

Supplemental Information

Chronic Doxycycline Treatment Ameliorates Motor Coordination Deficits and Modulates Striatal Inflammation in a Mouse Model of Tauopathy

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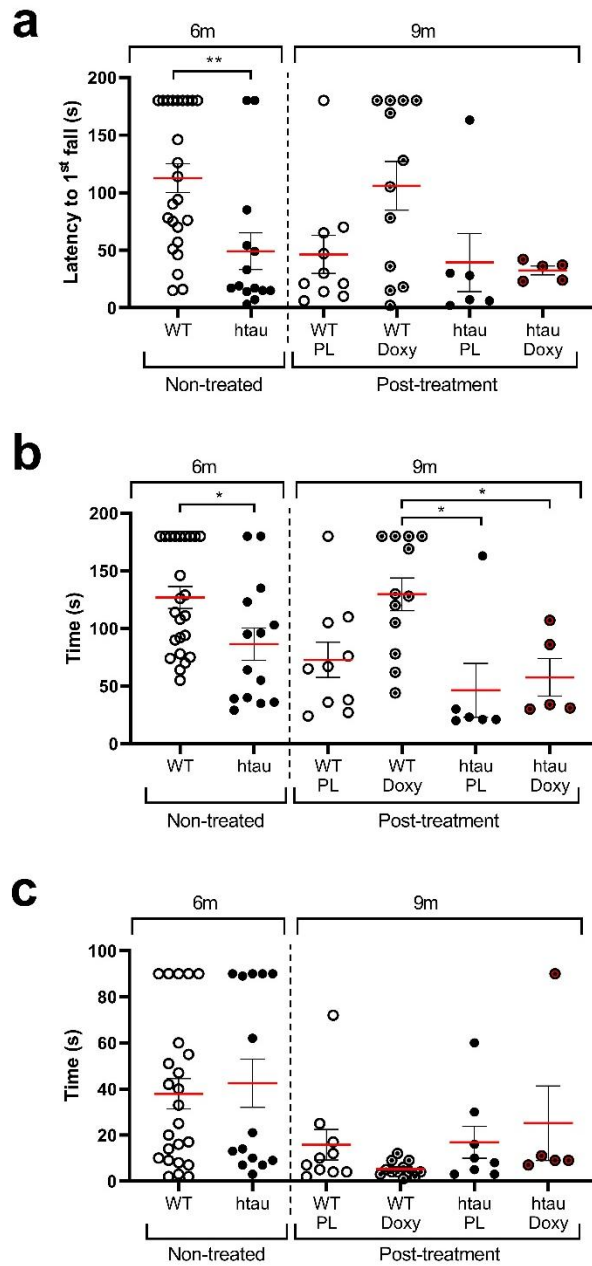


Figure S1 Additional measured parameters in Rotarod and seed-buried tasks. **(a-b)** Latency to the first fall from treadmill **(a)** and maximum time on the rod were registered. 6m Non-treated: WT $n = 24$, htau $n = 14$. 9m Treated: WT PL $n = 10$, WT Doxy $n = 12$, htau PL $n = 6$, htau Doxy $n = 5$; $**p < 0.01$, statistical tests: 6m unpaired t-test and 9m Two-way ANOVA followed by Tukey's post hoc test. Data are shown as scatter dot plots, with mean $\pm$ SEM. **(c)** Time to find unburied seed to control motivation among groups. 6m Non-treated: WT $n = 24$, htau $n = 14$. 9m: WT PL $n = 10$, WT Doxy $n = 12$, htau PL $n = 8$, htau Doxy $n = 5$; No significant differences were observed between groups. Statistical tests: 6m unpaired t-test and 9m Two-way ANOVA followed by Tukey's post hoc test. Data are shown as scatter dot plots, with mean $\pm$ SEM.

