

SUPPLEMENTAL DATA

Ivermectin attenuates nicotine-induced reward-like behaviors in mice

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Table 1. Acquisition score comparisons among treatment groups (Second)

(I) grup	Mean ± SD	(J) grup	Mean Difference (I-J)	<i>p</i>	Adjusted <i>p</i> -value (Benjamini–Hochberg FDR)	95% Confidence Interval	
						Lower Bound	Upper Bound
VEC	482.50 ± 37.86 ^b	NIC	130,62	0,001		-	-48,91
					0,007	212,34	
		IVM	-35,83	0,670		-	51,52
		1			0,957	123,19	
		IVM	-15,83	0,957		-	71,52
NIC	613.12 ± 56.83 ^a	5			0,957	103,19	
		VEC	130,62	0,001	0,007	48,91	212,34
		IVM	94,792	0,019	0,038	13,08	176,50
		1					
		IVM	114,792	0,004	0,012	33,08	196,50
IVM 1	518.33 ± 57.96 ^b	5					
		VEC	35,83	0,670	0,957	-51,52	123,19
		NIC	-94,792	0,019	0,038	-	-13,08
						176,50	
		IVM	20,00	0,919	0,957	-67,35	107,35
IVM 5	498.33 ± 61.21 ^b	5					
		VEC	15,83	0,957	0,957	-71,52	103,19
		NIC	-114,792	0,004	0,012	-	-33,08
						196,50	
		IVM	-20,00	0,919	0,957	-	67,35
		1				107,35	

Adjusted *p*-values were calculated using the Benjamini–Hochberg false discovery rate (BH-FDR) method within each predefined family of tests. a. b: Different letters indicate statistically significant differences among the means. $\eta^2 = 0.532$; $p = 0.001$. Abbreviations: VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg; FDR: False discovery rate; n: Group sample size; SD: Standard deviation; F: F statistics; η^2 : Effect size.

Table 2. Comparison of reinstatement and pre-test values within each treatment group (Mean $\pm$ Standard Deviation)

Groups	n	Pre-Test	Reinstatement	t (1. df)	η^2	p
<i>VEC</i>	6	441.83 $\pm$ 35.02	457.16 $\pm$ 52.95	-0.592. 10	0.03 4	0.567
<i>NIC</i>	8	463.75 $\pm$ 42.93	560.00 $\pm$ 45.56	-4.348. 14	0.57 5	< 0.001
<i>IVM 1</i>	6	456.66 $\pm$ 45.59	492.50 $\pm$ 50.01	-1.297. 10	0.14 4	0.224
<i>IVM 5</i>	6	462.66 $\pm$ 41.50	456.33 $\pm$ 48.81	0.242. 10	0.00 6	0.814

Abbreviations: VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg; n: Group sample size; SD: Standard deviation; t: T statistics; η^2 : Effect size.

Post Test

Table 3. Post hoc multiple comparisons of group differences in Post test

(I) grup	Mean $\pm$ SD	(J) grup	Mean Difference (I-J)	<i>p</i>	Adjusted p-value (Benjamini–Hochberg FDR)	95% Confidence Interval	
						Lower Bound	Upper Bound
VEC	482.50 $\pm$ 37.86 ^b	NIC	-130.62	0.001		-	-41.15
					0.004	220.10	
		IVM	-106.33	0.106		-	-10.68
		1			0.211	201.99	
NIC	613.12 $\pm$ 56.83 ^a	IVM	-143.66	0.001		-	-48.01
		5			0.004	239.32	
		VEC	130.62	0.001	0.004	41.15	220.10
		IVM	24.29	0.930	0.964	-65.19	113.77
IVM 1	588.83 $\pm$ 86.77 ^{ab}	1					
		IVM	-13.04	0.964	0.964	-	76.44
		5				102.52	
		VEC	106.33	0.106	0.211	10.68	201.99
IVM 5	626.17 $\pm$ 46.70 ^a	NIC	-24.29	0.930	0.964	-	65.19
		1				113.77	
		IVM	-37.33	0.791	0.964	-	58.32
		5				132.99	
IVM 5	626.17 $\pm$ 46.70 ^a	VEC	143.66	0.001	0.004	48.01	239.32
		NIC	13.04	0.964	0.964	-76.44	102.52
		IVM	37.33	0.791	0.964	-58.32	132.99
		1					

η^2 : 0.502, (Welch ANOVA) $p < 0.001$, Homogeneity test: Levene homogeneity test < 0.05 , Multiple within-group comparisons: Games howell. Adjusted p-values were calculated using the Benjamini–Hochberg false discovery rate (BH-FDR) method within each predefined family of tests. Abbreviations: VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg; SD: Standard deviation; FDR: False discovery rate; CI: Confidence interval.

