

The Results of a Multi-Company Validation of the ActualHCA™ Home Cage Monitoring System for Rodent CNS Safety Pharmacology Studies

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Introduction: Traditional core battery CNS safety pharmacology assessment relies on the FOB/Inwin, a subjective behavioral screen including a panoply of rodent-specific parameters that are difficult to translate to human outcomes. Home cage monitoring systems objectively measure continuous rodent behavior, day and night, over multiple days. The welfare benefits of the approach, which allows group housing and non-invasive monitoring, are established. However, the value of these data in CNS safety assessment compared to those obtained from the FOB/Inwin remains largely untested.

The aim of this collaborative work was to assess the potential of rodent home cage monitoring, using a few key parameters (locomotor activity, rearing and body temperature), to better predict risk over traditional FOB/Inwin. Three compounds (GSK, JNJ and AZ), for which findings have previously been reported in FOB/Inwin and clinically (GSK and AZ), were tested using the home cage monitoring system and the data were analyzed.

Methods: For each test condition, six male Han Wistar rats (sourced from Charles River UK Limited, Margate) were implanted with temperature sensitive RFID transponders (BioMark USA) and housed 3 per cage. Cages were placed inside the home cage monitoring system and recorded for 10 days to provide an initial 4 days baseline measurement followed by the dose/treatment day and then followed by a further 5 days post-dose. In addition to the dosing procedure, a blood sample was taken within 24hrs. Both events are indicated on the plots. On post-dose day 3, the cages were changed.

For each animal, 3 days of pre-dose data are averaged to form a 24hr baseline profile. The continuous profiles (24hr or 5-day) show the behavioural profile recorded plotted alongside the baseline for reference. The bar graphs focus on specific time windows highlighted by a yellow overlay. The bars represent the difference between the observed measurements and the pre-dose baseline within the window of interest. Data were analysed using one way ANOVA, followed by Dunnett's test. Statistical significance was set at $p < 0.05$. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and local policies on the Care, Welfare and Treatment of Animals.

