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Article

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Posted Date: October 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10784918/v1>

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Additional Declarations: No competing interests reported.

Self-grooming and locomotor activity after parecoxib administration in a prenatal valproic acid rat model

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Abstract

Prenatal valproic acid (VPA) exposure models selected neurodevelopmental behavioural domains, but no single rodent assay represents autism spectrum disorder as a whole. We analysed individual-animal records from male Sprague-Dawley offspring in Control, VPA, parecoxib 10 mg/kg and 20 mg/kg groups, with eight recorded animals per group. Offspring were weaned at PND21, began postnatal dosing at PND35 and received daily intraperitoneal parecoxib or saline for 21 days. Behavioural testing began about 1 h after the final administration. Each 15-min open-field and self-grooming session used the first 5 min for adaptation and the final 10 min for analysis. Self-grooming frequency differed among groups (one-way ANOVA $F_{3,28} = 33.94$, $p < 0.001$), as did duration (Welch ANOVA $F_{3,14.82} = 23.54$, $p < 0.001$); parecoxib groups showed lower values than VPA. Total open-field distance also differed among groups (one-way ANOVA $F_{3,28} = 26.44$, $p < 0.001$); both parecoxib groups showed higher recorded distance than VPA. Centre-zone outcomes were less consistent and treated as exploratory. These findings support behavioural differences associated with the parecoxib groups in the VPA model, but do not establish clinical efficacy, a dose-response relationship, target engagement or a molecular mechanism.

Keywords: valproic acid, self-grooming, repetitive behaviour, open-field test, locomotor activity, retrospective analysis

047 1 Introduction

048
049 Autism spectrum disorder is a heterogeneous neurodevelopmental condition that
050 includes social-communication differences and restricted or repetitive patterns of
051 behaviour. Animal models therefore represent selected constructs rather than the full
052 clinical phenotype. Prenatal valproic acid (VPA) exposure is widely used to study neu-
053 rodevelopmental alterations, but its behavioural profile depends on gestational timing,
054 dose, route, strain, sex, developmental stage and the assay battery [1–5]. Recent work
055 has consequently emphasized model heterogeneity, protocol dependence and the need
056 to interpret each behavioural endpoint according to the construct it measures [6–8].

057 Self-grooming provides an operational readout related to repetitive or stereotyped
058 behaviour when frequency or cumulative duration differs between groups. It does not
059 measure social communication, social preference, cognition or the broader heterogene-
060 ity of autism-related phenotypes [9–11]. The open-field test provides complementary
061 information about locomotor and exploratory activity. Total distance, centre distance,
062 centre entries and time in the centre are related but non-interchangeable outcomes, and
063 centre-zone measures are sensitive to apparatus dimensions, illumination, test dura-
064 tion and tracking settings [12, 13]. Total distance should therefore be interpreted as
065 recorded locomotor or exploratory activity rather than as a direct measure of anxiety.

066 Neuroinflammation has been implicated in the neurodevelopmental alterations
067 associated with prenatal VPA exposure and in the pathophysiology of autism-related
068 phenotypes more broadly [14]. Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme in
069 prostaglandin synthesis and a central node of inflammatory signalling in the brain. In
070 rodent models, inhibition of prostaglandin endoperoxide synthase 2 (PTGS2)/COX-
071 2 attenuated hippocampal neuroinflammation and improved cognitive function in a
072 postoperative cognitive dysfunction model [15], and the selective COX-2 inhibitor
073 parecoxib reduced lipopolysaccharide-induced microglial activation in vitro [16]. These
074 observations provide a pharmacological rationale for examining whether COX-2 inhi-
075 bition modifies behavioural readouts in the prenatal VPA model. The present study
076 therefore reports an exploratory two-dose parecoxib comparison in the VPA model,
077 with self-grooming and open-field outcomes interpreted strictly at the behavioural
078 level.

079 Recent preclinical studies using VPA and other ASD-related models commonly
080 combine behavioural assays with social testing, cognition, histology, circuit measure-
081 ments, inflammatory markers, mitochondrial measures or other biological readouts
082 [17–22]. Such designs can address complementary biological questions, but they also
083 require clear separation between observed behaviour and proposed mechanism. The
084 present study adds a documented two-dose parecoxib comparison to the available
085 behavioural evidence, while retaining a clear boundary around what behaviour alone
086 can establish. Self-grooming and open-field records cannot determine target engage-
087 ment or a molecular pathway, even when the compound identity and dose are
088 known.

089 This study aimed to characterize behavioural differences across the recorded
090 parecoxib groups in individual-animal records from a completed prenatal VPA rat
091 experiment. The analysis retains individual observations, distinguishes the principal
092

behavioural outcomes from exploratory centre-zone measures, and reports the available paired records without treating them as a time-adjusted comparison with the VPA group.

This study makes three contributions to the behavioural evidence base for the prenatal VPA model. First, it reports individual-animal behavioural records rather than group summaries alone, in line with recent calls to account for protocol dependence and model heterogeneity when interpreting VPA-related behavioural outcomes [1, 3, 6–8]. Second, it presents the complete recorded open-field profile (total distance, centre distance, centre entries and time in the centre) alongside self-grooming frequency and duration, so that the behavioural pattern is evaluated as a whole rather than through a single selected endpoint [9, 10, 12, 13]. Third, it adds descriptive 21-day body-weight monitoring and daily administration records as general health-monitoring context, a dimension that is often omitted from behavioural studies focused on a single assay. Together, these features provide a more complete description of the recorded experiment than the behavioural outcomes alone, while the evidence remains exploratory and does not establish clinical efficacy, a dose-response relationship, target engagement or a molecular mechanism.