Extinction 1

Table 4. Post hoc multiple comparisons of group differences in Extinction 1

(I) grup	Mean $\pm$ SD	(J) grup	Mean Difference (I-J)	<i>p</i>	Adjusted p-value (Benjamini–Hochberg FDR)	95% Confidence Interval	
						Lower Bound	Upper Bound
VEC	444.33 $\pm$ 40.87 ^b	NIC	-96.29	0.002		-	-31.502
					0.007	161.081	
		IVM 1	-32.16	0.579		-	37.096
					0.695	101.429	
NIC	540.63 $\pm$ 45.43 ^a	IVM 5	1.00	0.999		-68.263	70.263
					0.999		
		VEC	96.29	0.002		31.502	161.081
					0.007		
IVM 1	476.50 $\pm$ 50.44 ^{ab}	IVM	64.12	0.053		-0.664	128.914
					0.106		
		IVM 5	97.29	0.002		32.502	162.081
					0.007		
IVM 5	443.33 $\pm$ 33.28 ^b	VEC	32.16	0.579		-37.096	101.429
					0.695		
		NIC	-64.12	0.053		-	0.664
					0.106	128.914	
IVM 5	443.33 $\pm$ 33.28 ^b	IVM 5	33.16	0.555		-36.096	102.429
					0.695		
		VEC	-1.00	0.999		-70.263	68.263
					0.999		
IVM 5	443.33 $\pm$ 33.28 ^b	NIC	-97.29	0.002		-	-32.502
					0.007	162.081	
		IVM 1	-33.16	0.555		-	36.096
					0.695	102.429	

η^2 : 0.524, $p=0.001$, Homogeneity test: Levene homogeneity test >0.05 , Multiple within-group comparisons: Tukey. Adjusted p-values were calculated using the Benjamini–Hochberg false discovery rate (BH-FDR) method within each predefined family of tests. Abbreviations: VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg; SD: Standard deviation; FDR: False discovery rate; CI: Confidence interval.

Extinction 2

Table 5. Post hoc multiple comparisons of group differences in Extinction 2

(I) grup	Mean $\pm$ SD	(J) grup	Mean Difference (I-J)	<i>p</i>	95% Confidence Interval	
					Lower Bound	Upper Bound
VEC	444.33 $\pm$ 43.26	NIC	-46.67	0.167	-106.76	13.42
		IVM 1	-19.83	0.826	-84.07	44.41
		IVM 5	-25.50	0.692	-89.74	38.74
NIC	491.00 $\pm$ 30.27	VEC	46.67	0.167	-13.42	106.76
		IVM 1	26.83	0.609	-33.26	86.92
		IVM 5	21.17	0.763	-38.92	81.26
IVM 1	464.17 $\pm$ 47.83	VEC	19.83	0.826	-44.41	84.07
		NIC	-26.83	0.609	-86.92	33.26
		IVM 5	-5.667	0.995	-69.91	58.57
IVM 5	469.83 $\pm$ 40.29	VEC	25.50	0.692	-38.74	89.74
		NIC	-21.17	0.763	-81.26	38.92
		IVM 1	5.667	0.995	-58.57	69.91

η^2 : 0.178, $p=0.220$, Homogeneity test: Levene homogeneity test >0.05 . Abbreviations:

VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5:

Ivermectin 5 mg/kg; SD: Standard deviation; CI: Confidence interval.

