

Supplementary Information

Supplementary methods

The Supplementary Information contains complete descriptive and pairwise statistics for the six recorded behavioural outcomes, matched pre-administration and post-administration analyses in PC1 and PC2, distribution diagnostics and bounded sensitivity analyses. The animal procedures were approved by the MAHSA University Animal Ethics Committee (MUAEC; approval no. RMC/NOVEMBER/2025/AEC02; 21 November 2025). Prenatal VPA exposure was delivered as a single 600 mg/kg intraperitoneal injection on gestational day 12.5. Male offspring were weaned at PND21, began the postnatal administration schedule at PND35 and were tested after the final day-21 injection. PC1 denotes parecoxib 10 mg/kg and PC2 denotes parecoxib 20 mg/kg, both administered intraperitoneally once daily for 21 consecutive days. The parecoxib preparation was Dynastat, 40 mg per vial, registered in Malaysia under MAL20033926ARZ, with lot MF2144 and vial trace code PAA216488. At the recorded 20 mg/mL working concentration, the theoretical dose volumes were 0.5 mL/kg for PC1 and 1.0 mL/kg for PC2. Control and VPA offspring received 0.9% saline on the same schedule; the monitoring record used a nominal volume of 1 mL/100 g and recorded actual injection volumes against body weight. The analyses remain behavioural and do not establish target engagement or molecular mechanism.

Post-administration group comparisons used record-level rows with $n = 8$ male offspring per group. The source thesis stated that all 32 selected offspring contributed complete data to the principal analyses and that no animal met the withdrawal criteria. Tukey-adjusted comparisons were used after one-way ANOVA for self-grooming frequency and total distance. Games-Howell comparisons were used after Welch ANOVA for self-grooming duration and centre distance. Dunn-Holm comparisons were used after Kruskal-Wallis tests for centre entries and time in the centre. Mean-difference confidence intervals are reported for the parametric pairwise comparisons. Rank-biserial correlation is reported for rank-based comparisons. Exact source values are retained in the Source Data workbook. The experiment comprised 12 dams and 12 litters, and analyses were conducted on the recorded individual-offspring rows.

Behavioural testing began approximately 1 h after the final administration. Rats were acclimatized to the test room for 30 min. Tests were conducted at 22 ± 1 °C and 100–150 lux, and apparatuses were wiped with 75% alcohol after each use. Each behavioural session lasted 15 min, with the first 5 min used for adaptation and the final 10 min used for analysis. The self-grooming cage measured $30 \times 20 \times 20$ cm; the open-field arena measured $50 \times 50 \times 50$ cm, with a 25×25 cm centre zone. Self-grooming events included face washing, body or limb licking and fur grooming; consecutive actions separated by no more than 5 s were counted as one event. SMART 3.0 was used for tracking. Baseline-change coupling, activity-normalized centre-zone metrics, multidomain correlations, descriptive PCA, anchored scores, individual profiles, cross-domain paired-change associations and robustness displays are reported here as supplementary analyses. They were not treated as independent confirmatory endpoints. The pooled associations may partly reflect between-group separation. Because a change score mathematically contains the baseline value, baseline-change correlations are susceptible to mathematical coupling and regression-to-the-mean effects. These analyses are therefore descriptive and are not interpreted as evidence of a biological baseline-dependent response. Activity-normalized centre-zone metrics are exploratory normalization sensitivity analyses and may be affected by denominator-driven artefacts because total distance differs strongly among groups.

Supplementary Table S1. Descriptive statistics

Table 1: Descriptive statistics for post-administration outcomes. Values are calculated from record-level rows and are shown as mean, SD, median and interquartile range.

Dataset	Outcome	Group	<i>n</i>	Mean	SD	Median	Q1	Q3	Role
Self grooming	Frequency	Control	8	3.125	1.126	3.50	2.75	4.00	Supporting
Self grooming	Frequency	VPA	8	9.000	1.604	9.00	7.75	10.25	Supporting
Self grooming	Frequency	PC1	8	5.750	1.035	6.00	5.00	6.25	Supporting
Self grooming	Frequency	PC2	8	4.375	1.061	4.50	3.75	5.00	Supporting
Self grooming	Duration	Control	8	28.875	8.374	29.50	27.25	33.25	Principal
Self grooming	Duration	VPA	8	107.500	29.218	112.50	83.25	132.50	Principal
Self grooming	Duration	PC1	8	41.500	14.957	40.50	35.75	46.75	Principal
Self grooming	Duration	PC2	8	16.750	9.377	15.50	9.25	25.25	Principal
Open field	Total distance	Control	8	1992.214	382.263	1886.99	1827.96	1956.95	Supporting
Open field	Total distance	VPA	8	503.745	311.319	468.95	289.14	726.93	Supporting
Open field	Total distance	PC1	8	1321.729	367.807	1214.77	1065.81	1555.15	Supporting
Open field	Total distance	PC2	8	1523.194	298.382	1445.61	1282.34	1686.76	Supporting
Open field	Centre distance	Control	8	208.906	74.304	180.41	144.42	282.05	Exploratory
Open field	Centre distance	VPA	8	9.244	13.652	2.01	0.00	13.38	Exploratory
Open field	Centre distance	PC1	8	25.565	24.567	22.27	5.69	38.01	Exploratory
Open field	Centre distance	PC2	8	79.690	41.852	90.88	47.52	113.07	Exploratory
Open field	Centre entries	Control	8	12.875	3.227	13.00	11.25	13.50	Exploratory
Open field	Centre entries	VPA	8	2.250	3.576	0.50	0.00	2.75	Exploratory
Open field	Centre entries	PC1	8	2.500	2.268	2.00	1.00	3.25	Exploratory
Open field	Centre entries	PC2	8	5.000	1.690	5.00	4.00	6.25	Exploratory
Open field	Time in centre	Control	8	27.464	15.088	27.25	16.15	34.03	Exploratory
Open field	Time in centre	VPA	8	1.536	1.910	0.69	0.00	2.88	Exploratory
Open field	Time in centre	PC1	8	2.591	2.687	1.85	0.40	3.80	Exploratory
Open field	Time in centre	PC2	8	7.415	4.387	7.97	5.04	9.63	Exploratory