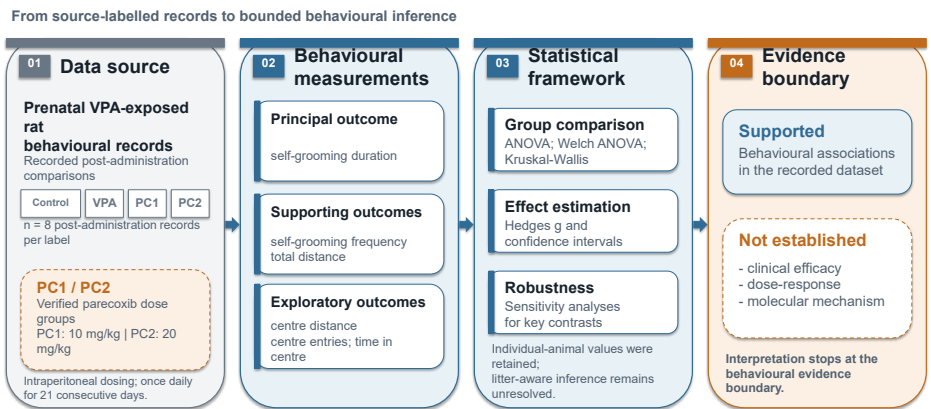


Fig. 1 Data-to-evidence framework for the behavioural analysis. The four modules show the study records, the behavioural measurements and reporting hierarchy, the statistical analyses, and the boundary of supported inference. Self-grooming duration was the principal outcome; self-grooming frequency and total distance were supporting outcomes; centre-zone measures were exploratory. PC1 denotes parecoxib 10 mg/kg and PC2 denotes parecoxib 20 mg/kg, both administered intraperitoneally for 21 consecutive days. The framework summarizes behavioural evidence and does not establish clinical efficacy, target engagement or molecular mechanism.

2 Results

2.1 Dataset overview and analysis scope

The post-administration records contained four groups: Control, VPA, PC1 (parecoxib 10 mg/kg) and PC2 (parecoxib 20 mg/kg). Each group contributed eight recorded animals to the group-level self-grooming and open-field analyses. Matched

pre-administration and post-administration self-grooming records in PC1 and PC2 gave 16 matched records for descriptive within-group analyses. The principal outcome was self-grooming duration; frequency and total distance were supporting outcomes; centre distance, centre entries and time in the centre were exploratory. The outcome hierarchy was established for reporting during analysis and was not prospectively preregistered. Core post-administration outcomes are summarized in Table 1; complete pairwise comparisons and sensitivity analyses are provided in the Supplementary Information.

2.2 Self-grooming outcomes

Self-grooming frequency differed among groups (one-way ANOVA $F_{3,28} = 33.94$, $p < 0.001$, $\omega^2 = 0.755$). Mean frequency was 3.12 events (SD 1.13) in Control, 9.00 (SD 1.60) in VPA, 5.75 (SD 1.04) in PC1 and 4.38 (SD 1.06) in PC2. Tukey-adjusted comparisons showed higher frequency in VPA than Control and lower frequency in both parecoxib groups than VPA (all $p < 0.001$); PC1 and PC2 did not differ ($p = 0.138$).

Self-grooming duration also differed among groups (Welch ANOVA $F_{3,14.82} = 23.54$, $p < 0.001$). Mean duration was 28.88 s (SD 8.37) in Control, 107.50 s (SD 29.22) in VPA, 41.50 s (SD 14.96) in PC1 and 16.75 s (SD 9.38) in PC2. Games-Howell comparisons showed higher duration in VPA than Control ($p = 0.001$), lower duration in both parecoxib groups than VPA (both $p = 0.001$), and lower duration in PC2 than PC1 ($p = 0.009$). Oriented Hedges g values for the VPA-PC1 and VPA-PC2 contrasts were 2.69 and 3.95, respectively. Figure 2 shows the individual observations and group distributions.

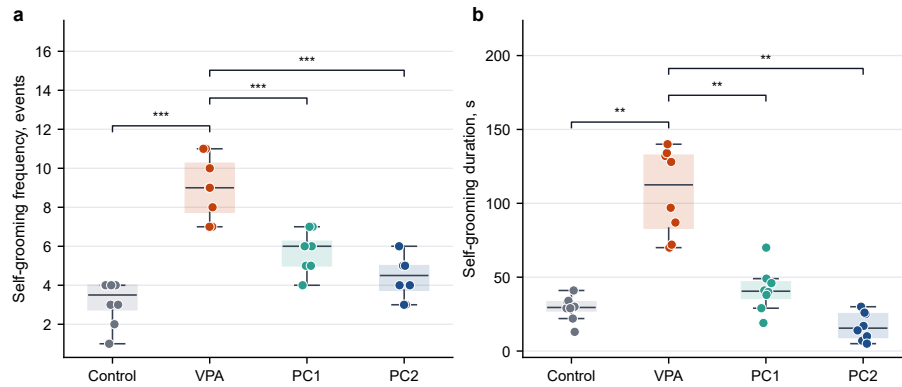


Fig. 2 Post-administration self-grooming outcomes. Panel (a) shows frequency and panel (b) shows cumulative duration. Points represent individual recorded animals ($n = 8$ per group); boxes show the interquartile range, centre lines show medians, and whiskers extend to the most extreme values within 1.5 times the interquartile range. Brackets show the adjusted comparisons displayed in the panels: Control versus VPA, VPA versus PC1 and VPA versus PC2. PC1 denotes parecoxib 10 mg/kg and PC2 denotes parecoxib 20 mg/kg. Asterisks denote adjusted $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). Exact adjusted p values, effect sizes and confidence intervals are provided in Supplementary Table S2 and Source Data.