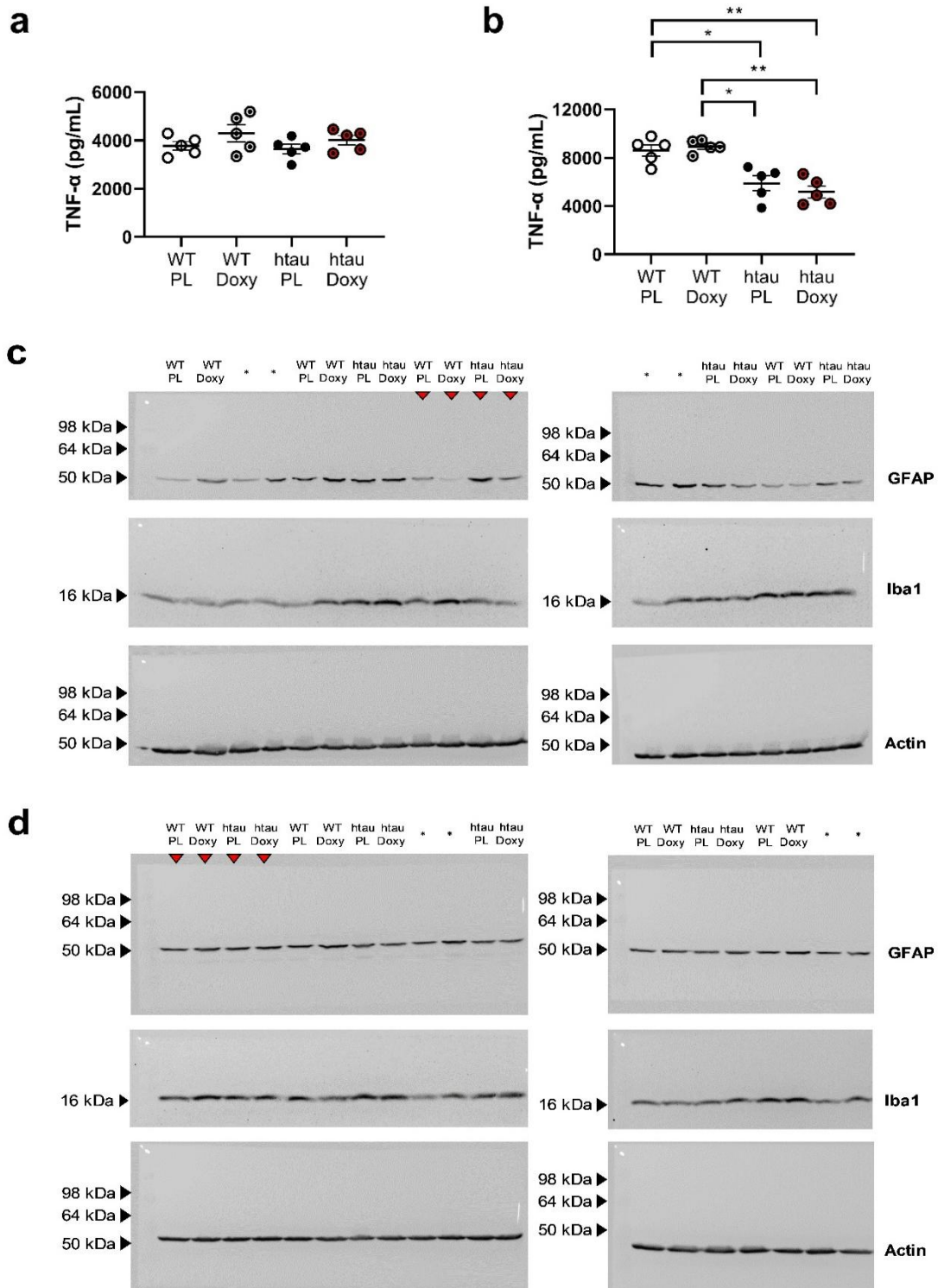


Figure S2 ELISA and Western Blot analysis from STR and OB. **(a-b)** TNF- α levels in STR **(a)** and OB **(b)** of 9-month-old WT and htau mice treated with Doxy or placebo, measured by ELISA. $n = 5$ per group; $*p < 0.05$, $**p < 0.01$. Statistical test: Two-way ANOVA followed by Tukey's post-hoc test. Data are presented as mean $\pm$ SEM. **(c-d)** Full blots used for quantification of GFAP and Iba1 contents in STR **(c)** and OB **(d)** of treated 9-month-old mice. Actin was used as a loading control for both proteins. (*) indicate samples not included in the analyses. Red arrows show the samples used for the panels shown in the main section.