Reinstatement

Table 6. Post hoc multiple comparisons of group differences in Reinstatement

(I) grup	Mean $\pm$ SD	(J) grup	Mean Difference (I-J)	<i>p</i>	Adjusted p-value (Benjamini–Hochberg FDR)	95% Confidence Interval	
						Lower Bound	Upper Bound
VEC	457.17 $\pm$ 52.95 ^b	NIC	-102.83	0.004		-	-29.243
					0.012	176.424	
		IVM 1	-35.33	0.605		-	43.338
					0.726	114.005	
		IVM 5	0.833	0.999		-77.838	79.505
					0.999		
		VEC	102.83	0.004		29.243	176.424
					0.012		
NIC	560.00 $\pm$ 45.57 ^a	IVM 1	67.500	0.080		-6.090	141.090
					0.160		
		IVM 5	103.66	0.004		30.076	177.257
					0.012		
IVM 1	492.50 $\pm$ 50.01 ^{ab}	VEC	35.33	0.605		-43.338	114.005
					0.726		
		NIC	-67.50	0.080		-	6.090
					0.160	141.090	
		IVM 5	36.16	0.587		-42.505	114.838
					0.726		
IVM 5	456.33 $\pm$ 48.82 ^b	VEC	-0.833	0.999		-79.505	77.838
					0.999		
		NIC	-103.66	0.004		-	-30.076
					0.012	177.257	
		IVM 1	-36.16	0.587		-	42.505
					0.726		
						114.838	

η^2 : 0.492, $p=0.002$, Homogeneity test: Levene homogeneity test >0.05 , Multiple within-group comparisons: Tukey. Adjusted p-values were calculated using the Benjamini–Hochberg false discovery rate (BH-FDR) method within each predefined family of tests. Abbreviations: VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg; SD: Standard deviation; FDR: False discovery rate; CI: Confidence interval.

LOCOMOTOR ACTIVITY TESTS

Post-conditioning phase

Table 7a. Effects of nicotine on the locomotor activity of mice receiving Grooming (sec)

(I) grup	Mean $\pm$ SD	(J) grup	Mean		95% CI		<i>p</i>
			Difference (I-J)	<i>p</i>	Lower Bound	Upper Bound	
VEC	2.00 $\pm$ 1.41	NIC	-1.375	0.312	-3.528	0.778	0.250
		IVM 1	-0.500	0.930	-2.802	1.802	
		IVM 5	0.000	1.000	-2.302	2.302	
NIC	3.38 $\pm$ 1.69	VEC	1.375	0.312	-.778	3.528	
		IVM 1	0.875	0.677	-1.278	3.028	
		IVM 5	1.375	0.312	-.778	3.528	
IVM 1	2.50 $\pm$ 1.05	VEC	0.500	0.930	-1.802	2.802	
		NIC	-0.875	0.677	-3.028	1.278	
		IVM 5	0.500	0.930	-1.802	2.802	
IVM 5	2.00 $\pm$ 1.41	VEC	0.000	1.000	-2.302	2.302	
		NIC	-1.375	0.312	-3.528	0.778	
		IVM 1	-0.500	0.930	-2.802	1.802	

η^2 : 0.167; Group sample sizes VEC. IVM1 and IVM5: 6. NIC: 8; SD: Standard deviation; CI: Confidence interval; VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg.

Table 7b. Effects of nicotine on the locomotor activity of mice receiving Sniffing (sec)

<i>I) grup</i>	Med (min:max)	Mean Rank	<i>p</i>
VEC	2 (5:0)	11.5	0.771
NIC	2 (5:0)	12.63	
IVM 1	2 (5:2)	14.75	
IVM 5	3 (4:1)	15.42	

In the post-conditioning phase, the Sniffing behaviour did not conform to a normal distribution ($p < 0.05$), therefore the non-parametric Kruskal-Wallis H test was applied.

Abbreviations: VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg.

Table 7c. Effects of nicotine on the locomotor activity of mice receiving distance moved (cm)

(I) grup	Mean $\pm$ SD	(J) grup	Mean	<i>p</i>	95% CI		<i>p</i>
			Difference (I-J)		Lower Bound	Upper Bound	
VEC	3261 $\pm$ 734	NIC	-866.62	0.425	-2416.71	683.46	0.462
		IVM 1	-239.16	0.978	-1896.28	1417.95	
		IVM 5	-427.33	0.890	-2084.45	1229.78	
NIC	4127 $\pm$ 1166	VEC	866.62	0.425	-683.46	2416.71	
		IVM 1	627.45	0.679	-922.63	2177.55	
		IVM 5	439.29	0.860	-1110.80	1989.38	
IVM 1	3500 $\pm$ 1124	VEC	239.16	0.978	-1417.95	1896.28	
		NIC	-627.45	0.679	-2177.55	922.63	
		IVM 5	-188.16	0.989	-1845.28	1468.95	
IVM 5	3688 $\pm$ 994	VEC	427.33	0.890	-1229.78	2084.45	
		NIC	-439.29	0.860	-1989.38	1110.80	
		IVM 1	188.16	0.989	-1468.95	1845.28	

η^2 : 0.108; Group sample sizes VEC. IVM1 and IVM5: 6. NIC: 8; SD: Standard deviation; CI: Confidence interval; VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg.

