Non invasive and automated acquisition of physiological and developmental parameters in rat neonates

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Introduction

Methodologies that are currently used for non clinical assessment of the safety of pharmaceuticals on neonatal development are focused on morphology and behaviour functional endpoints. The potential undesirable pharmacodynamic effects of new pharmaceuticals on cardiovascular, respiratory and central nervous system functions, which are considered as vital organ systems, are investigated in adult animals as part of the core battery of safety pharmacology studies. Due to the lack of practical and validated methods, the potentially adverse functional effects of pharmaceuticals on these vital organ systems are not currently investigated in neonates during reproductive toxicity studies for effects on pre- and post-natal development or during toxicity studies in juvenile animals. The PhenoPups device (Fig. 1 and Fig. 2) provides a novel approach to evaluation of a range of physiological and behavioural parameters in neonatal rats. Using this approach, changes in heart rate, respiratory parameters, body temperature, vocalization and actimetry can be rapidly and conveniently evaluated. The PhenoPups approach is intended for use with rat and mouse pups from post-natal day (PND) 1 to PND 5 (rats) or weaning (mice). The aim of the present study was to assess the ability of this test system to detect disorders in rat pups exposed prenatally to chlorpromazine hydrochloride (CPZ), a known behavioral teratogen.

Figure 1: The PhenoPups device

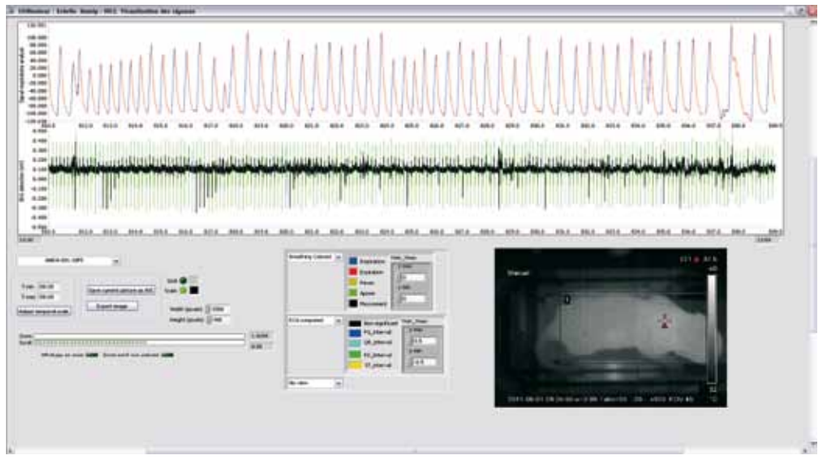

Figure 2: Simultaneous recording of physiological parameters

Materials and methods

CPZ was administered to groups of pregnant Sprague-Dawley rats (3 per dose-level) by the oral route at 0, 1, 5 or 25 mg/kg/day (groups 1 to 4), daily from day 6 to day 17 post-coitum (pc); one high-dose dam was sacrificed because of absence of delivery. Three untreated pregnant rats served as controls. Overall, the PhenoPups evaluation was performed in 100 rat pups from the litters of these dams.

The testing battery was performed on pups on PND 1 and PND 5. The parameters evaluated were respiratory minute volume, heart rate, body temperature, ultrasonic vocalization and general activity. These parameters were measured simultaneously, non-invasively and automatically in warmed chambers (at 32°C) in four unrestrained rat pups at a time (Fig. 3):

- The pups were placed individually in (four) whole-body flow plethysmographs for monitoring of respiratory function.

Figure 3: Flow-through plethysmograph with electrodes embedded in the floor

- Heart rate was measured by means of electrodes embedded in the chamber floor
- Body temperature was measured by infrared thermography.
- General activity (locomotion) was assessed in an openfield apparatus using a camera system; body posture was also monitored via body contact with the chamber floor (Fig. 4).

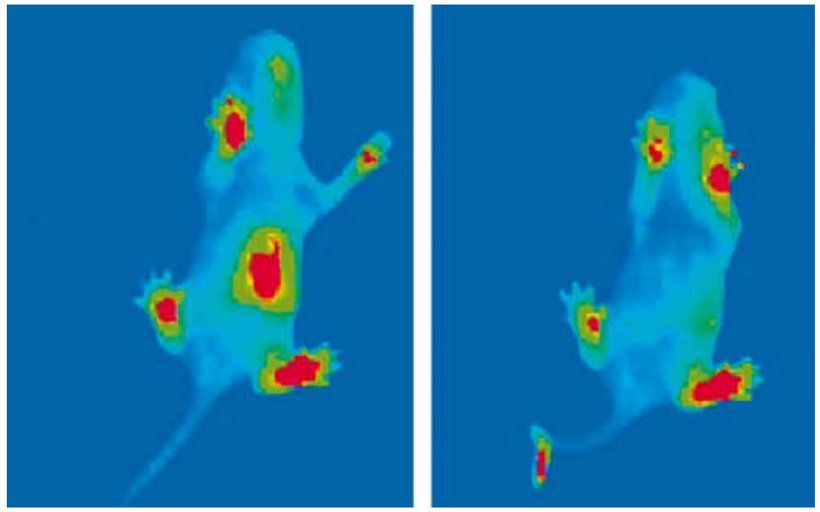

Figure 4: Locomotion assessment by camera recording of openfield gel floor deformation under body contact

- Ultrasonic vocalization (a potential measure of stress) was recorded and quantified.