2.3 Open-field locomotor activity

Total open-field distance differed among groups (one-way ANOVA $F_{3,28} = 26.44$, $p < 0.001$, $\omega^2 = 0.705$). Mean distance was 1992.21 recorded tracking units (SD 382.26) in Control, 503.75 (SD 311.32) in VPA, 1321.73 (SD 367.81) in PC1 and 1523.19 (SD 298.38) in PC2. Tukey-adjusted comparisons showed lower distance in VPA than Control and higher distance in both parecoxib groups than VPA (all $p < 0.001$); PC1 and PC2 did not differ ($p = 0.645$). Figure 3 presents total distance together with the exploratory centre-zone outcomes.

Centre-zone measures were less consistent. Centre distance differed overall (Welch ANOVA $F_{3,13.85} = 22.18$, $p < 0.001$), but PC1 did not differ from VPA after Games-Howell adjustment ($p = 0.397$); PC2 exceeded VPA ($p = 0.007$). Centre entries and time in the centre also differed overall (Kruskal-Wallis $H = 20.70$, $p < 0.001$, and $H = 22.04$, $p < 0.001$, respectively), but neither PC1 nor PC2 differed from VPA after Dunn-Holm adjustment. These outcomes are retained as exploratory and are not interpreted as direct anxiety measures. All four open-field outcomes are displayed in Fig. 3; expanded centre-zone displays and activity-normalized sensitivity analyses are provided in Supplementary Figures S1 and S4.

Table 1 Core post-administration behavioural outcomes. Values are mean (SD), $n = 8$ recorded animals per group. PC1 denotes parecoxib 10 mg/kg and PC2 denotes parecoxib 20 mg/kg. The table contains the principal outcome and the two supporting outcomes. Overall p values are from one-way ANOVA for self-grooming frequency and total distance and Welch ANOVA for self-grooming duration. Complete adjusted pairwise comparisons, effect sizes and confidence intervals are provided in Supplementary Table S2.

Outcome	Control	VPA	PC1	PC2	Overall p
Self-grooming frequency, events	3.12 (1.13)	9.00 (1.60)	5.75 (1.04)	4.38 (1.06)	< 0.001
Self-grooming duration, s	28.88 (8.37)	107.50 (29.22)	41.50 (14.96)	16.75 (9.38)	< 0.001
Total distance, recorded tracking units	1992.21 (382.26)	503.75 (311.32)	1321.73 (367.81)	1523.19 (298.38)	< 0.001

2.4 Supporting paired analyses

Matched pre-administration and post-administration self-grooming records were analysed for PC1 and PC2. Within PC1, the mean frequency change was -3.88 events (95% CI -5.79 to -1.96 ; Holm-adjusted $p = 0.002$); within PC2, the mean change was -4.88 events (95% CI -6.39 to -3.36 ; Holm-adjusted $p < 0.001$). For duration, the corresponding changes were -93.88 s (95% CI -140.72 to -47.03 ; $p = 0.004$) in PC1 and -68.38 s (95% CI -111.06 to -25.69 ; $p = 0.007$) in PC2. These paired analyses are descriptive within-group summaries rather than evidence of a causal treatment response. The matched trajectories are shown in Fig. 4; baseline-change coupling is reported in Supplementary Table S5 and Supplementary Fig. S3 because the change score mathematically contains the baseline value.