Supplementary Table S2. Pairwise comparisons

Table 2: Multiplicity-adjusted pairwise comparisons. Mean differences are Group 1 minus Group 2; key contrasts are reported as PC1 or PC2 minus VPA where applicable. Confidence intervals are 95% intervals from the corresponding Tukey or Games-Howell comparison when available. Effect estimates and effect-size intervals are retained from the record-level analysis.

Outcome	Method	Group 1	Group 2	Mean difference (95% CI)	Effect estimate (95% CI)	<i>p</i> _{adj}
Frequency	Tukey	Control	VPA	-5.88 [-7.36, -4.39]	Hedges <i>g</i> = -4.01 [-6.46, -3.34]	< 0.001

Outcome	Method	Group 1	Group 2	Mean difference (95% CI)	Effect estimate (95% CI)	p_{adj}
Frequency	Tukey	PC1	VPA	-3.25 [-4.70, -1.80]	Hedges $g = -2.28$ [-4.09, -1.57]	< 0.001
Frequency	Tukey	PC2	VPA	-4.63 [-6.08, -3.17]	Hedges $g = -3.22$ [-5.45, -2.49]	< 0.001
Frequency	Tukey	PC1	PC2	1.38	Hedges $g = 1.24$ [0.37, 2.61]	0.138
Duration	Games-Howell	Control	VPA	-78.63 [-112.90, -44.35]	Hedges $g = -3.46$ [-6.52, -2.73]	0.001
Duration	Games-Howell	PC1	VPA	-66.00 [-101.24, -30.76]	Hedges $g = -2.69$ [-5.19, -1.88]	0.001
Duration	Games-Howell	PC2	VPA	-90.75 [-125.09, -56.41]	Hedges $g = -3.95$ [-7.06, -3.19]	0.001
Duration	Games-Howell	PC1	PC2	24.75 [6.16, 43.34]	Hedges $g = 1.87$ [1.13, 3.63]	0.009
Total distance	Tukey	Control	VPA	1488.47 [1114.63, 1862.31]	Hedges $g = 4.04$ [3.29, 8.62]	< 0.001
Total distance	Tukey	PC1	VPA	817.98 [452.58, 1183.39]	Hedges $g = 2.27$ [1.64, 3.83]	< 0.001
Total distance	Tukey	PC2	VPA	1019.45 [692.46, 1346.44]	Hedges $g = 3.16$ [2.45, 5.06]	< 0.001
Total distance	Tukey	PC1	PC2	-201.46	Hedges $g = -0.57$ [-1.84, 0.34]	0.645
Centre distance	Games-Howell	Control	VPA	–	Hedges $g = 3.53$ [3.08, 6.35]	0.001
Centre distance	Games-Howell	PC1	VPA	16.32 [-13.61, 46.25]	Hedges $g = 0.78$ [-0.08, 1.90]	0.397
Centre distance	Games-Howell	PC2	VPA	70.45 [21.24, 119.65]	Hedges $g = 2.14$ [1.26, 5.37]	0.007
Centre distance	Games-Howell	PC1	PC2	-54.13	Hedges $g = -1.49$ [-3.63, -0.59]	0.038
Centre entries	Dunn-Holm	Control	VPA	–	Rank-biserial $r = 1.00$ [1.00, 1.00]	< 0.001
Centre entries	Dunn-Holm	PC1	VPA	–	Rank-biserial $r = 0.28$ [0.33, 0.84]	0.728
Centre entries	Dunn-Holm	PC2	VPA	–	Rank-biserial $r = 0.50$ [0.25, 1.00]	0.290
Centre entries	Dunn-Holm	PC1	PC2	–	Rank-biserial $r = -0.66$ [-1.00, -0.16]	0.379
Time in centre	Dunn-Holm	Control	VPA	–	Rank-biserial $r = 1.00$ [1.00, 1.00]	< 0.001
Time in centre	Dunn-Holm	PC1	VPA	–	Rank-biserial $r = 0.33$ [0.23, 0.81]	0.504
Time in centre	Dunn-Holm	PC2	VPA	–	Rank-biserial $r = 0.78$ [0.38, 1.00]	0.103
Time in centre	Dunn-Holm	PC1	PC2	–	Rank-biserial $r = -0.66$ [-1.00, -0.19]	0.237

Supplementary Table S3. Matched pre-record and post-record analyses

Table 3: Matched self-grooming analyses in PC1 and PC2. These analyses are descriptive within-group summaries.