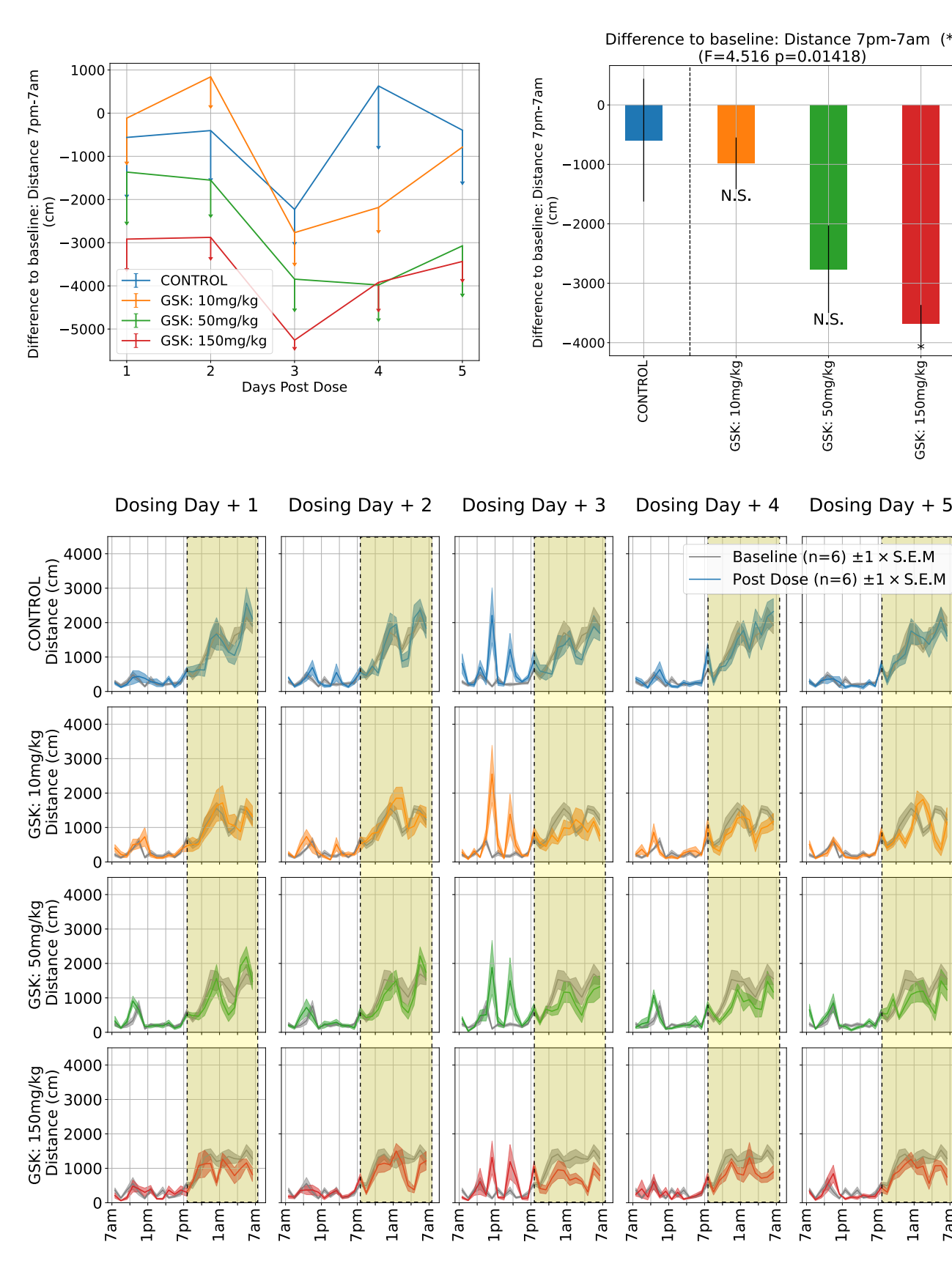
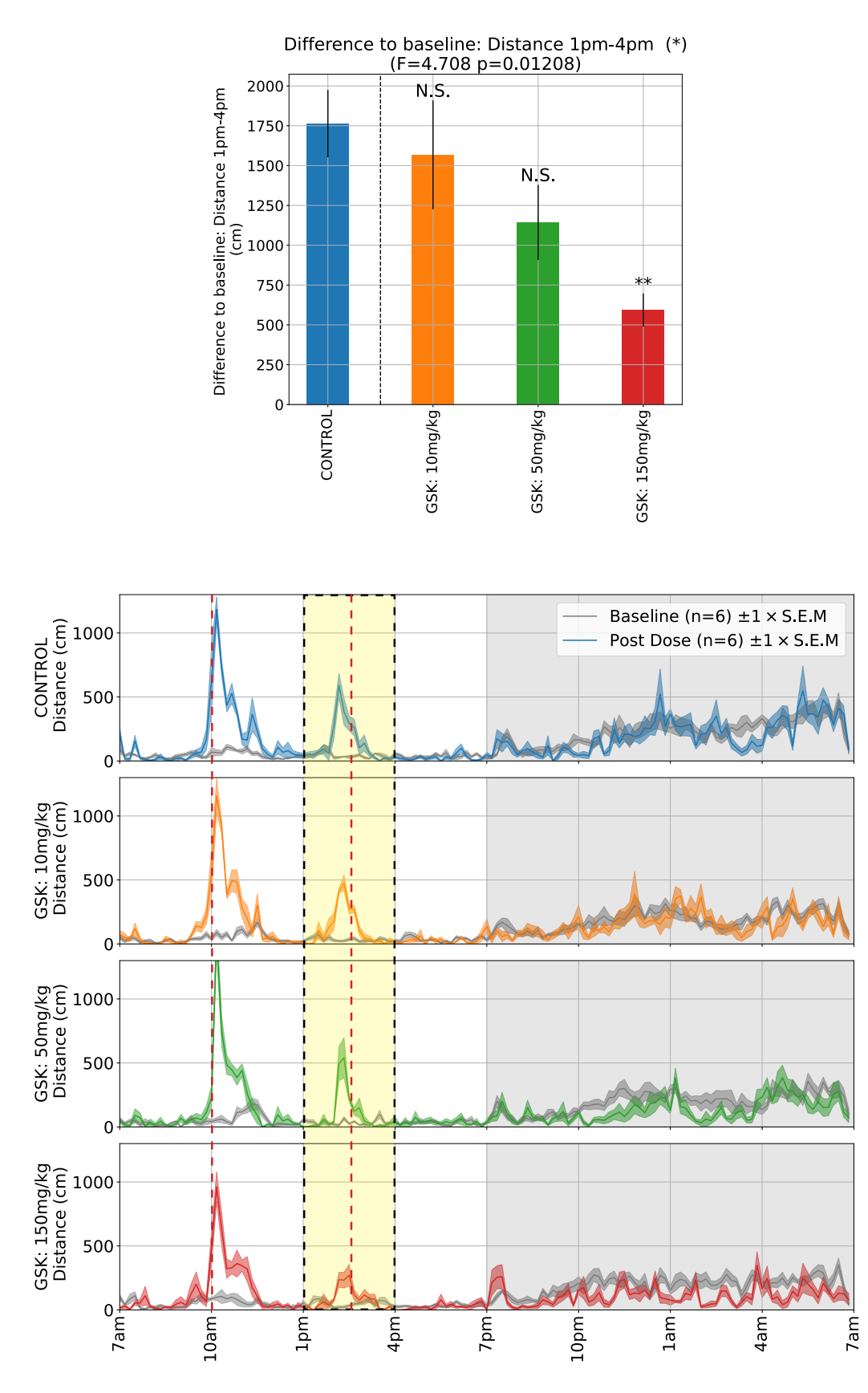