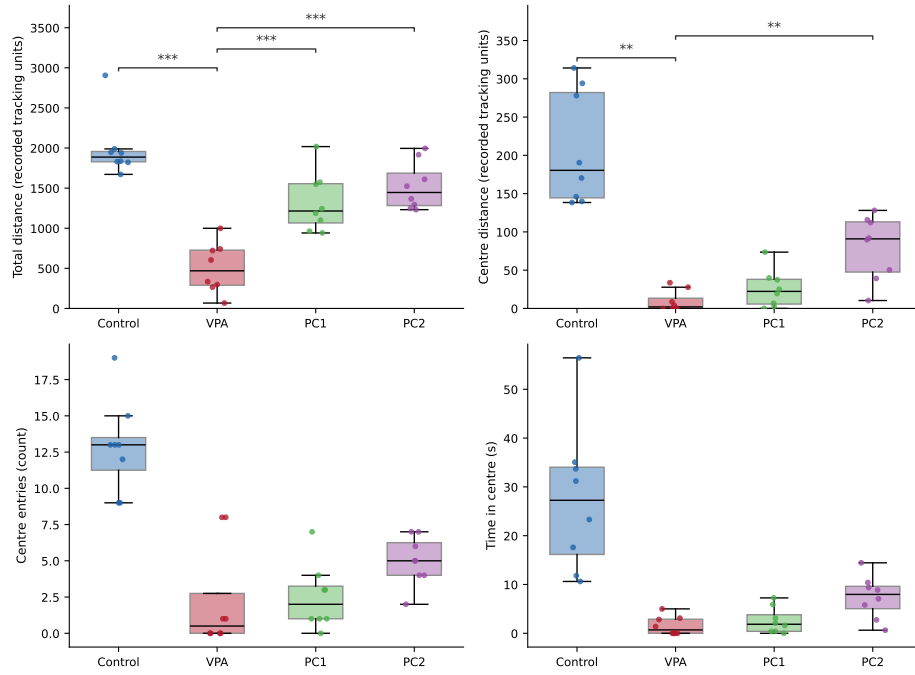


Fig. 3 Open-field outcomes in the four recorded groups. Panel (a) shows total distance, panel (b) shows centre distance, panel (c) shows centre entries and panel (d) shows time in the centre. Points represent individual recorded animals ($n = 8$ per group); boxes show the interquartile range, centre lines show medians, and whiskers extend to the most extreme values within 1.5 times the interquartile range. Total distance is expressed in recorded tracking units on the exported recorded scale. In panel (a), brackets show Tukey-adjusted comparisons for Control versus VPA, VPA versus PC1 and VPA versus PC2 (all $p < 0.001$). In panel (b), brackets show Games-Howell-adjusted comparisons for Control versus VPA ($p = 0.001$) and VPA versus PC2 ($p = 0.007$); the VPA-versus-PC1 comparison was not significant ($p = 0.397$) and is not displayed. No VPA-versus-parecoxib pairwise comparison was significant for centre entries or time in the centre after Dunn-Holm adjustment, and panels (c) and (d) therefore show no comparison brackets. PC1 denotes parecoxib 10 mg/kg and PC2 denotes parecoxib 20 mg/kg. Centre-zone outcomes are exploratory and are not interpreted as direct anxiety measures.

2.5 Core effect-size summary

The core effect-size pattern was concentrated in self-grooming frequency, self-grooming duration and total distance. For the PC1-versus-VPA contrasts, oriented Hedges g values were 2.28, 2.69 and 2.27 for frequency, duration and total distance, respectively. The corresponding PC2-versus-VPA values were 3.22, 3.95 and 3.16. Centre-zone effect estimates were less uniform and are not included in the main effect-size summary. Figure 5 displays the core estimates with bootstrap 95% intervals. Bounded sensitivity checks and distribution diagnostics are provided in Supplementary Table S4, whereas the separate robustness-estimation display is provided in Supplementary Fig. S5.

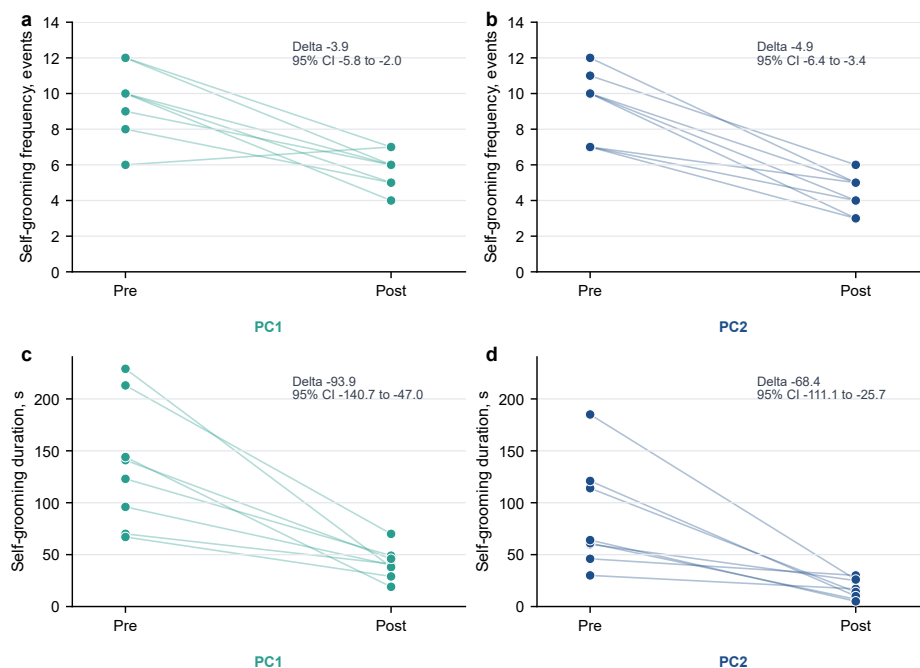


Fig. 4 Matched pre-administration and post-administration self-grooming records in PC1 and PC2. PC1 denotes parecoxib 10 mg/kg and PC2 denotes parecoxib 20 mg/kg. Panels (a) and (b) show frequency in PC1 and PC2, respectively; panels (c) and (d) show cumulative duration. Lines connect values from the same recorded animal ($n = 8$ per group). Delta values are mean post-minus-pre changes with 95% confidence intervals. The display is descriptive.

2.6 Body-weight monitoring during the administration period

Body weight was recorded daily, providing 22 time points for each of the 32 recorded animals: a pre-dose baseline on Day 0 followed by the 21 administration days (Day 1–21), with no missing weight records. Body weight increased steadily in all four groups. From Day 0 to Day 21, mean body weight rose from 109.38 g (SD 5.01) to 251.62 g (SD 11.89) in Control, from 99.88 g (SD 8.53) to 195.00 g (SD 12.02) in VPA, from 110.62 g (SD 8.99) to 210.75 g (SD 9.22) in PC1 and from 117.75 g (SD 9.47) to 224.12 g (SD 8.71) in PC2. The VPA group had the lowest mean body weight at most time points, whereas PC2 had the highest baseline weight. These weight records are reported as general health-monitoring context; they are descriptive and are not interpreted as evidence of a treatment-specific effect on growth. Fig. 6 shows the group-level weight trajectories. Daily administration was completed for all animals, and the recorded clinical observations were predominantly unremarkable; a summary of the administration and observation records is provided in Supplementary Table S8.