Outcome	Analysis	Group or source	Statistic	df	<i>p</i>	Effect or change (95% CI)
Frequency	Repeated-measures time	PC1 + PC2	$F = 72.059$	1,15	< 0.001	partial $\eta^2 = 0.828$
Frequency	Repeated-measures group	PC1 vs PC2	$F = 2.098$	1,14	0.170	partial $\eta^2 = 0.130$
Frequency	Time $\times$ group	PC1 vs PC2	$F = 0.937$	1,14	0.349	partial $\eta^2 = 0.063$
Duration	Repeated-measures time	PC1 + PC2	$F = 36.882$	1,15	< 0.001	partial $\eta^2 = 0.711$
Duration	Repeated-measures group	PC1 vs PC2	$F = 6.071$	1,14	0.027	partial $\eta^2 = 0.302$
Duration	Time $\times$ group	PC1 vs PC2	$F = 0.905$	1,14	0.358	partial $\eta^2 = 0.061$
Frequency	Paired change	PC1	t	7	0.002	-3.88 [-5.79, -1.96]; paired $d_z = -1.69$
Frequency	Paired change	PC2	t	7	< 0.001	-4.88 [-6.39, -3.36]; paired $d_z = -2.70$
Duration	Paired change	PC1	t	7	0.004	-93.88 [-140.72, -47.03]; paired $d_z = -1.68$
Duration	Paired change	PC2	t	7	0.007	-68.38 [-111.06, -25.69]; paired $d_z = -1.34$

Supplementary Table S4. Sensitivity and diagnostic analyses

Table 4: Bounded sensitivity analyses and distribution diagnostics. Baseline-aware analyses were restricted to PC1 and PC2. This table contains both sensitivity checks and residual diagnostics; the separate robustness-estimation display is provided in Supplementary Figure S5.

Outcome	Analysis	Statistic	<i>p</i>	Interpretation boundary
Frequency	10,000-permutation ANOVA	$F = 33.937$	< 0.001	Supports the retained omnibus frequency result; fixed seed = 20260727.
Frequency	PC1 vs PC2 baseline check	$t = 0.377$	0.712	Descriptive baseline comparison within the two labels only.
Frequency	PC1 vs PC2 change check	$t = 0.968$	0.350	Does not estimate a time-adjusted effect against VPA.
Frequency	Baseline-adjusted post comparison	$t = -2.492$	0.027	ANCOVA sensitivity restricted to PC1 and PC2.
Duration	PC1 vs PC2 baseline check	$t = 1.795$	0.095	Descriptive baseline comparison within the two labels only.
Duration	PC1 vs PC2 change check	$t = -0.951$	0.358	Does not estimate a time-adjusted effect against VPA.
Duration	Baseline-adjusted post comparison	$t = -3.122$	0.008	ANCOVA sensitivity restricted to PC1 and PC2.
Frequency	Residual normality diagnostic	Shapiro $p = 0.326$	–	Diagnostic only; does not establish population normality at $n = 8$ per group.

Outcome	Analysis	Statistic	p	Interpretation boundary
Duration	Residual normality diagnostic	Shapiro	– $p = 0.693$	Diagnostic only; variance heterogeneity supported Welch inference.
Total distance	Residual normality diagnostic	Shapiro	– $p = 0.010$	Diagnostic only; the retained omnibus test remained one-way ANOVA with permutation sensitivity for frequency only.
Centre distance	Residual normality diagnostic	Shapiro	– $p = 0.408$	Diagnostic only; variance heterogeneity supported Welch inference.

Supplementary Table S5. Baseline-change coupling

Table 5: Descriptive associations between the available pre-record value and the post-minus-pre change in matched PC1 and PC2 records. Because the change score mathematically contains the baseline value, these correlations are susceptible to mathematical coupling and regression-to-the-mean effects. They are not interpreted as evidence of a biological baseline-dependent response.

Outcome	n	Spearman ρ	Spearman p	Linear slope	Linear intercept	Linear p	Interpretation boundary
Frequency	16	-0.791	< 0.001	-0.865	3.787	< 0.001	Descriptive association in matched PC1 and PC2 records; it does not establish a time-adjusted intervention effect.
Duration	16	-0.926	< 0.001	-0.854	13.007	< 0.001	Descriptive association in matched PC1 and PC2 records; it does not establish a time-adjusted intervention effect.

Supplementary Table S6. Locomotion-normalized centre-zone sensitivity

Table 6: Summary of locomotion-normalized centre-zone analyses. Values are mean (SD) by group; overall tests are Kruskal-Wallis tests and pairwise values are Dunn-Holm adjusted. All metrics are exploratory. Distance is expressed on the recorded exported scale. The ratios may also be affected by denominator-driven artefacts because total distance differs strongly among groups.

Metric	Control	VPA	PC1	PC2	Overall p	VPA versus parecoxib-group interpretation
Centre distance fraction (%)	10.62 (3.91)	2.89 (3.69)	2.30 (2.48)	5.38 (3.23)	0.002	PC1 $p_{\text{adj}} = 0.873$; PC2 $p_{\text{adj}} = 0.306$.
Centre entries per 1,000 recorded tracking units	6.64 (2.03)	19.24 (41.80)	2.16 (2.07)	3.30 (1.01)	0.006	PC1 $p_{\text{adj}} = 0.631$; PC2 $p_{\text{adj}} = 0.306$.
Centre time per 1,000 recorded tracking units	14.35 (8.71)	11.63 (25.70)	2.34 (2.60)	4.94 (3.43)	0.008	PC1 $p_{\text{adj}} = 0.873$; PC2 $p_{\text{adj}} = 0.855$.