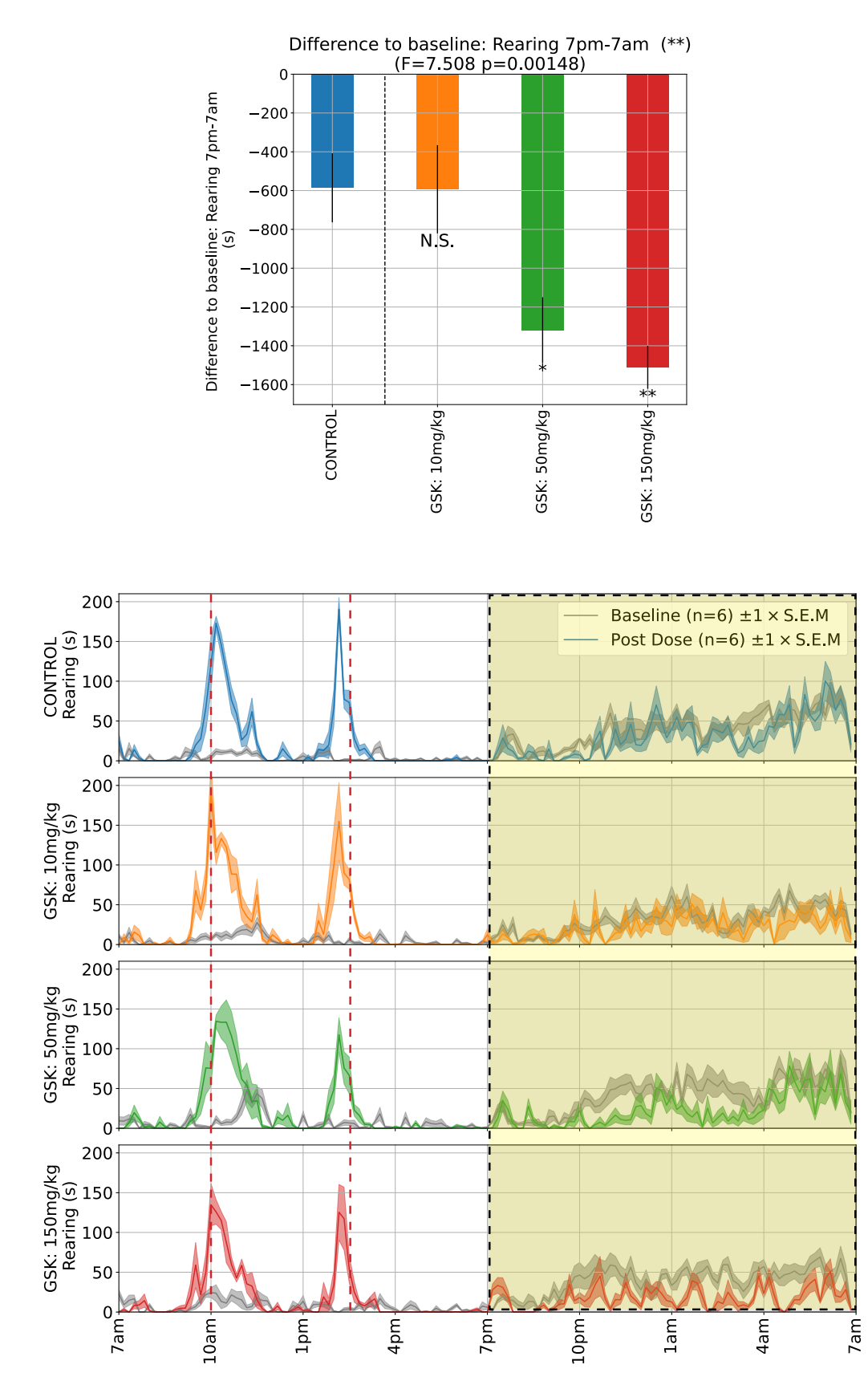
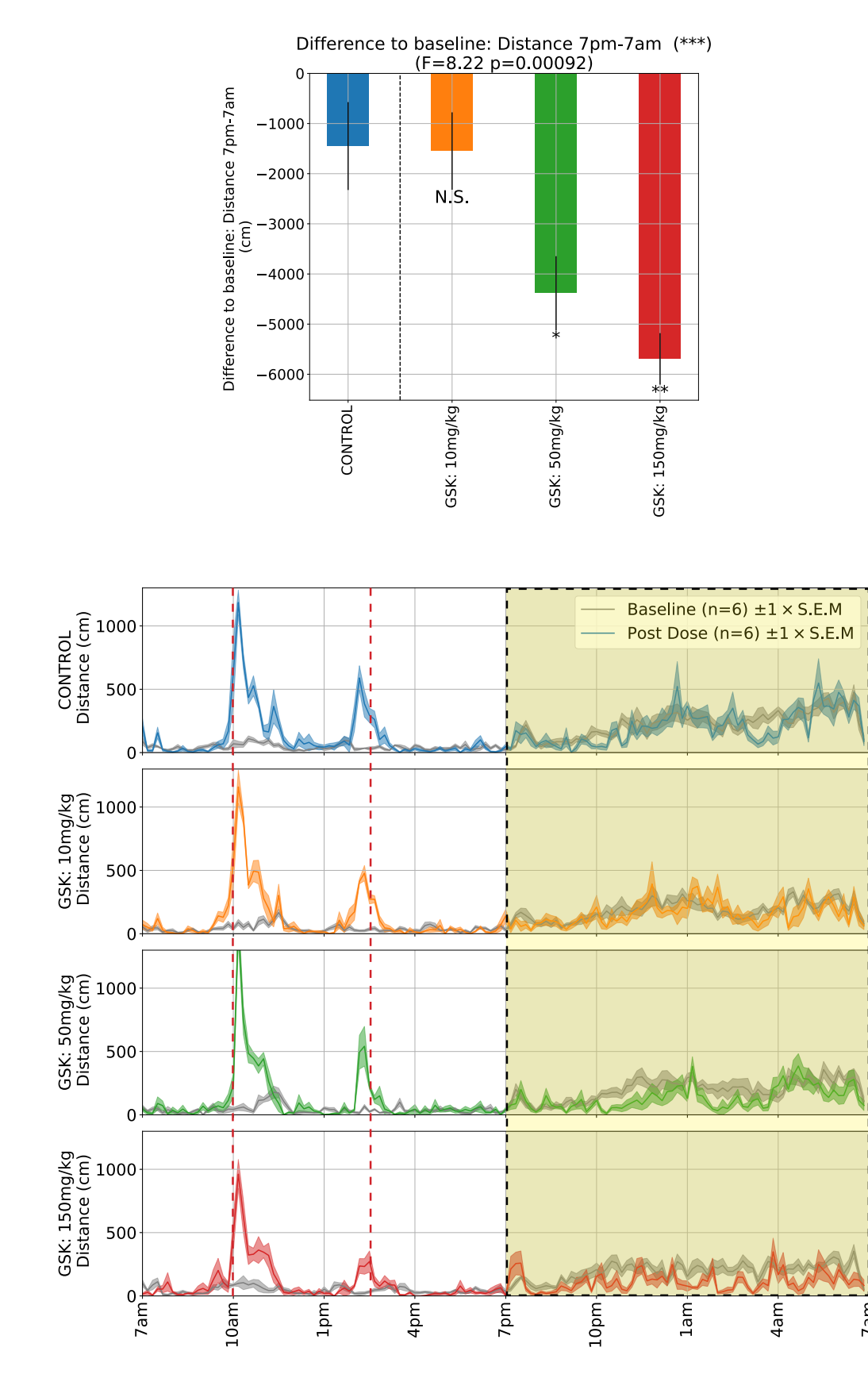
Activity – dosing day (dark phase)