LOCOMOTOR ACTIVITY TESTS

Reinstatement test

Table 8a. Effects of nicotine on the locomotor activity of mice receiving Grooming (sec)

(I) grup	Mean ± SD	(J) grup	Mean		95% CI		<i>p</i>
			Difference (I-J)	<i>p</i>	Lower Bound	Upper Bound	
VEC	2.17 ± 1.47	NIC	-0.833	0.879	-3.949	2.283	0.898
		IVM 1	-0.666	0.944	-3.998	2.664	
		IVM 5	-0.500	0.975	-3.831	2.831	
NIC	3.00 ± 2.20	VEC	0.833	0.879	-2.283	3.949	
		IVM 1	0.166	0.999	-2.949	3.283	
		IVM 5	0.333	0.991	-2.783	3.449	
IVM 1	2.83 ± 2.48	VEC	0.666	0.944	-2.664	3.9982	
		NIC	-0.166	0.999	-3.283	2.949	
		IVM 5	0.166	0.999	-3.164	3.498	
IVM 5	2.66 ± 1.97	VEC	0.500	0.975	-2.831	3.831	
		NIC	-0.333	0.991	-3.449	2.783	
		IVM 1	-0.166	0.999	-3.498	3.164	

η^2 : 0.026; Group sample sizes VEC. IVM1 and IVM5: 6. NIC: 8; SD: Standard deviation; CI: Confidence interval; VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg.

Reinstatement test

Table 8b. Effects of nicotine on the locomotor activity of mice receiving Sniffing (sec)

(I) grup	Mean ± SD	(J) grup	Mean		95% CI		<i>p</i>
			Difference (I-J)	<i>p</i>	Lower Bound	Upper Bound	
VEC	2.333 ± 2.07	NIC	-1.041	0.730	-3.829	1.746	0.666
		IVM 1	-0.166	0.999	-3.147	2.813	
		IVM 5	0.000	1.000	-2.980	2.980	
NIC	3.38 ± 1.85	VEC	1.041	0.730	-1.746	3.829	

		IVM 1	0.875	0.819	-1.913	3.663
		IVM 5	1.041	0.730	-1.746	3.829
IVM 1	2.50 ± 1.38	VEC	0.166	0.999	-2.813	3.147
		NIC	-0.875	0.819	-3.663	1.913
		IVM 5	0.166	0.999	-2.813	3.147
IVM 5	2.33 ± 2.07	VEC	0.000	1.000	-2.980	2.980
		NIC	-1.041	0.730	-3.829	1.746
		IVM 1	-0.166	0.999	-3.147	2.813

η²: 0.067; Group sample sizes VEC. IVM1 and IVM5: 6. NIC: 8; SD: Standard deviation; CI: Confidence interval; VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg.

Table 8c. Effects of nicotine on the locomotor activity of mice receiving Distance moved (cm)

(I) grup	Mean ± SD	(J) grup	Mean Difference (I-J)	p	95% CI		p
					Lower Bound	Upper Bound	
VEC	3199 ± 510	NIC	-656.9	0.557	-2032.9	719.1	0.505
		IVM 1	-713.5	0.544	-2184.5	757.5	
		IVM 5	-351.1	0.910	-1822.2	1119.8	
NIC	3855 ± 1447	VEC	656.9	0.557	-719.1	2032.9	
		IVM 1	-56.58	0.999	-1432.6	1319.4	
		IVM 5	305.7	0.926	-1070.2	1681.7	
IVM 1	3912 ± 328	VEC	713.5	0.544	-757.5	2184.5	
		NIC	56.58	0.999	-1319.4	1432.6	
		IVM 5	362.3	0.902	-1108.7	1833.3	
IVM 5	3550 ± 634	VEC	351.1	0.910	-1119.8	1822.2	
		NIC	-305.7	0.926	-1681.7	1070.2	
		IVM 1	-362.3	0.902	-1833.3	1108.7	

η²: 0.099; Group sample sizes VEC. IVM1 and IVM5: 6. NIC: 8; SD: Standard deviation; CI: Confidence interval; VEC: Vehicle control; NIC: Nicotine; IVM 1: Ivermectin 1 mg/kg; IVM 5: Ivermectin 5 mg/kg.