Supplementary Table S7. Cross-domain associations in matched PC1 and PC2 records

Table 7: Exploratory associations between paired self-grooming changes and open-field or anchored-score measures in the 16 matched PC1 and PC2 records. Holm correction was applied across the six candidate associations shown. These correlations are descriptive and do not establish causality, a time-adjusted intervention effect or a common mechanism.

X variable	Y variable	n	Spearman ρ	Raw p	Holm p	Interpretation boundary
Frequency change, post minus pre	Total distance	16	0.196	0.468	1.000	Exploratory association in matched PC1 and PC2 records; not a causal or time-adjusted intervention analysis.
Frequency change, post minus pre	Centre distance	16	0.037	0.891	1.000	Exploratory association in matched PC1 and PC2 records; not a causal or time-adjusted intervention analysis.
Duration change, post minus pre	Total distance	16	0.009	0.974	1.000	Exploratory association in matched PC1 and PC2 records; not a causal or time-adjusted intervention analysis.
Duration change, post minus pre	Centre distance	16	0.585	0.017	0.103	Exploratory association in matched PC1 and PC2 records; not a causal or time-adjusted intervention analysis.
Duration change, post minus pre	Primary-key control-like score	16	0.168	0.535	1.000	Exploratory association in matched PC1 and PC2 records; not a causal or time-adjusted intervention analysis.
Frequency change, post minus pre	Duration change, post minus pre	16	0.078	0.775	1.000	Exploratory association in matched PC1 and PC2 records; not a causal or time-adjusted intervention analysis.

Supplementary Table S8. Administration and clinical-observation summary

Table 8: Administration and clinical-observation records during the 21-day dosing period. Daily dosing records were complete for all animals (21 days $\times$ 8 animals = 168 injection records per group). Clinical observations were recorded once daily per animal; counts are numbers of animal-day records. These records are descriptive monitoring documentation and are not interpreted as a systematic tolerability or adverse-event assessment.

Group	Treatment	Nominal dose	Dosing days	Injection records	Clinical observations (animal-day counts)
Control	0.9% saline	1 mL/100 g body weight	Day 1–21	168 (8 animals $\times$ 21)	Normal 176; no other observations recorded
VPA	0.9% saline	1 mL/100 g body weight	Day 1–21	168 (8 animals $\times$ 21)	Normal 168; mildly ruffled fur 2; mild piloerection 2; slightly reduced activity 2; reduced activity 1; ruffled fur 1
PC1	Parecoxib	10 mg/kg	Day 1–21	168 (8 animals $\times$ 21)	Normal 176; no other observations recorded
PC2	Parecoxib	20 mg/kg	Day 1–21	168 (8 animals $\times$ 21)	Normal 172; alert 2; reduced activity 2

Supplementary Figure S1. Standardized centre-zone outcomes

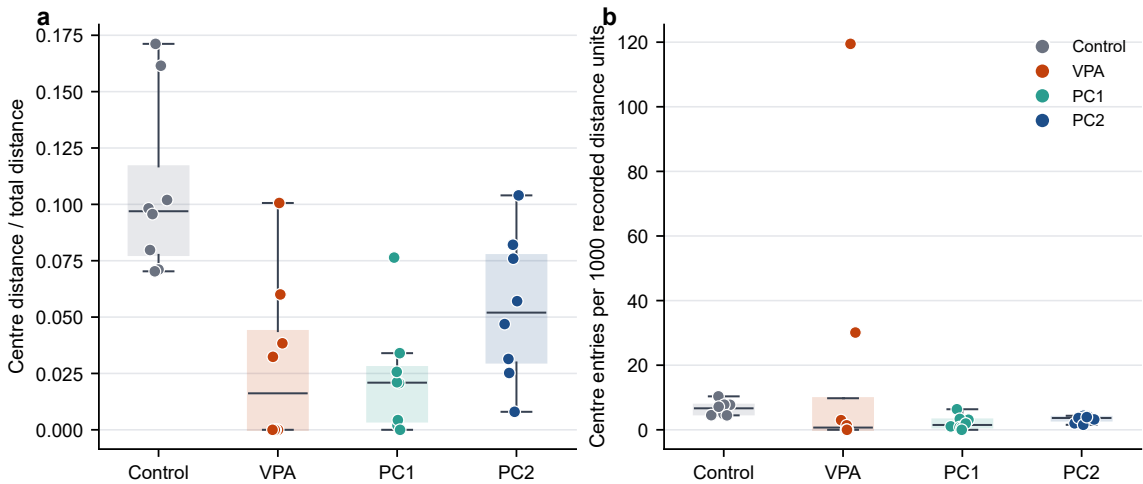


Figure 1: Standardized centre-zone outcomes shown for descriptive comparison. Standardization does not change the inferential hierarchy or convert centre-zone measures into a single construct.