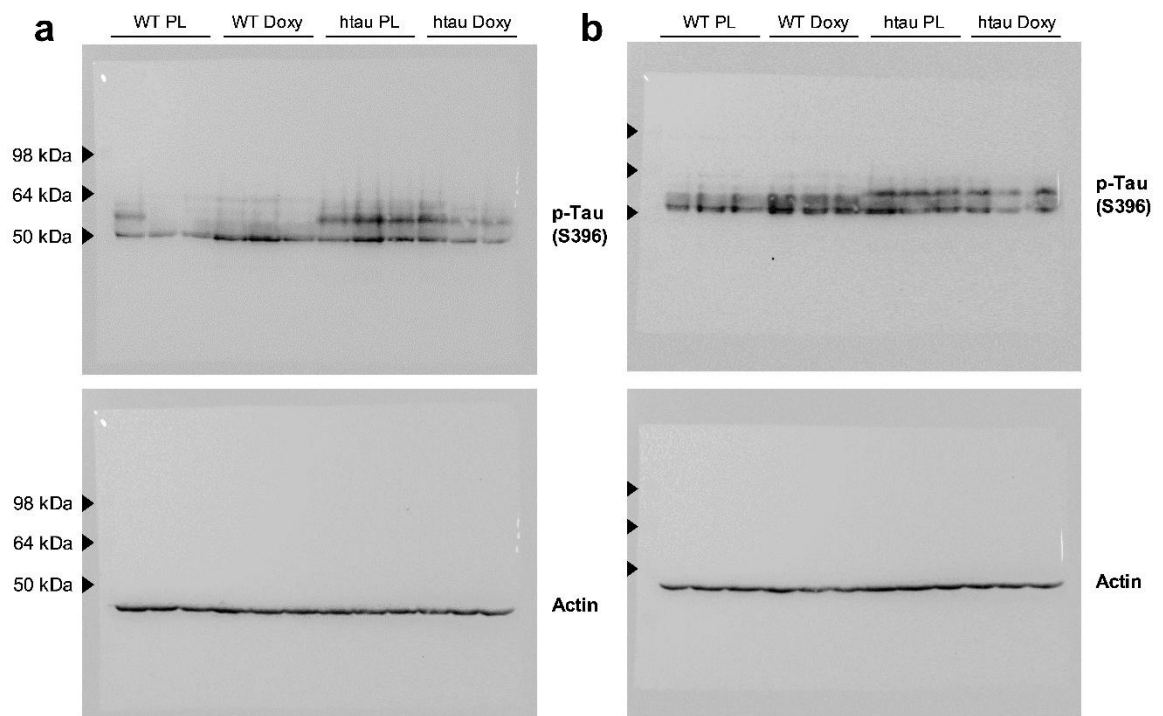


Figure S3 Western Blot analysis of p-tau levels from STR and OB. **(a-b)** Full bots used for quantification of p-tau (S396) contents in STR **(a)** and OB **(b)** of treated 9-month-old mice. Actin was used as a loading control.