Rearing – dosing day (dark phase)

Activity – dosing day (post blood sample)

Activity – five days post dose (dark phase)

GSK



The compound is a 4-aminoquinoline antimalarial that demonstrates low to moderate clearance and excellent oral bioavailability with linear pharmacokinetics. In the clinic, a seizure was observed in one subject following a single dose stopping progression of the compound's development. In a previous repeat dose investigative toxicology study, CNS effects were evident at the 150 mg/kg dose, however, no CNS effects were observed in the definitive GLP general toxicity or Irwin studies up to 50 mg/kg. In the present study, rats received a single oral dose of either vehicle (1% w/v) aqueous methylcellulose or GSK compound (10, 50, or 150 mg/kg). A dose dependent reduction in activity was observed in the 50 and 150 mg/kg groups during the dark phase on dosing day and persisting for 5 more days post-dose. These results suggest a greater sensitivity for continuous home cage monitoring in assessing activity measures over standard GLP studies.

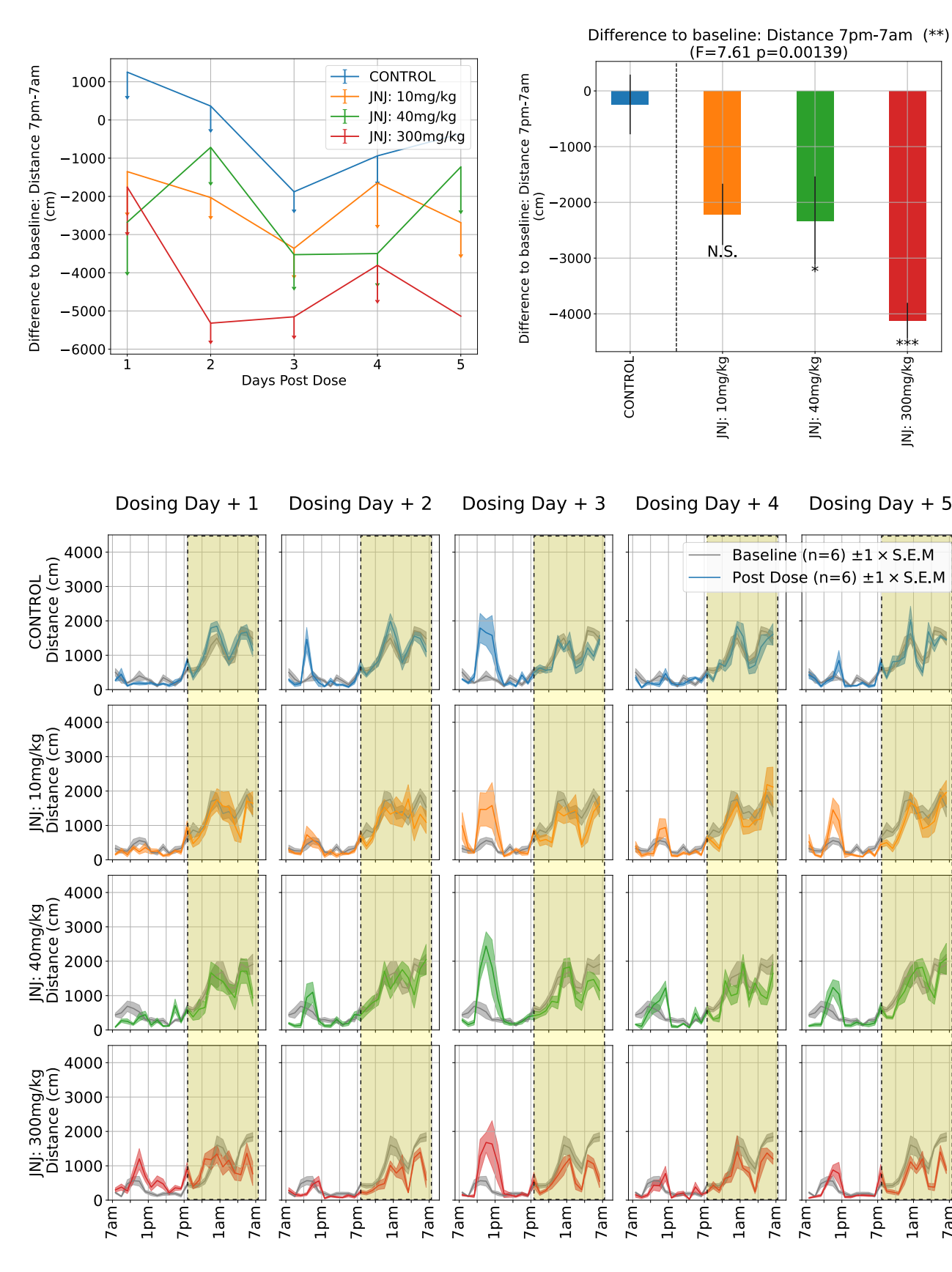
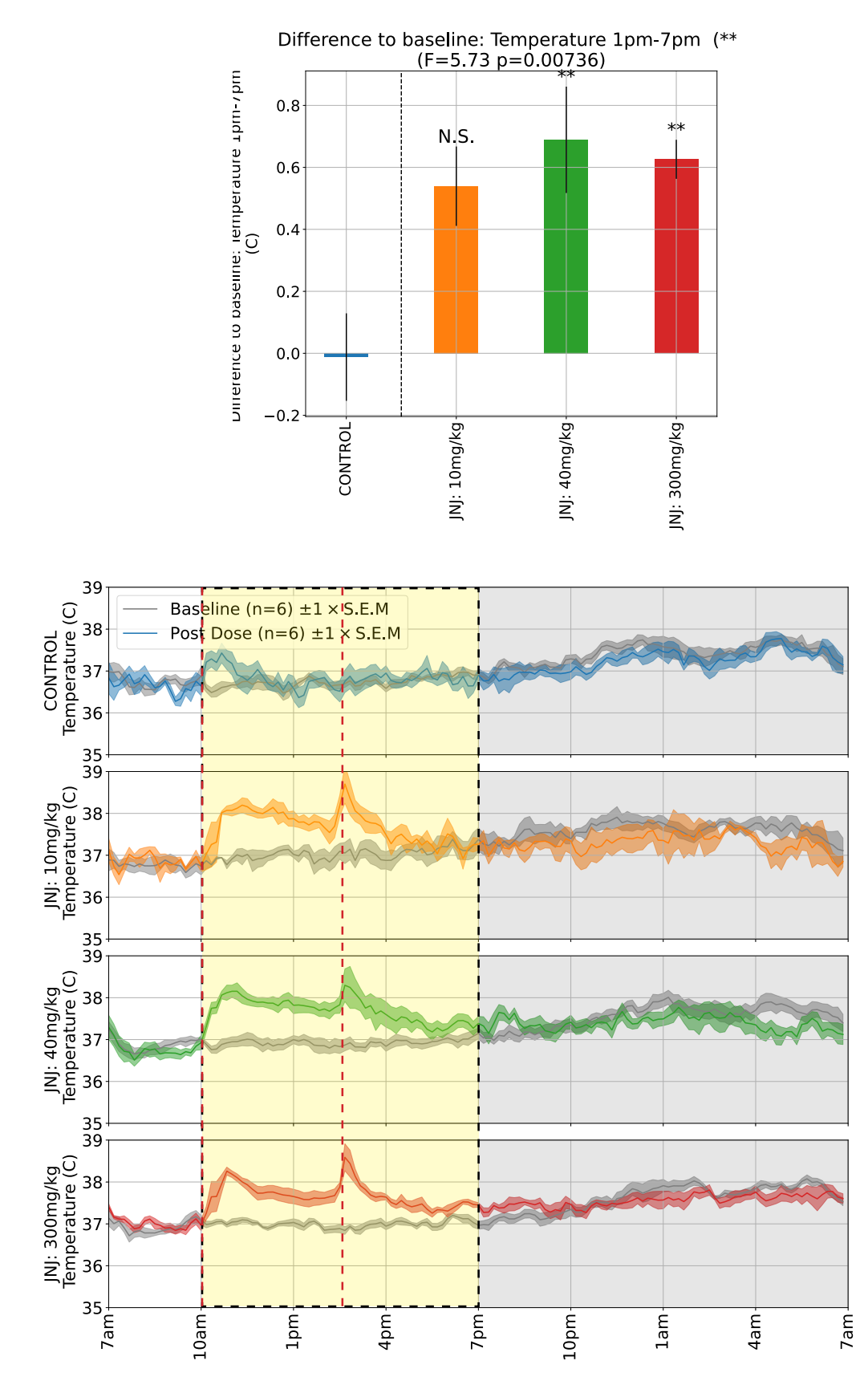
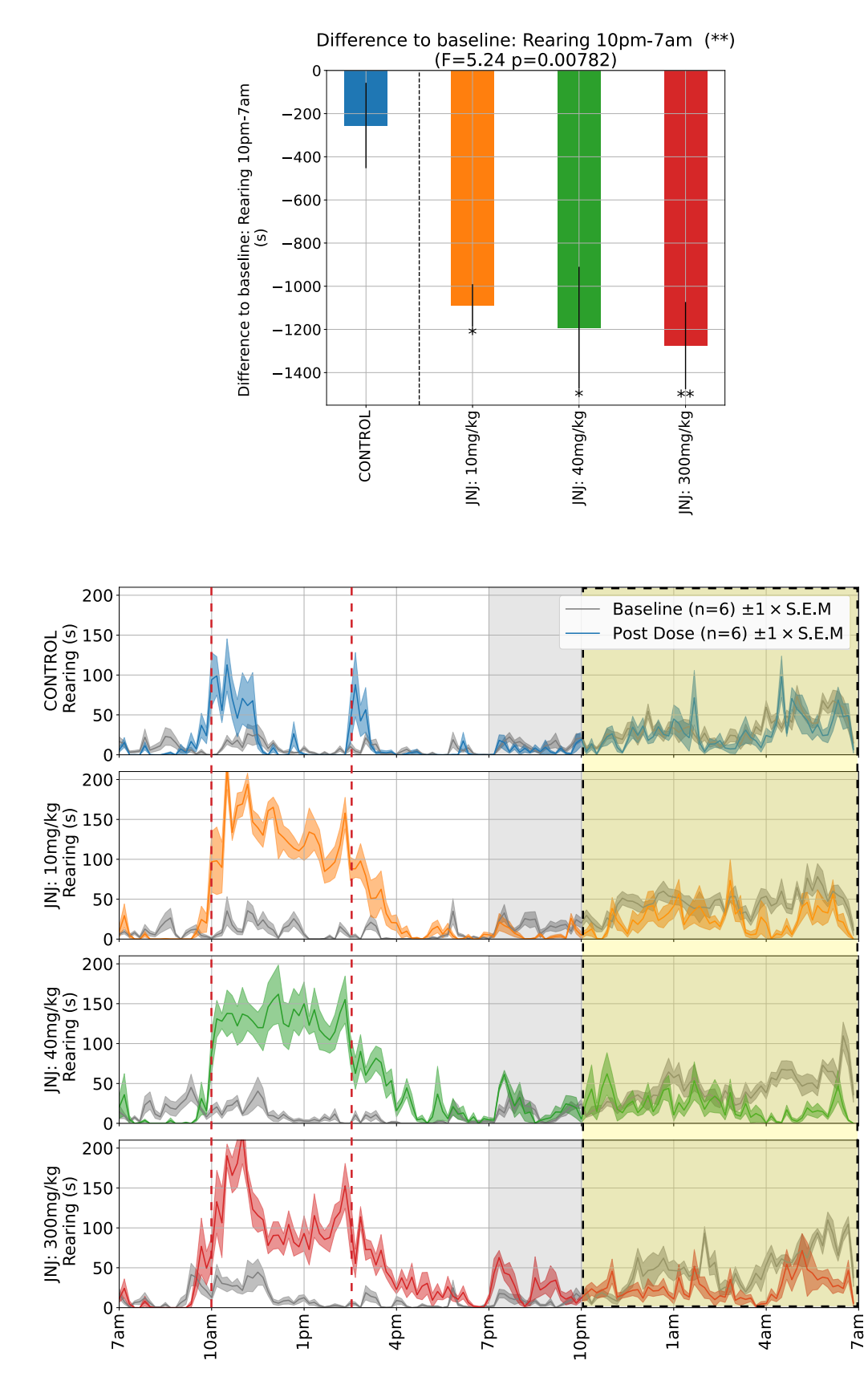
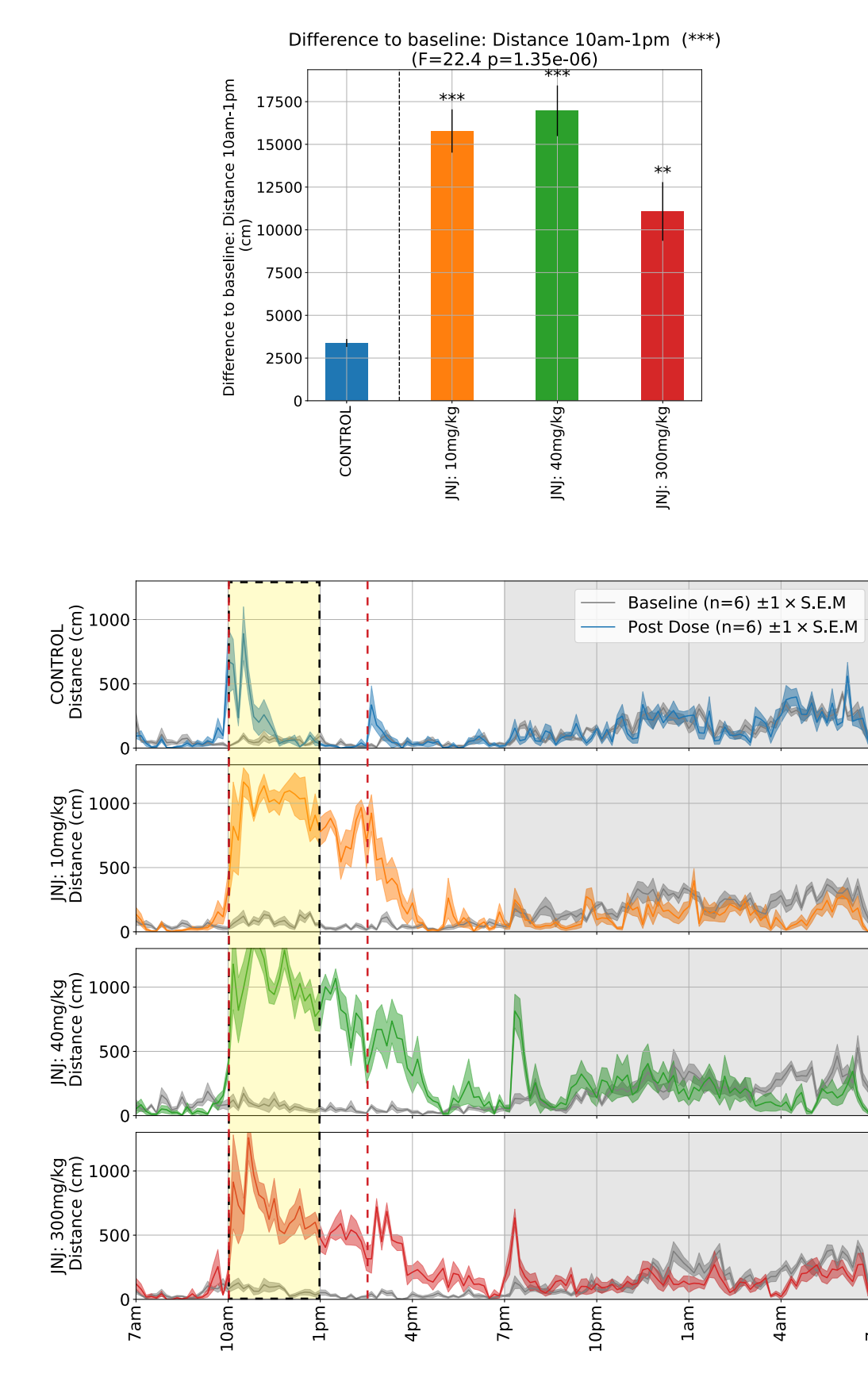
Activity – 3h post dose