Supplementary Figure S2. Distribution diagnostics

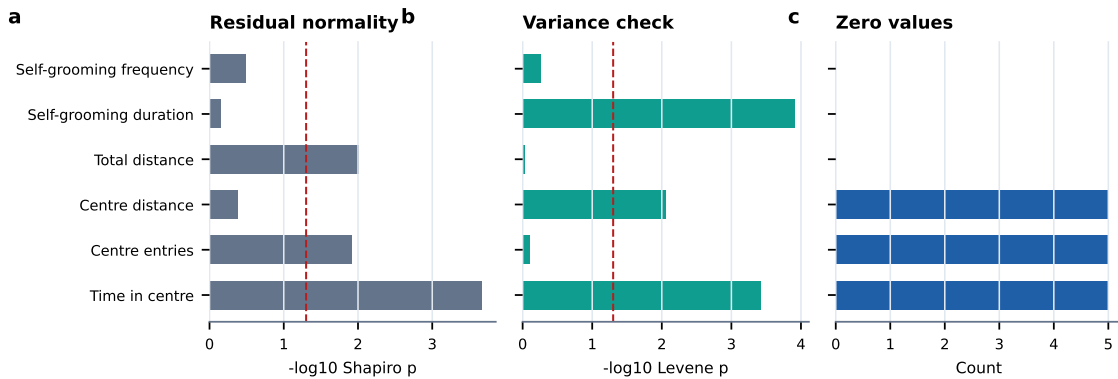


Figure 2: Distribution and residual diagnostics used to document the retained Welch or rank-based procedures.

Supplementary Figure S3. Baseline-change coupling

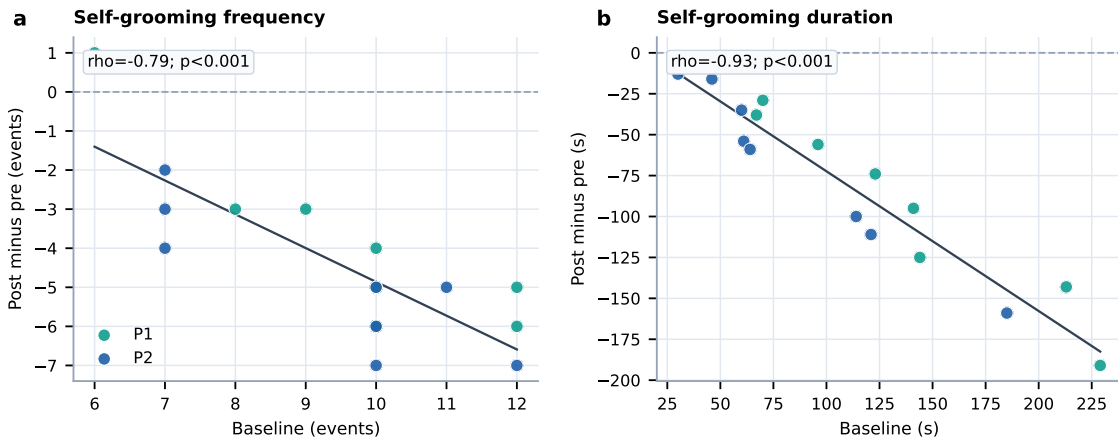


Figure 3: Relationship between available pre-record values and post-minus-pre changes for PC1 and PC2. Because the change score contains the baseline value, these relationships are descriptive and are not interpreted as evidence of a biological baseline-dependent response. Matched trajectories are shown in the main manuscript as Fig. 4.

Supplementary Figure S4. Locomotion-normalized centre-zone outcomes

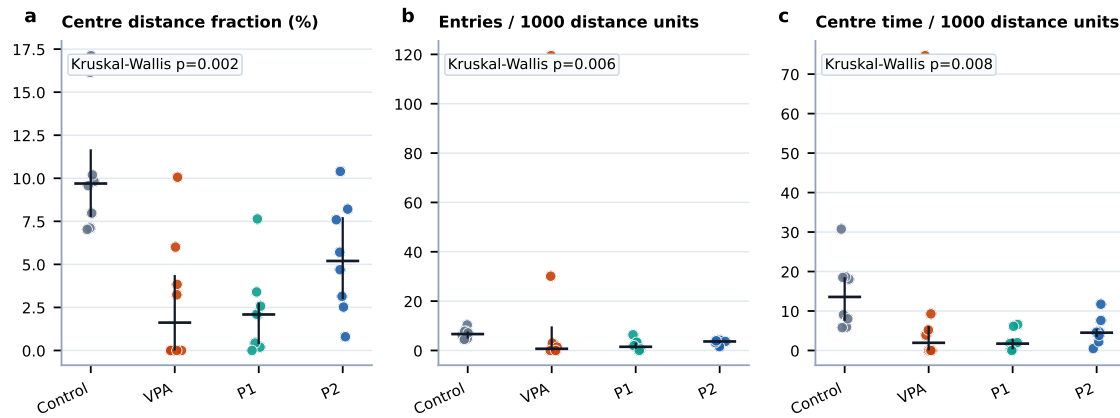


Figure 4: Exploratory centre-zone measures displayed relative to total recorded distance. These ratios are descriptive normalization sensitivity analyses and are not treated as additional confirmatory endpoints.