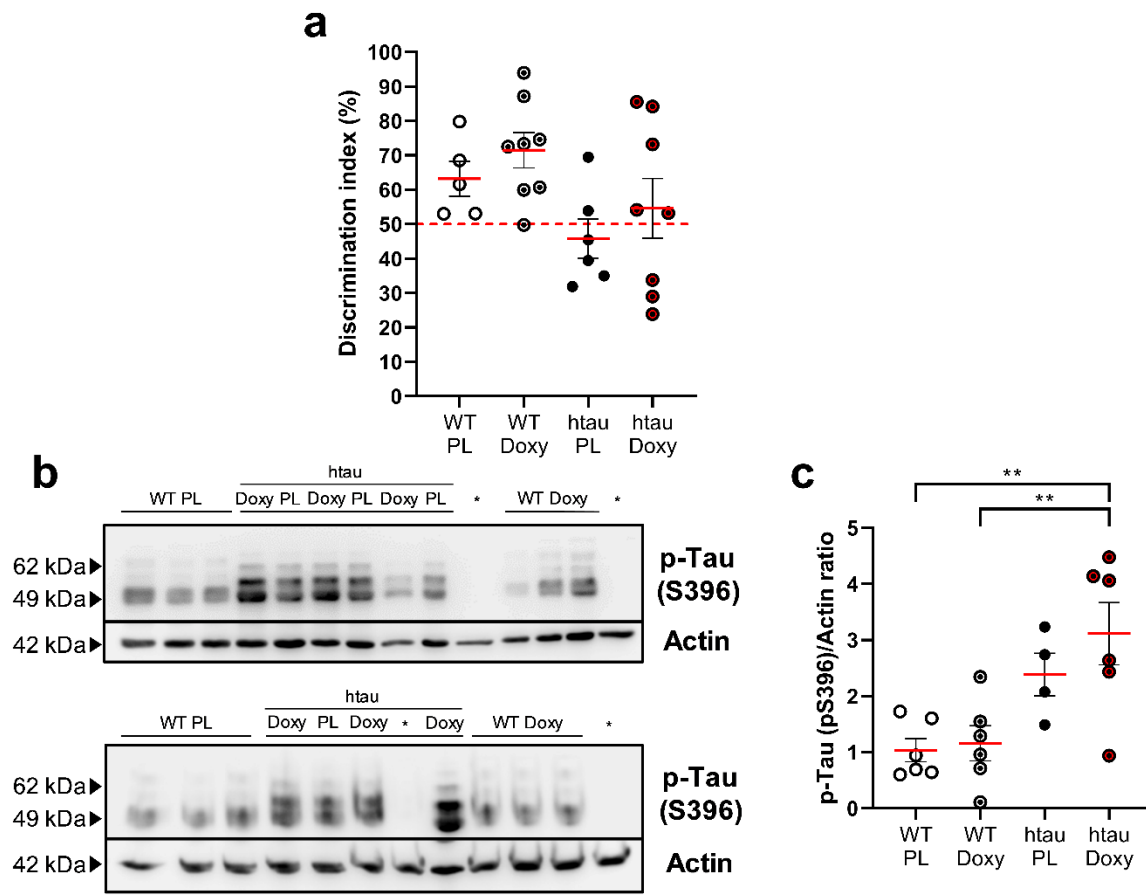


Figure S4 Effects of long-lasting, chronic doxycycline treatment (3m to 12m) on cognitive impairment and phosphotau accumulation in the prefrontal cortex. **(a)** Novel Object recognition task. Briefly, in the training phase, mice were allowed to explore identical objects for 10 min, and 5 hr later, mice were tested for 5 min in the same chamber with a familiar and a novel object. Discrimination index was calculated as the time spent exploring the novel object divided by the total time spent exploring both objects (familiar and novel). 12m Post-treated: WT PL n = 5, WT Doxy = 8, htau PL = 6, htau Doxy n = 8; No significant differences were observed between groups. Statistical test: Two-way ANOVA followed by Tukey's post hoc test. Data are shown as scatter dot plots, with mean $\pm$ SEM. **(b)** Western Blot analysis of p-tau levels from PFC of 12m, treated mice. **(c)** Quantification of p-tau levels in PFC. Actin was used as a loading control. (*) indicate samples not included in the analyses. WT PL n = 5, WT Doxy = 8, htau PL = 6, htau Doxy n = 8. ** $p < 0.01$. Statistical test: Two-way ANOVA followed by Tukey's post-hoc test. Data are presented as mean $\pm$ SEM.

Experimental group	WT PL												WT Doxy												htau PL												htau Doxy												
Mouse #	0	2	6	16	0	2	6	30	0'	30	1	3	2	10	11	30	31	50	0	2	10	11	20	30	13	65	83	90	91	13	30	52	76	65	74	85	2	11											
Sex	♀	♀	♀	♀	♂	♂	♂	♂	♀	♂	♂	♂	♀	♀	♀	♀	♀	♀	♂	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀	♂	♂	♂	♂	♀	♀	♀	♀	♂	♂										
Rotarod 6m	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Rotarod 9m	X	X	X	X	X	X	X	X	X	X	-	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-	X	-	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Olfaction 6m	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Olfaction 9m	X	X	X	X	X	X	X	X	X	X	-	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ELISA	X	X	X	-	X	X	-	-	-	-	-	-	-	X	X	-	-	-	-	X	X	X	-	-	X	-	X	-	-	-	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
WB Iba1/GFAP	X	X	X	-	X	X	-	-	-	-	-	-	-	X	X	-	-	-	-	X	X	X	-	-	X	-	X	-	-	-	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
WB p-Tau	X	X	X	-	X	X	-	-	-	-	-	-	-	X	X	-	-	-	-	X	X	X	-	-	X	-	X	-	-	-	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table S1 Mice used through the study, related to Methods, crosses indicate the experiments performed on each mouse included in the study, as approved by the Institutional Animal Care and Use Committee. Whenever possible the same breeds of mice were used for behavioral and post-mortem analyses. Mouse # (mouse number) in *italics* were only used for behavioral experiments (not end point) and following the 3R rules were assigned to further experiments.