Rearing – last 9h dosing day (dark phase)

Temperature – 9h post dose

Activity – five days post dose (dark phase)

JNJ



The compound is a high affinity antagonist at human adenosine receptors which was selected as a drug candidate for the treatment of Parkinson's disease. In this study, rats received a single oral dose of the JNJ compound (10, 40 and 300 mg/kg/10 mL) and were monitored for 6 days. Following administration, the effects included i) increased horizontal and vertical locomotor activity, which was associated with a slight increase in body temperature during the daytime, at each dose; ii) decreased horizontal and vertical locomotor activity in the dark phase, at each dose; iii) decreased horizontal and vertical locomotor activity in the dark phase of the subsequent 5 days at 40 and 300 mg/kg. The hyperactivity and increased body temperature for the 3 doses are in line with the modified Irwin test data. However, the modified Irwin test does not include observations during the dark phase and hypoactivity was not detected.

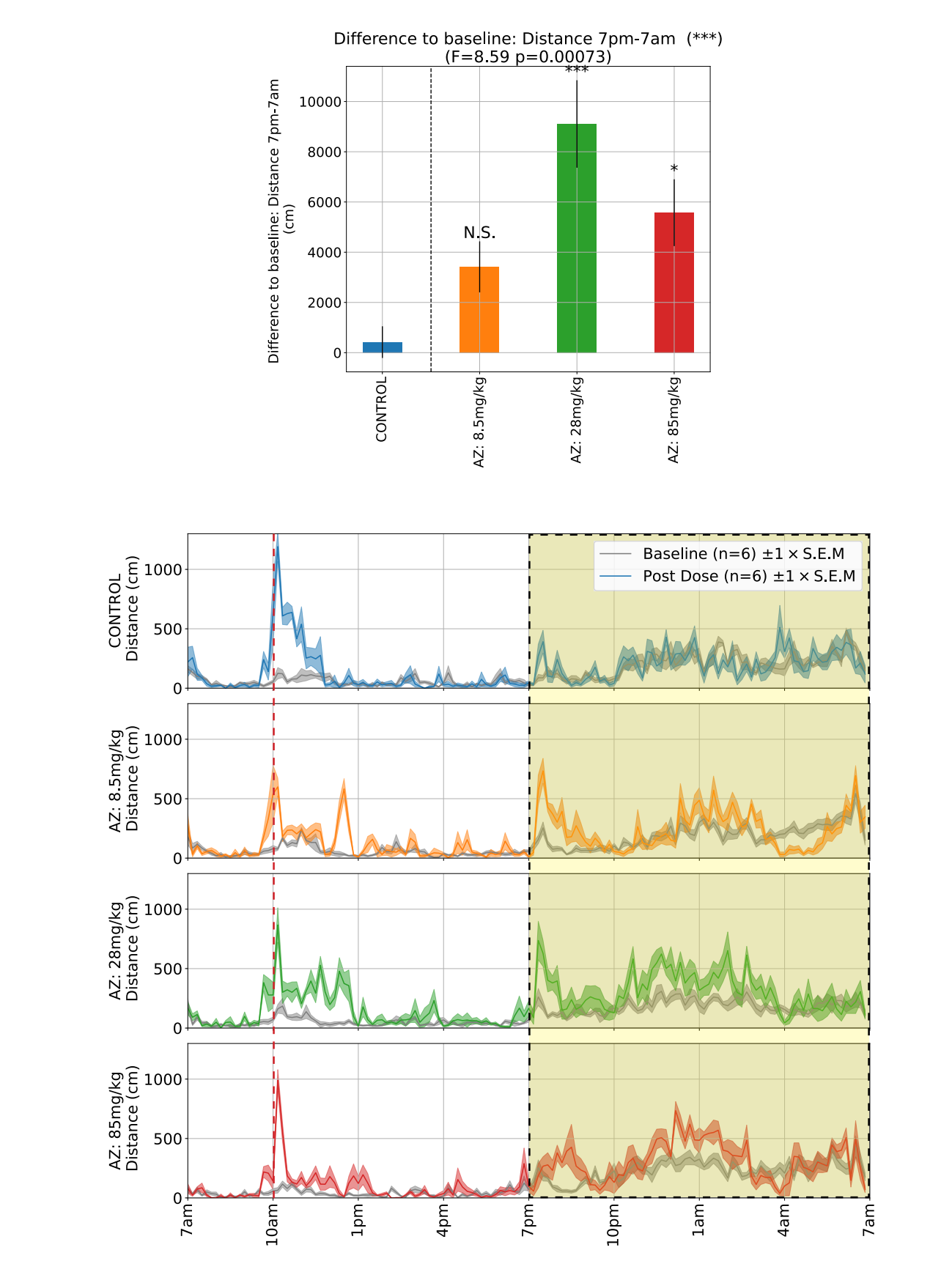
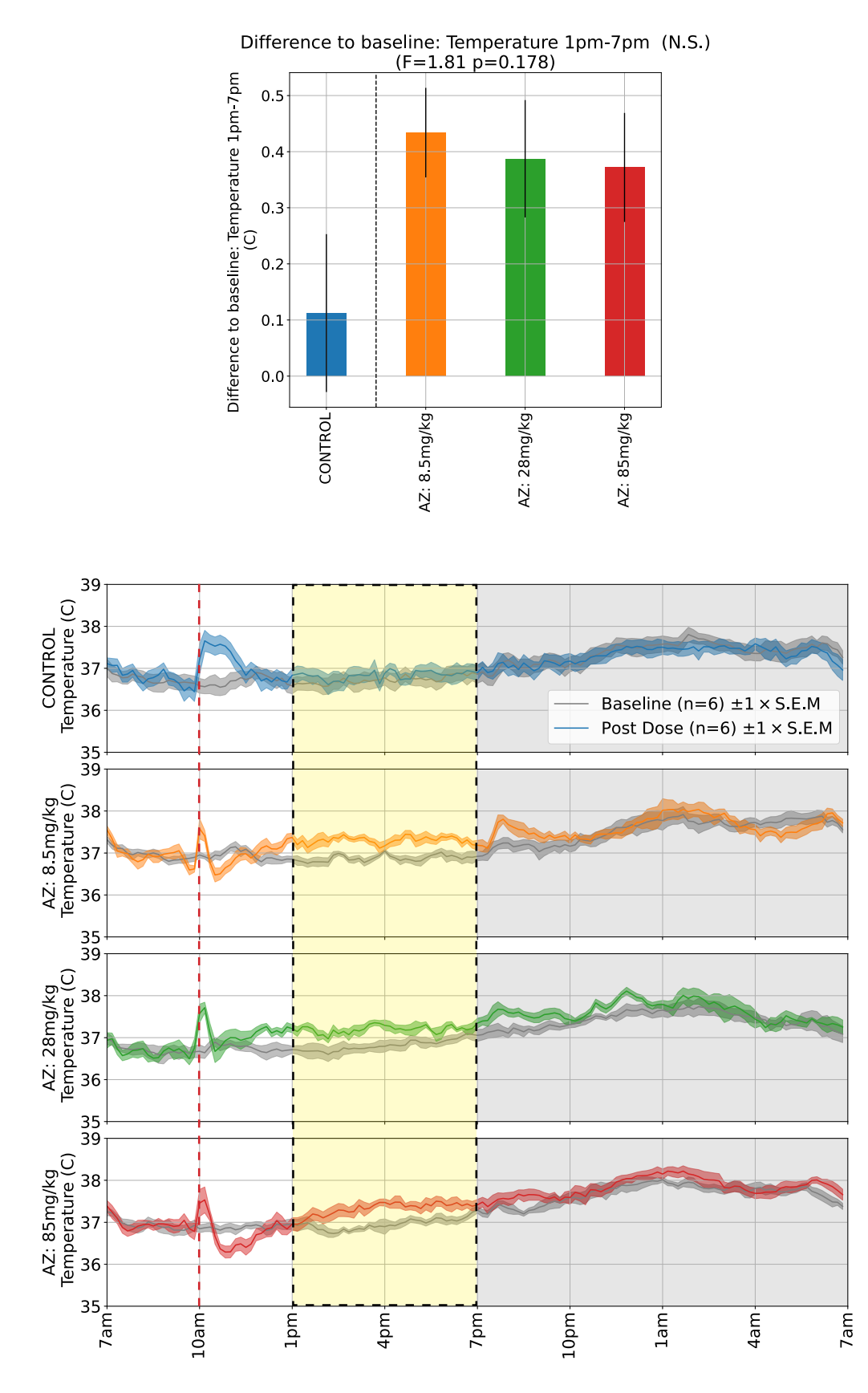
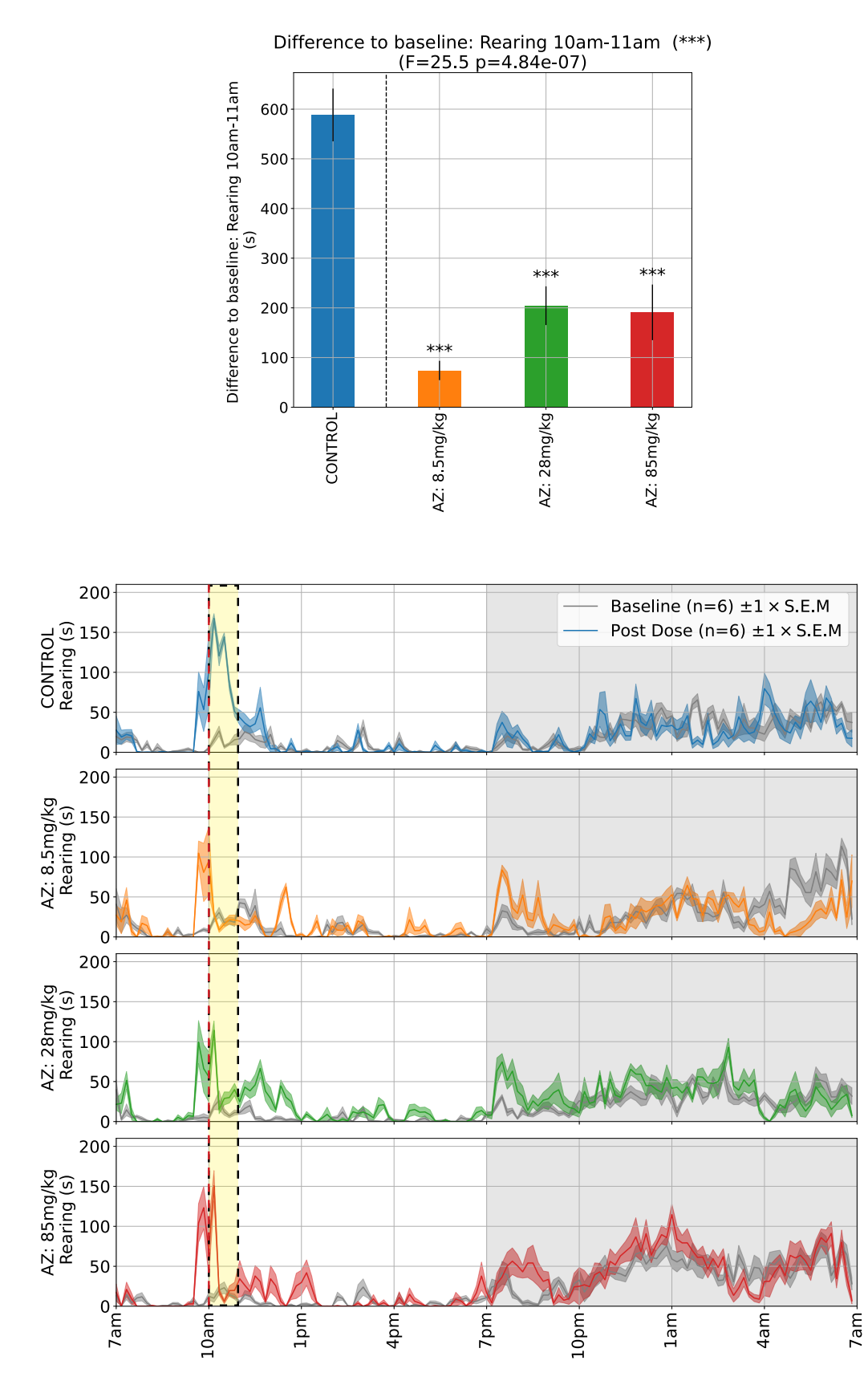
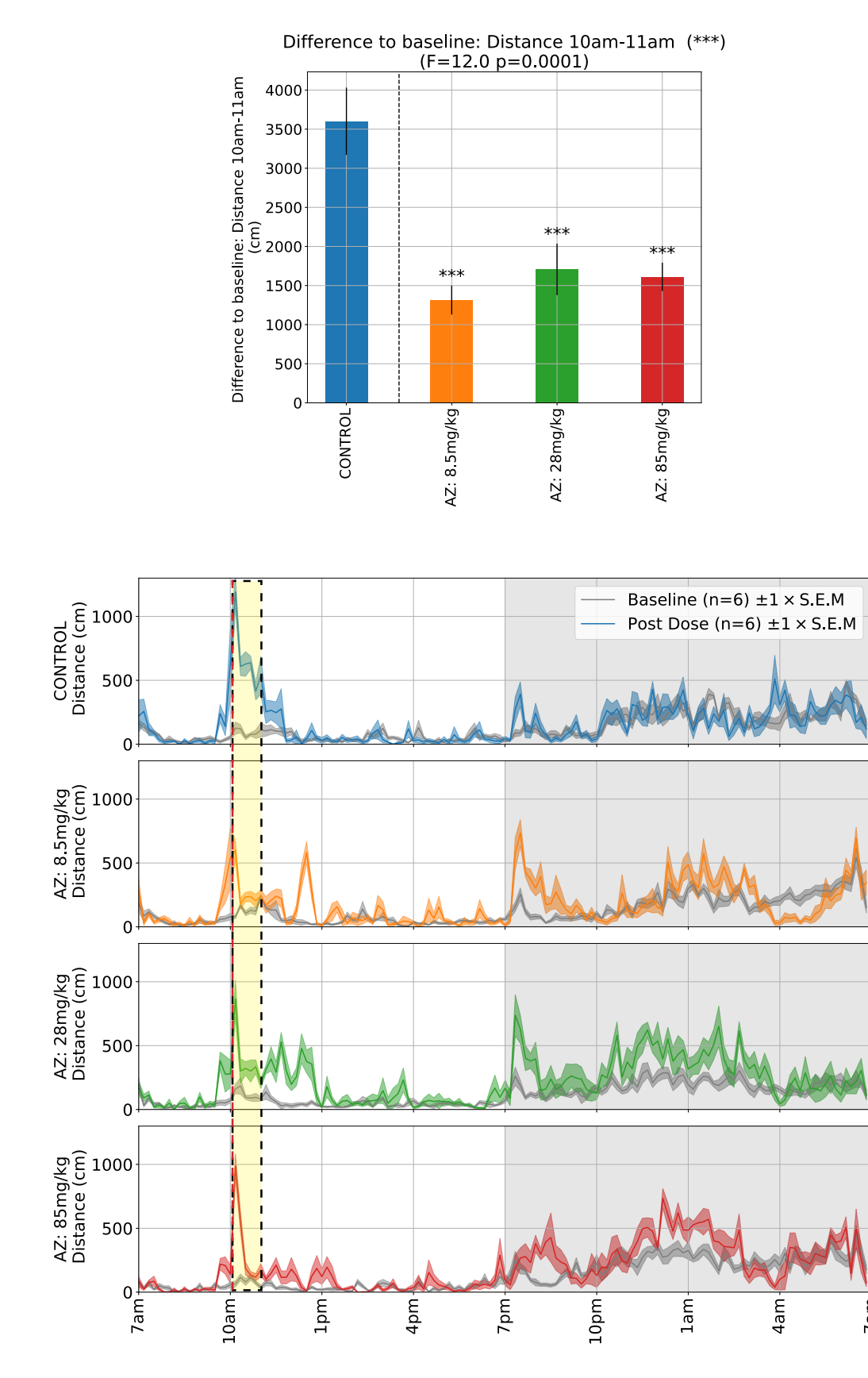
Activity – 1h post dose