Supplementary Figure S5. Robustness estimation

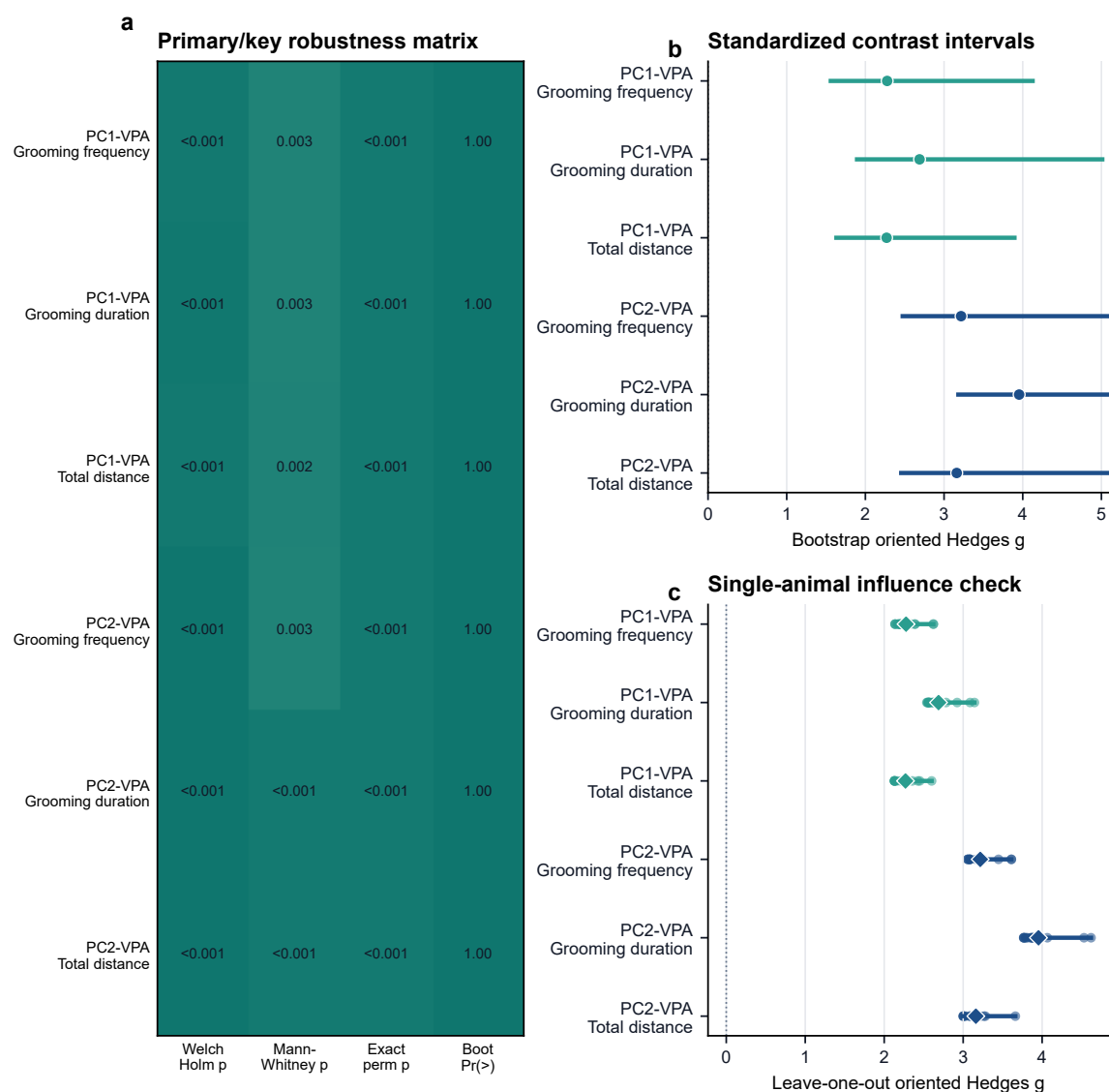
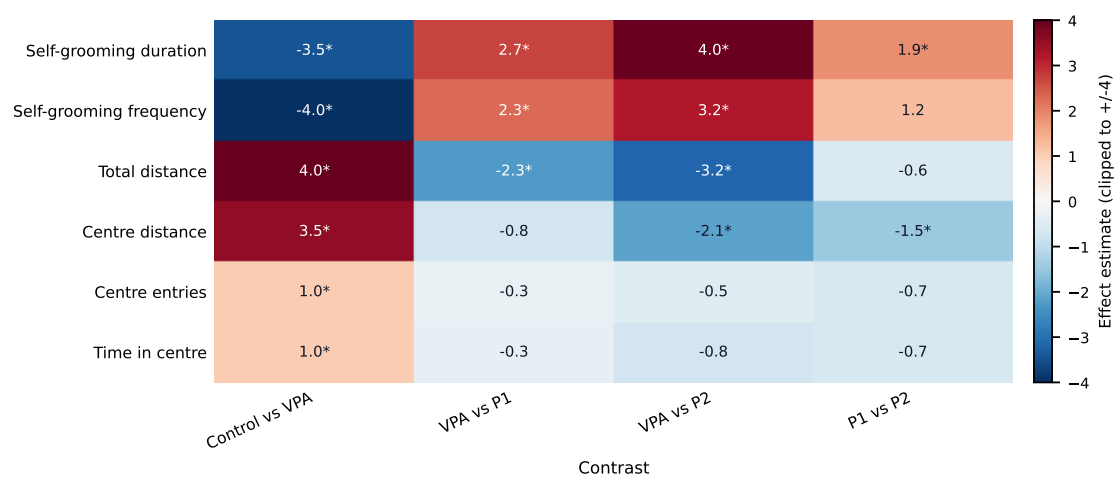


Figure 5: Robustness analyses for the core PC1-versus-VPA and PC2-versus-VPA contrasts. The analyses evaluate stability of the recorded behavioural differences under alternative procedures and do not resolve intervention identity, dose, litter structure or mechanism.

Supplementary Figure S6. Contrast direction summary



* adjusted $p < 0.05$. Effect type differs by endpoint and is listed in the source table.

Figure 6: Direction of selected group contrasts across the retained behavioural outcomes. Each endpoint is displayed separately for descriptive comparison.

