increased in TASTPM mice (genotype: $F_{(1,39)} = 0.00$; $p = 0.96$) or altered by blockade of novelty-induced CRFR1 or CRFR2 activation in either genotype (Treatment: $F_{(1,39)} = 1.01$; $p = 0.75$). C) In TASTPM mice, the defecation rate increased with repeated exposure to novelty ($F_{(19,760)} = 2.81$; $p < 0.001$), but was decreased by blocking novelty-induced CRFR1 activation and increased by antagonism of novelty-induced NMDAR activation ($p = 0.03$ and $p = 0.003$ versus vehicle-treated TASTPM), an effect attenuated by co-treatment with CP154,526 ($p = 0.16$ versus vehicle-treated TASTPM, Treatment: $F_{(3,40)} = 10.48$; $p < 0.001$). $^{*}p < 0.05$, $^{+}p < 0.05$ versus vehicle-treated TASTPM.