Figure	Graph	Parameter	Test	Values
Figure 1	1b, 6m	Number of falls in Rotarod task <i>Groups: WT vs. htau</i>	Unpaired student's t-test	t = 3.757 df = 36 p = 0.0006
Figure 1	1b, 9m	Number of falls in Rotarod task <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	F = 7.652 p = 0.0098
Figure 1	1c	Latency to find the seed in seed-buried task <i>Groups: WT vs. htau</i>	Two-way ANOVA with RM	F = 19.14 p = 0.0001
Figure 1	1d	Latency to find the seed in seed-buried task <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Three-way ANOVA with RM; Treatment factor	F = 10.88 p = 0.0024
Figure 2	2a	IL-10 concentration in striatum homogenates (pg/mL) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	F = 5.922 p = 0.0271
Figure 2	2b	IL-10 concentration in olfactory bulb homogenates (pg/mL) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	F = 0.2031 p = 0.6582
Figure 2	2e	GFAP/Actin ratio in striatum homogenates (arbitraty units) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	F = 2.577 p = 0.1344

Figure 2	2f	GFAP/Actin ratio in olfactory bulb homogenates (arbitrary units) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 1.257$ $p = 0.2842$
Figure 2	2g	Iba1/Actin ratio in striatum homogenates (arbitrary units) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 0.1619$ $p = 0.6945$
Figure 2	2h	Iba1/Actin ratio in olfactory bulb homogenates (arbitrary units) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 0.1356$ $p = 0.7191$
Figure 3	3c	p-tau/Actin ratio in striatum homogenates (arbitrary units) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 0.4546$ $p = 0.5129$
Figure 3	3d	p-tau/Actin ratio in olfactory bulb homogenates (arbitrary units) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 0.0951$ $p = 0.7631$
Figure S1	S1a, 6m	Latency to first fall in Rotarod task <i>Groups: WT vs. htau</i>	Unpaired student's t-test	$t = 3.115$ $df = 36$ $p = 0.0036$
Figure S1	S1a, 9m	Latency to first fall in Rotarod task <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 1.422$ $p = 0.2428$
Figure S1	S1b, 6m	Maximum time on the rod in Rotarod task <i>Groups: WT vs. htau</i>	Unpaired student's t-test	$t = 2.457$ $df = 36$ $p = 0.0190$
Figure S1	S1b, 9m	Maximum time on the rod in Rotarod task <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 3.511$ $p = 0.0711$
Figure S1	S1c, 6m	Latency to find unburied seed in seed-buried task <i>Groups: WT vs. htau</i>	Unpaired student's t-test	$t = 0.3869$ $df = 36$ $p = 0.7011$
Figure S1	S1c, 9m	Latency to find unburied seed in seed-buried task <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 0.0251$ $p = 0.8751$
Figure S2	S2a	TNF- α concentration in striatum homogenates (pg/mL) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	$F = 3.380$ $p = 0.0846$

Figure S2	S2b	TNF- α concentration in olfactory bulb homogenates (pg/mL) <i>Groups: WT PL vs. WT Doxy vs. htau PL vs. htau Doxy</i>	Two-way ANOVA; Treatment factor	F = 0.1540 p = 0.6999
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Table S2. Statistical analyses used through the study, related to Figures and Methods, indicating the statistical tests used, p-values and statistics values per graph for all figures. Statistical tests used for comparing groups: Unpaired student's t-test (two groups, one independent variable), Two-way ANOVA (two independent variables) or Three-way ANOVA (three independent variables).