Supplementary Figure S7. Multidomain structure

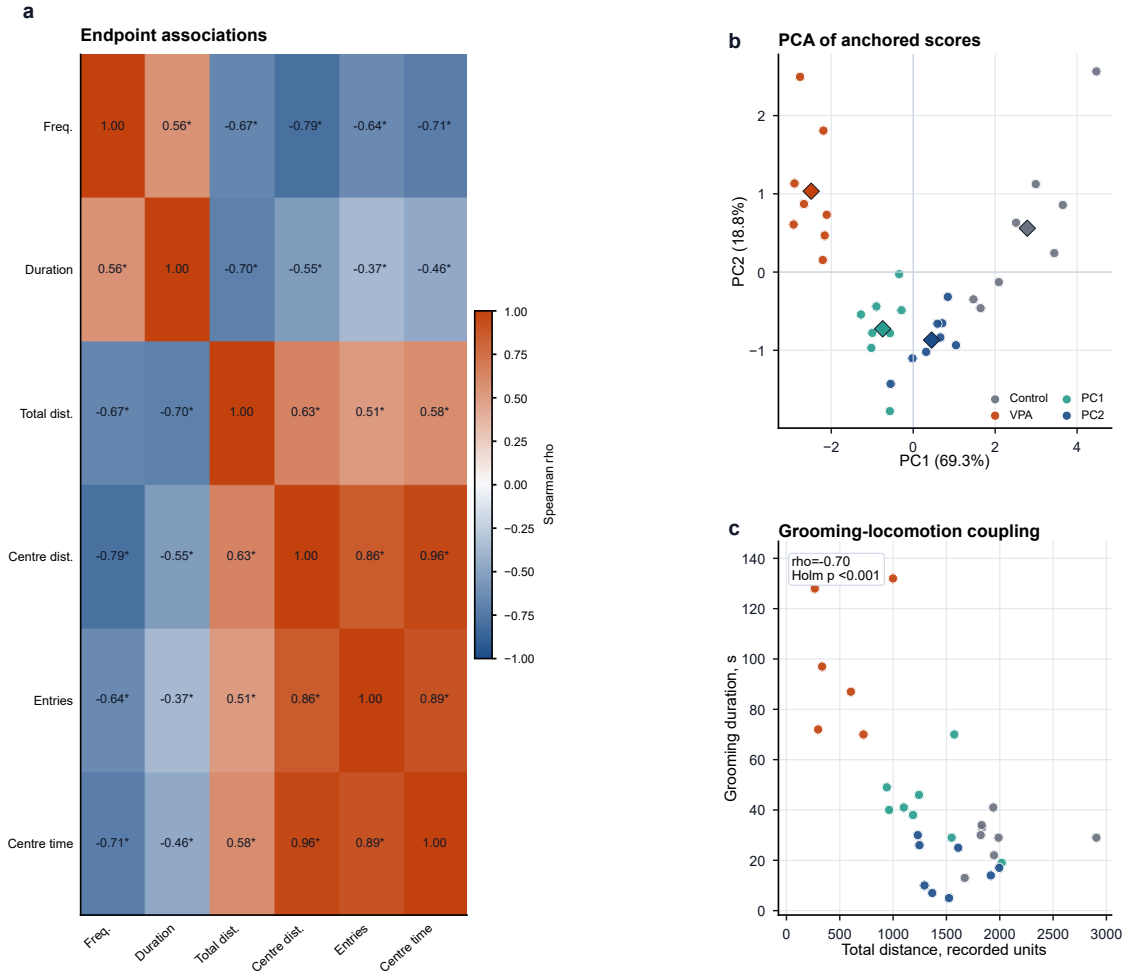


Figure 7: Descriptive multidomain correlation and principal-component visualization. Pooled associations may partly reflect between-group separation and should not be interpreted as evidence of within-group behavioural coupling. PCA is descriptive and is not independent validation.