Rearing – 1h post dose

Temperature – 3-9h post dose

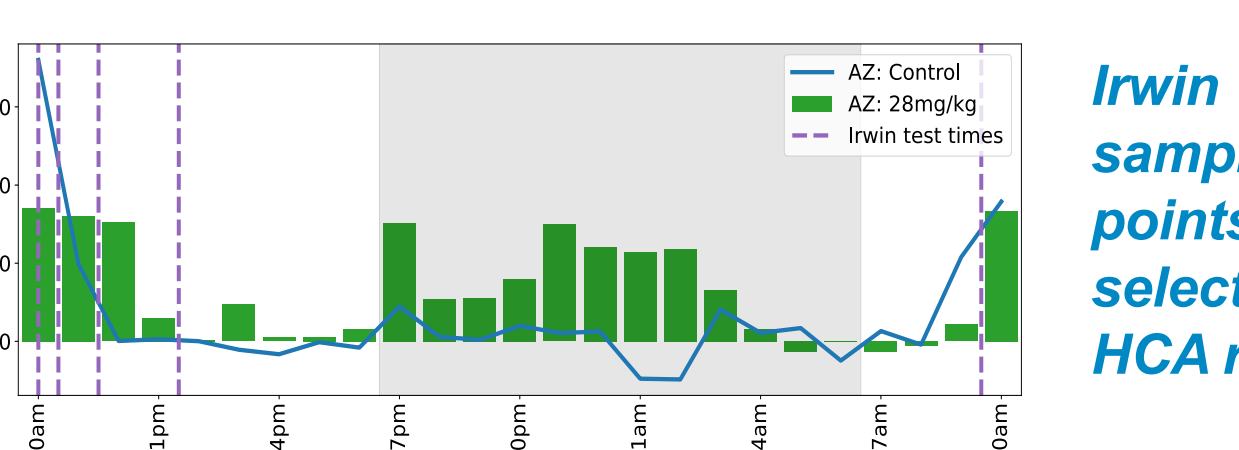
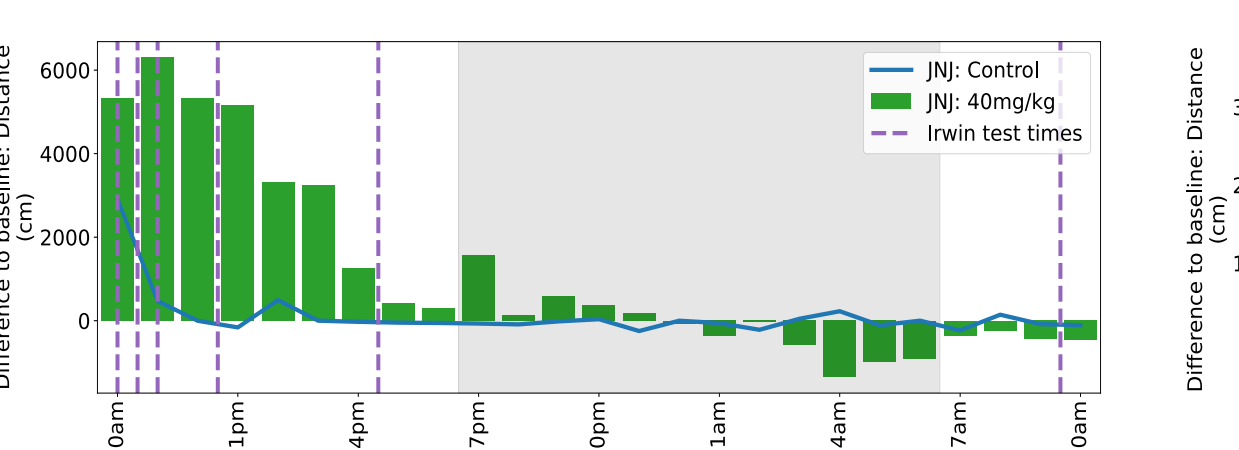
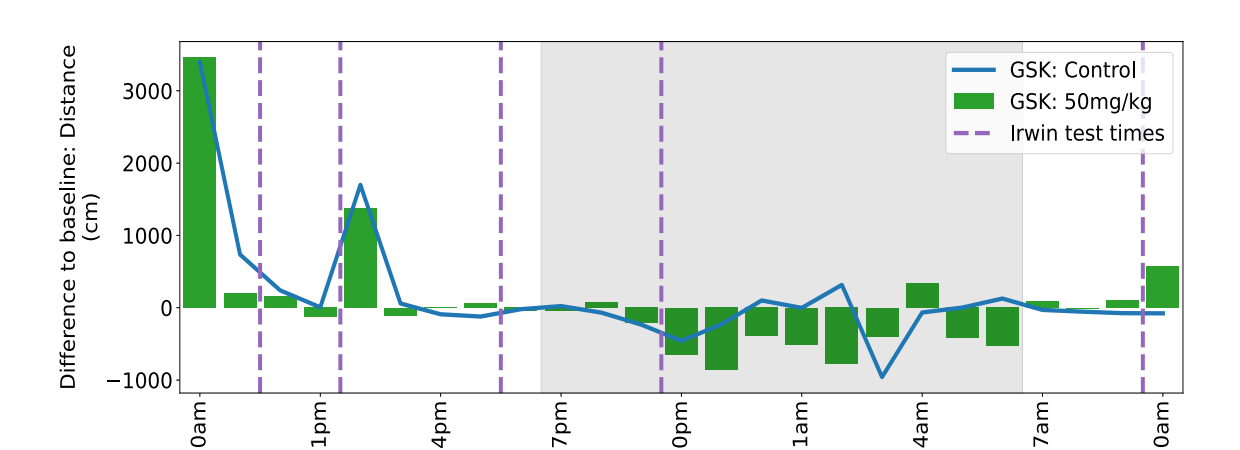
Activity – dosing day (dark phase)

AZ



The compound is a negative allosteric modulator at the metabotropic glutamate receptor subtype 5, developed for multiple neuroscience and gastrointestinal indications. Clinical development was discontinued due to dose-limiting anxiety and hallucination. Here the effects of one orally administered dose (at 0, 8.5, 28 and 85 mg/kg/5 mL) were monitored for 6 days. The AZ compound: i) reduced both horizontal and vertical locomotor activity 1h post-dose, at each dose; ii) increased body temperature 3-6h post-dose, at each dose; iii) increased horizontal locomotor activity in the dark phase at 28, 85 mg/kg. The results are in line with the Irwin assessment data, where reduced spontaneous activity was detected within 1h post-dose, at each dose. However, the results were not consistent with those of a separate locomotor study that showed a dose-dependent increase in horizontal (but not vertical) locomotor activity, largely attributed to increases during the first 10 minutes post-dose. This discrepancy remains unexplained.

Conclusions: All three test compounds clearly affected behavioral activity and in some cases at concentrations below that observed in previous Irwin tests. The continuous behavioural profile obtained from home cage monitoring graphically demonstrates the natural behavioral variability in animals over time and clearly highlights a sampling risk in using snapshot observations at arbitrary times. Effects that were not originally detected in FOB/Inwin tests may well be due to the sampling effect, which is particularly acute during the dark phase (which is generally not observed). In addition to the continuous non-evoked behavioral profiles, we show that routine interventions, such as blood sampling and cage changes, can be used to evoke behavioral responses with measurable



effects reported for two of the test compounds. In summary, continuous collection of a few key parameters using home cage monitoring represents an

animal welfare refinement and could be used to improve sensitivity in repeat dose toxicology studies alongside clinical observations or in place of FOB/Inwin.

Irwin sampling points for selected HCA results.