3 Discussion

This analysis identified behavioural differences associated with the recorded parecoxib groups in self-grooming and recorded locomotor activity. Relative to Control, the VPA

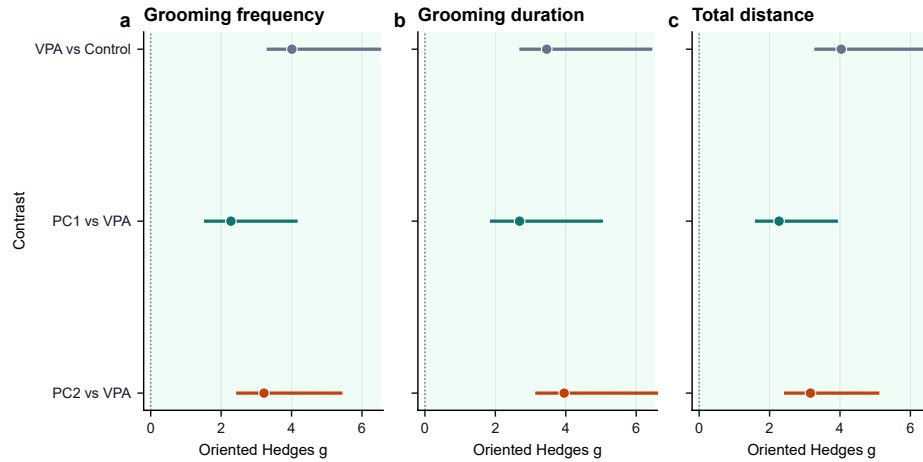


Fig. 5 Core endpoint effect sizes. Panels (a)–(c) show oriented Hedges g estimates for self-grooming frequency, self-grooming duration and total distance, respectively. Each panel contains the three retained reporting contrasts: VPA versus Control, PC1 versus VPA and PC2 versus VPA. For the VPA-versus-Control contrast, positive values were oriented in the observed VPA-labelled direction relative to Control. For the PC1-versus-VPA and PC2-versus-VPA contrasts, positive values indicate a difference in the direction of the Control-labelled mean. Points are oriented Hedges g estimates and horizontal bars show bootstrap 95% intervals. Centre-zone endpoints and the PC1-versus-PC2 contrast are not included in this core summary. This sign convention is statistical and does not imply efficacy, normalization, dose response or mechanism.

group had higher self-grooming frequency and duration and lower total distance. Relative to VPA, the 10-mg/kg and 20-mg/kg parecoxib groups had lower self-grooming values and higher total distance. The paired records within these groups showed lower post-administration self-grooming values; however, because matched Control and VPA baselines were not available, these within-group comparisons cannot be interpreted as time-adjusted treatment effects.

Self-grooming was the clearest behavioural signal. Its frequency and cumulative duration can serve as operational readouts related to repetitive behaviour, but they do not capture social communication, cognition or autism spectrum disorder as a whole. The present data therefore support a domain-specific behavioural association rather than a broad claim about the full phenotype.

Total distance provides a distinct locomotor or exploratory signal. The higher PC1 and PC2 values relative to VPA are consistent with altered activity under the recorded test conditions. Total distance can also reflect motor capacity, arousal, habituation and apparatus settings, and should not be treated as a direct anxiety outcome. The centre-zone measures were inconsistent across endpoints, which further supports keeping them exploratory rather than combining them into a single construct.

Because COX-2 inhibition has been associated with reduced neuroinflammatory signalling in rodent models, examining whether parecoxib modifies behavioural readouts in the prenatal VPA model was scientifically reasonable. However, the present study did not measure COX-2 expression, inflammatory mediators or any other biological endpoint, and the behavioural records alone cannot indicate target engagement or a molecular pathway.