Evaluation was completed in 20 minutes, after which the pups were returned to their littermates and mother. Pup body weight was also monitored over this period.

Results

In pregnant dams, treatment with CPZ resulted in hypoactivity and reflux at dosing in 2/3 females receiving the high dose-level of 25 mg/kg/day. Mean body weight gain and mean food consumption were not affected by CPZ.

Endpoints measured in pups born from females treated with vehicle (group 1; 3 litters), or CPZ at 1 mg/kg/day (group 2; 3 litters), 5 mg/kg/day (group 3; 3 litters) or 25 mg/kg/day (group 4; 2 litters) are summarized in Fig 5. The mean pup body weight was slightly lower in the offspring of rats treated at 25 mg/kg/day compared with controls.

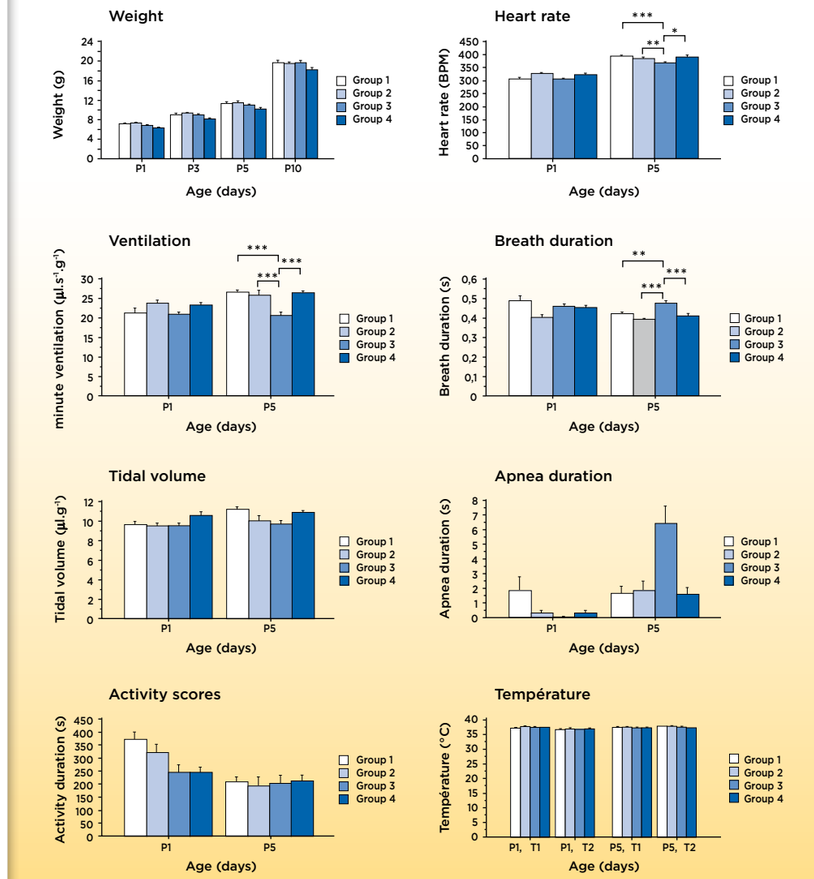

Figure 5: Endpoints in pups born from control and CPZ-treated females

No statistically significant changes in any functional parameter were observed on PND 1, although activity scores were lower in all pups born in treated groups, with a dose relationship, in comparison with controls.

At evaluation on PND 5, heart rate and respiratory minute volume were significantly lower and breath duration and apnea duration were significantly longer in pups born from females treated at 5 mg/kg/day than in other groups.

Discussion and conclusions

The PhenoPups device is an automated instrument which allows functional investigations under controlled conditions in unrestrained rat and mouse pups from PND 1 onwards. This device permits safety pharmacology investigations in neonates during pre- and postnatal reproductive toxicity studies, in which pups are exposed through their treated mothers, or juvenile toxicity studies, in which pups are treated directly. Overall, this approach allows rapid and convenient monitoring of physiological and behioural parameters in neonatal rat pups using a battery of non-invasive tests from as early as PND 1, as demonstrated by these results with CPZ.