Supplementary Figure S8. Individual endpoint profiles

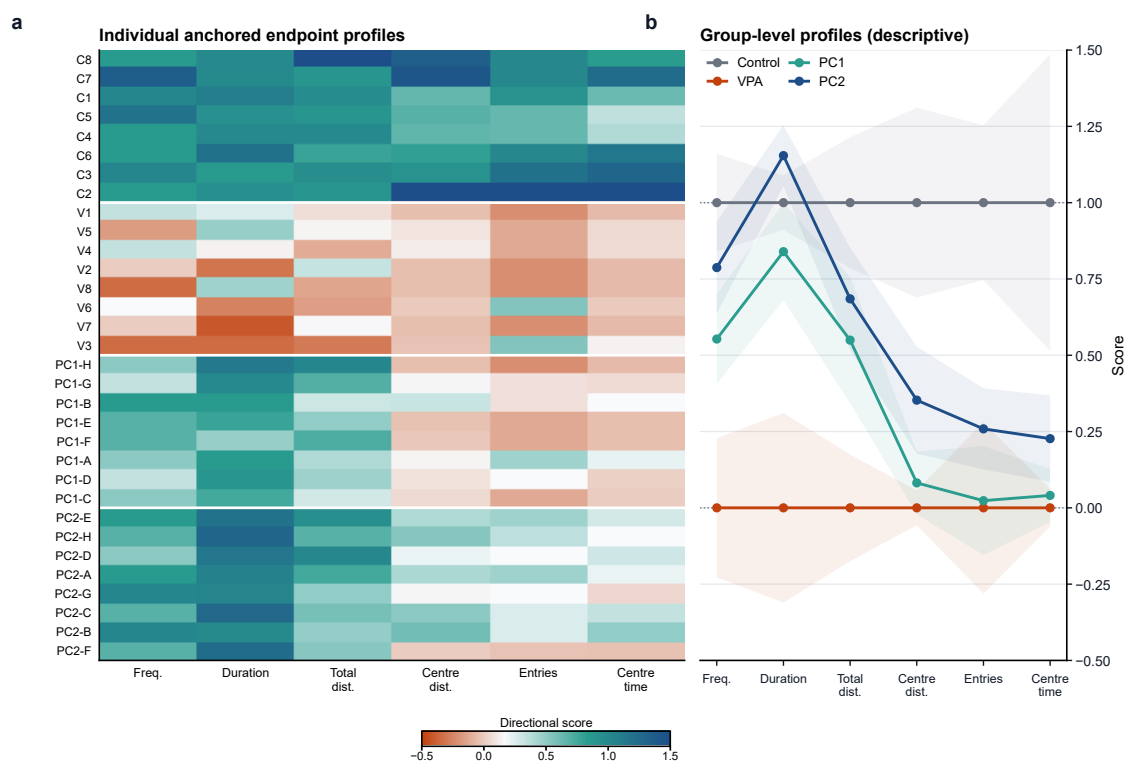


Figure 8: Individual and group-level endpoint profiles based on anchored descriptive scores. The scores are an orientation device and do not represent causal response, recovery, responder classification or dose.

Supplementary Figure S9. Cross-domain paired-change associations

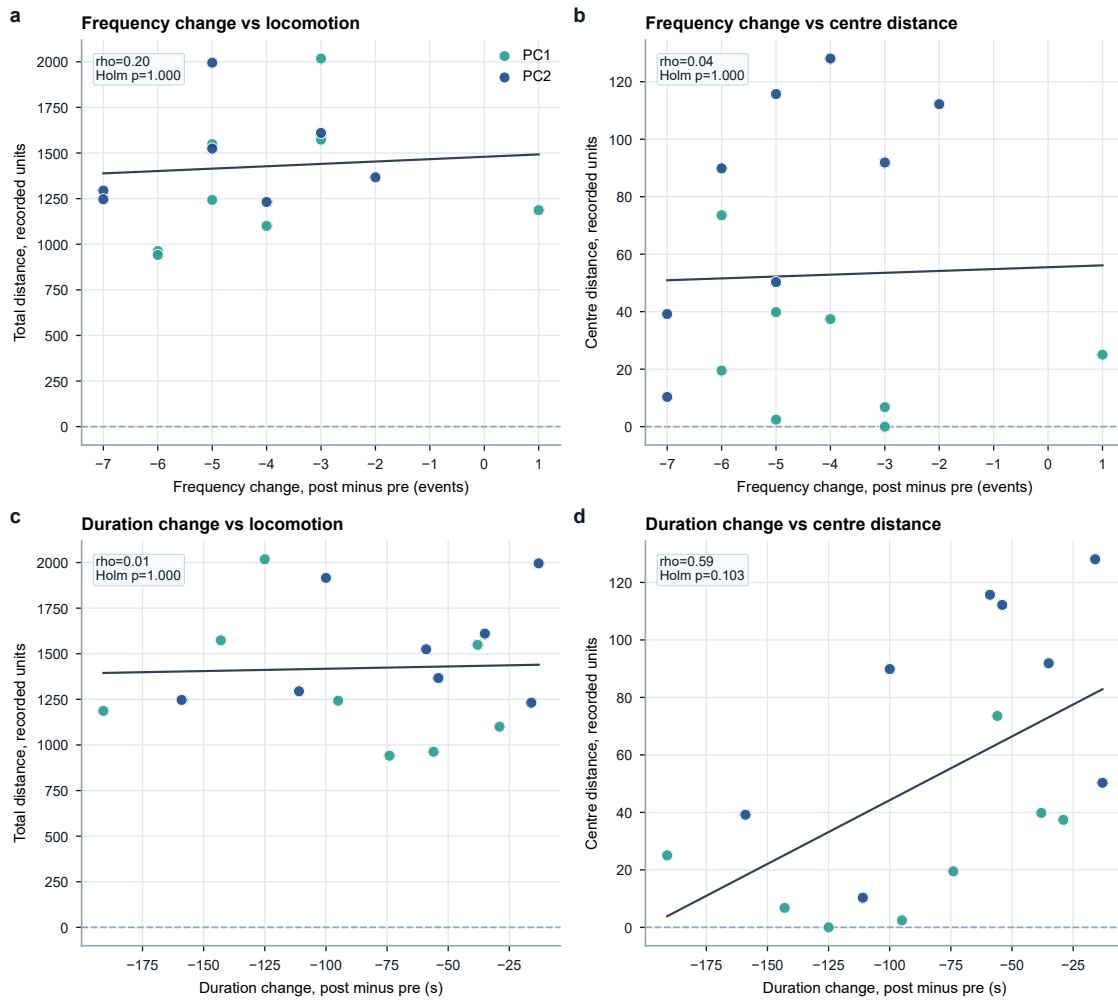


Figure 9: Exploratory cross-domain associations in the matched PC1 and PC2 records. The associations are descriptive, may be influenced by the limited matched sample, and do not establish causality, a time-adjusted intervention effect or a common mechanism.