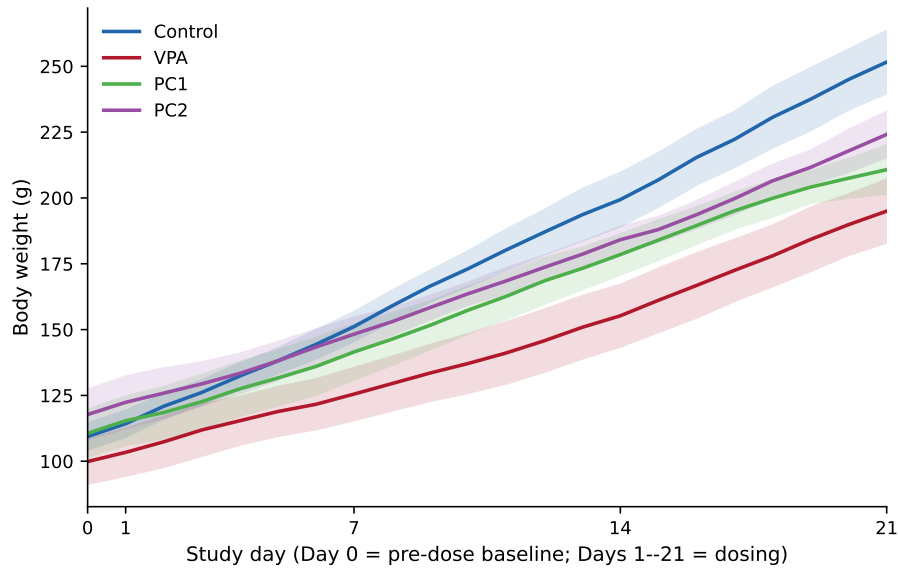


Fig. 6 Body weight during the 21-day administration period. Lines show group means and shaded bands show ± 1 SD for Control, VPA, PC1 (parecoxib 10 mg/kg) and PC2 (parecoxib 20 mg/kg), $n = 8$ recorded animals per group. Day 0 is the pre-dose baseline recorded before the first injection; dosing days are Day 1–21, so PND35 corresponds to dosing day 1 and the final day-21 injection corresponds to approximately PND55. Weight records are descriptive health-monitoring context and are not interpreted as evidence of a treatment-specific effect on growth.

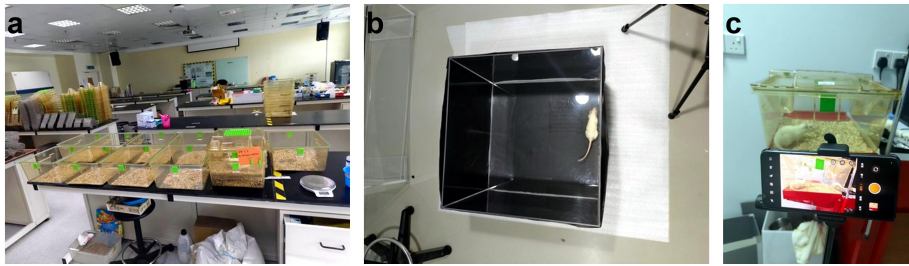


Fig. 7 Representative experimental environment and behavioural testing setups. Panel (a) shows the animal housing and experimental preparation environment, panel (b) shows the open-field apparatus during behavioural testing and panel (c) shows the behavioural recording setup. Photographs are illustrative of the recorded experimental conditions and were taken during the original experiment.

A further limitation is that injection volume differed between the groups. The theoretical injection volume was 0.5 mL/kg for PC1 and 1.0 mL/kg for PC2, whereas the monitoring record for the Control and VPA groups used a nominal saline volume of 1 mL/100 g (10 mL/kg). Treatment condition and injection volume were therefore not fully matched, and the retained records do not document a secondary dilution step for the parecoxib groups. This volume difference represents a potential procedural

415 confound that cannot be excluded from the observed behavioural differences; accord-
416 ingly, the two parecoxib doses should not be described as evidence of a dose-response
417 relationship.

418 The analysis included eight recorded offspring per group, but litter-level clustering
419 was not modelled because the exact contribution of each litter to the analysed groups
420 could not be reconstructed from the retained records. Litter-aware inference therefore
421 remains unresolved, and the animals should not be regarded as fully independent
422 biological replicates.

423 Baseline body weight also differed descriptively among the recorded groups on Day
424 0, before postnatal parecoxib administration, with mean values of 109.38 g in Control,
425 99.88 g in VPA, 110.62 g in PC1 and 117.75 g in PC2. Because Day 0 precedes the
426 first parecoxib injection, this pre-existing group imbalance may reflect litter-, cohort-
427 or other baseline differences and further limits treatment-specific interpretation of the
428 observed outcomes.

429 The behavioural records were collected in staged cohorts under nominally similar
430 housing, dosing, observation and test conditions, but batch-level allocation details were
431 not fully retained, so a cohort-related contribution to the observed group differences
432 cannot be formally excluded. In addition, behavioural testing began approximately 1
433 h after the final day-21 administration, so the design cannot fully distinguish effects
434 associated with repeated administration from possible acute effects of the final dose.

435 Taken together, these results document behavioural differences associated with
436 parecoxib administration in a prenatal VPA rat study, concentrated in self-grooming
437 frequency, self-grooming duration and total distance. They do not establish clinical
438 efficacy, a dose-response relationship, target engagement or a molecular mechanism,
439 and they should be interpreted as behavioural associations within the limits described
440 above.

441

442 4 Conclusion

443

444 This analysis identified behavioural differences associated with the recorded parecoxib
445 groups in a prenatal VPA rat study. The strongest contrasts involved self-grooming
446 frequency, self-grooming duration and total distance. The findings support behavioural
447 modulation associated with parecoxib administration, but they do not establish clinical
448 efficacy, a dose-response relationship or a molecular mechanism.

449

450 5 Methods

451

452 5.1 Ethics statement

453

454 All experimental procedures involving animals were reviewed and approved
455 by the MAHSA University Animal Ethics Committee (MUAEC; approval no.
456 RMC/NOVEMBER/2025/AEC02; 21 November 2025). All procedures were per-
457 formed in accordance with the relevant institutional and national guidelines and
458 regulations for the care and use of laboratory animals. Reporting was prepared with
459 reference to the ARRIVE 2.0 guidelines [23].

460

5.2 Study design and evidence scope

This study was an exploratory analysis of existing individual-animal behavioural records from a completed master’s research project at MAHSA University. The animal-level records were collected in 2026 and had not been reported in a previous publication according to the supplied study documentation. No prospective intervention assignment was performed in the present report. The post-administration records contained Control, VPA, PC1 and PC2 groups, with eight recorded male offspring per group for self-grooming and open-field outcomes. Rat identifiers were retained in the cleaned tables. The source thesis stated that all 32 selected male offspring contributed complete data to the principal analyses and that no animal met the withdrawal criteria. The supervisor provided authorization for publication of the dataset.

5.3 Data provenance

The source records comprised the laboratory records for behaviour, body weight, food intake, water intake and injection monitoring generated during the MAHSA University project in 2026. The present manuscript reports behavioural analyses and descriptive body-weight monitoring from these previously collected, unpublished animal-level records. The supplied documentation states that the supervisor authorized publication.

5.4 Experimental unit and litter structure

The breeding cohort comprised 12 Sprague-Dawley dams, the same two Sprague-Dawley breeder males used throughout the cohort and 12 litters. Each of the four groups was supplied by three group-specific dams and three litters. Control dams received saline on gestational day 12.5, whereas the dams contributing to VPA, PC1 and PC2 offspring received prenatal VPA. Offspring from a given litter entered only one preassigned group. Male offspring were selected within the corresponding group until eight animals were available per group. The retained records did not allow the exact number of analysed offspring contributed by each litter to be reconstructed; litter-aware inference therefore remains unresolved, and the primary analyses were performed at the level of the recorded animal. The records were collected in staged cohorts under the same housing, dosing, observation and behavioural-test conditions. Individual offspring records were used for the behavioural analyses.

5.5 Animals and housing

The animals were Sprague-Dawley rats. All analysed offspring were male, with 32 offspring distributed across four groups ($n = 8$ per group). The supplied records identify the animal source as a molecular biology laboratory at Universiti Kebangsaan Malaysia (UKM). Offspring were weaned and separated at 3 weeks of age. The post-natal administration schedule began at PND35 and continued for 21 consecutive days. Behavioural testing was performed approximately 1 h after the final day-21 injection, corresponding to approximately PND55 when PND35 is counted as the first dosing day. Rats were housed two per cage with animals from the same group at $22 \pm 2^\circ\text{C}$

507 and approximately $50 \pm 10\%$ relative humidity, under a 12-h light/12-h dark cycle
508 with lights on from 07:00 to 19:00. Standard laboratory chow and water were available
509 ad libitum, and cages were cleaned twice weekly. Body weight was recorded daily in
510 grams for each animal: a pre-dose baseline on Day 0, corresponding to the day before
511 the first injection, followed by Day 1–21 of the administration period. These records
512 are used descriptively as health-monitoring context.

513

514 **5.6 Prenatal VPA exposure**

515

516 Pregnant dams received a single intraperitoneal injection of sodium valproate on ges-
517 tational day 12.5 at 600 mg/kg. The product was Epilim Intravenous sodium valproate
518 (400 mg per vial; Sanofi S.r.l., Italy; product code MAL19950587AZ). Each vial was
519 reconstituted with the supplied 4 mL of Water for Injection to a working concentra-
520 tion of 100 mg/mL, corresponding to an injection volume of 6 mL/kg maternal body
521 weight. Control dams received an equal-volume intraperitoneal injection of 0.9% saline
522 on the same gestational day, based on the stated VPA injection volume.

523

524 **5.7 Recorded administration groups and allocation**

525 PC1 and PC2 were parecoxib sodium groups, corresponding to 10 mg/kg and
526 20 mg/kg, respectively. The supplied product record identifies the preparation
527 as Dynastat parecoxib sodium, 40 mg per vial, registered in Malaysia under
528 MAL20033926ARZ. The record lists Pfizer (Malaysia) Sdn. Bhd. as the registration
529 holder and Pharmacia & Upjohn, Kalamazoo, Michigan, USA, as the manufacturer,
530 with lot MF2144, a manufacture date of November 2024, an expiry date of October
531 2027 and vial trace code PAA216488. Parecoxib was dissolved in 2 mL of 0.9% saline
532 to a working concentration of 20 mg/mL. The theoretical intraperitoneal dose volumes
533 were 0.5 mL/kg for PC1 and 1.0 mL/kg for PC2, calculated from the daily body weight
534 and recorded to the practical syringe increment used during dosing. Although the
535 commercial label lists intravenous or intramuscular administration, the animal exper-
536 iment used intraperitoneal injection. Parecoxib or saline was administered once daily
537 for 21 consecutive days at approximately 10:00. Control and VPA offspring received
538 0.9% saline on the same daily schedule, with the monitoring record using a nominal
539 volume of 1 mL/100 g and recording the actual volume against body weight.

540

541 **5.8 Behavioural test conditions and sequence**

542

543 Behavioural testing began approximately 1 h after the final administration on day 21.
544 Rats were acclimatized to the behavioural testing room for 30 min before testing. Tests
545 were conducted in a soundproof, light-shielded room at $22 \pm 1^\circ\text{C}$ with light intensity
546 recorded as 100–150 lux. The open-field test was performed first and was followed
547 immediately by the self-grooming test, without a prespecified inter-test interval. Test
548 apparatuses were wiped with 75% alcohol after each use to reduce residual odour cues.
549 Representative photographs of the animal environment and behavioural testing setups
550 are provided in Fig. 7.

551

552

5.9 Self-grooming assessment	553
Each rat was placed individually in a transparent self-grooming cage measuring	554
30 × 20 × 20 cm. The session lasted 15 min, with the first 5 min used for adaptation	555
and the final 10 min used for analysis. Self-grooming included face washing with the	556
forepaws, licking of the trunk, limbs or tail and fur grooming with the teeth. Isolated	557
licking episodes lasting no more than 5 s without continuity were excluded from the	558
operational definition. Frequency was counted by complete grooming events, with con-	559
secutive grooming actions separated by intervals of no more than 5 s treated as one	560
event. Cumulative duration was recorded in seconds. Matched pre-administration and	561
post-administration records were analysed for PC1 and PC2, with eight records per	562
group. Behavioural recording and tracking used SMART 3.0. Frequency and duration	563
were analysed exactly as recorded. Blinding status of the behavioural scoring was not	564
documented in the retained records.	565
	566
5.10 Open-field assessment	567
The open-field arena measured 50 × 50 × 50 cm, with a 25 × 25 cm centre zone. Each	568
session lasted 15 min, with the first 5 min used for adaptation and the final 10 min	569
used for analysis. SMART 3.0 was used for behavioural tracking. The cleaned table	570
contained total distance, centre distance, centre entries and time in the centre for eight	571
animals per group. Total distance was treated as a locomotor or exploratory outcome.	572
Centre distance, centre entries and time in the centre were treated as exploratory	573
outcomes.	574
	575
	576
5.11 Euthanasia and terminal blood collection	577
After the behavioural assessments were completed, rats were deeply anaesthetised by	578
intraperitoneal injection of a ketamine–xylazine mixture. The working solution was	579
prepared from ketamine (100 mg/mL), xylazine (100 mg/mL) and sterile saline in a	580
10:1:9 volume ratio and was administered at 2 mL/kg body weight, corresponding	581
to ketamine 100 mg/kg and xylazine 10 mg/kg. After confirmation of loss of the	582
pedal withdrawal reflex, the thorax was opened and terminal blood was collected by	583
cardiac puncture, and death was confirmed by permanent cessation of respiration and	584
heartbeat. The cerebellum and medulla oblongata were then collected for a separate	585
study and were not included in the analyses of the present study.	586
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5.12 Analysis hierarchy and data processing	589
Source worksheets were converted into tidy individual-animal tables with one row	590
per animal, group and time point. Group labels and identifiers were standardized	591
without changing numeric values. Duplicate group-identifier records were screened,	592
and no duplicates were present in the cleaned tables. The reporting hierarchy was	593
established during the retrospective analysis for presentation: self-grooming duration	594
was the principal outcome, self-grooming frequency and total distance were support-	595
ing outcomes, and centre-zone measures were exploratory. This hierarchy was not	596
prospectively preregistered.	597
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5.13 Statistical analysis

This analysis included all eligible individual-animal records available from the completed experiment; therefore, no prospective sample-size calculation was performed for the present report. All tests were two-sided with $\alpha = 0.05$. Group-level post-administration analyses used $n = 8$ recorded male offspring per group, and individual observations are shown in the figures. Descriptive statistics are reported as mean (SD) in the main table. One-way ANOVA with Tukey-adjusted comparisons was used for self-grooming frequency and total distance. Welch ANOVA with Games-Howell-adjusted comparisons was used for self-grooming duration and centre distance. Kruskal-Wallis tests with Dunn-Holm comparisons were used for centre entries and time in the centre. The retained procedures and distribution diagnostics are documented in Supplementary Table S4; no unreported assumption checks or exclusion rules are claimed.

Self-grooming frequency was additionally checked with a 10,000-permutation ANOVA using the recorded fixed seed of 20260727. Effect sizes included ω^2 , oriented Hedges g , rank-biserial correlation and paired d_z , as applicable. Bootstrap intervals for effect-size displays used 5,000 resamples in the analysis code. For the VPA-versus-Control contrast, effect-size signs were oriented in the observed VPA-labelled direction relative to Control. For PC1-versus-VPA and PC2-versus-VPA contrasts, signs were oriented so that positive values indicated a difference in the direction of the Control-labelled mean. Paired pre-administration and post-administration analyses were restricted to PC1 and PC2 and were treated as descriptive sensitivity analyses. Holm adjustment was used for paired within-label comparisons.

Baseline-change coupling, pooled multidomain correlations, descriptive principal-component analysis, anchored scores, activity-normalized centre-zone metrics and cross-domain paired-change correlations were retained in the Supplementary Information. Because the change score mathematically contains the baseline value, baseline-change correlations are susceptible to mathematical coupling and regression-to-the-mean effects. These analyses are descriptive and are not interpreted as evidence of a biological baseline-dependent response. The pooled associations may partly reflect between-group separation and therefore should not be interpreted as evidence of within-group behavioural coupling. 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. PCA was used only for descriptive visualization and was not treated as independent validation or confirmatory evidence.

Analyses and figures were generated with Python using pandas, SciPy, statsmodels and Matplotlib.

6 Data availability

The datasets analysed during the current study, including raw self-grooming, open-field and body-weight records, cleaned analysis tables, statistical code, figure source data and a data dictionary, are available from the corresponding author on reasonable

request. A deposition in a public repository with a persistent identifier is planned before publication.

7 Code availability

The statistical analysis and figure-generation code used in this study is available from the corresponding author on reasonable request.

Author contributions

Y. Guo performed the behavioural assessments, analysed the data, prepared the figures and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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