Maternal separation and gastrointestinal transit time in neonate rats

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Abstract

Gastrointestinal transit times (GItts) were compared in separate litters of 10- and 15-day-old Sprague Dawley rats using barium sulphate. By tracking the leading front of the bolus on radiographs, the gastrocaecal transit times in pups were estimated. To measure the total GItt, the duration from orogastric gavage until an observable defecation of barium sulphate was recorded. The gastrocaecal times for 10-day-old pups maintained with their dam (n = 5) ranged from 4-5 h and those removed from the dam ranged from 2.5–5 h. For 15-day-old pups with their dam (n = 6) and without dam (n = 5), gastrocaecal times ranged from 4–6 h and 3.5–5 h, respectively. Ten-day-old pups that remained with the dam had a GItt of 13.8 ± 0.9 h and those kept in the absence of the dam had a time of 9.3 ± 0.7 h. This decrease (P < 0.05) in GItt in the absence of the dam was age-dependent in 10-day-old pups, and was not observed (P > 0.05) in 15-day-old pups. The results provide a basis, for the design of future studies involving neonate rat metabolism, to include maternal presence.

Keywords: Sprague Dawley, gastrointestinal transit time, barium sulphate

This study aims to investigate the acute effect of maternal separation on GItt in neonatal rat pups by a non-surgical method without the need of anaesthetics. Therefore, 10- and 15-day-old Sprague Dawley rats in the presence or absence of their mother were gavage fed barium sulphate and its passage was monitored non-invasively using X-ray radiography. Rats were selected for experimental ease as they are larger at 10 days than mice.

Studies were conducted in accordance with the University of Ottawa’s Animal Care Committee under protocol BMI-77. One litter of 10-day-old (n = 10) and one separate litter of 15-day-old (n = 11) Sprague Dawley rat pups (Charles River, Canada) weighing 19–28 g (10-day-old rats, 21.51 ± 1.61 g; 15-day-old rats, 27.40 ± 1.00 g; P < 0.05) were housed in polycarbonate rat cages and placed into an empty Ohio Medical Products Armstrong Isolation Servo-Care Incubator Model 190ASC (Ohmeda Inc, Liberty Corner, NJ, USA) at 37°C and fasted for 2 h to empty the stomach cavity. Cages provided ample space for pup activity and were outfitted with a food and water source for the dam.

Rats were individually identified and divided into two groups: maintained with their mother or placed in an
incubator without the mother. All pups were gavage fed
250 µL of barium sulphate (Liquid Polibar Plus, 53% w/v)
(EZEM Corp, Montreal Quebec, Canada) that was sus-
pended in ddH2O (1:1, v/v). For X-ray radiography,
animals were placed into a holding grid to ensure serial
tracking of bolus movement. X-ray radiographs (Hitachi
DGC-1010, 34 kV, 2 mA) (Hitachi Co, Tokyo, Japan) were
taken at 10, 20 and 30 min, followed by subsequent
exposures every 30 min thereafter. At 4 h, images were cap-
tured every hour for the duration of the experiment. All
aged pups separated from their mother were handled at
hourly intervals by weighing and feeding 0.25 mL of
Enfamil A + infant formula (Mead Johnson Nutritionals,
Evansville, IN, USA). In addition, saline solution (Baxter,
Deerfield, IL, USA) was injected subcutaneously to
hydrate pups in the absence of their mother.

For the present investigation, gastrocaecal and GItt were
defined as the duration for the leading edge of the barium
sulphate to enter the caecum and exit the anus, respectively.
X-ray radiographic images were analysed to determine
these time points. Gastrocaecal times were reported as a
range. GItts were reported as the mean ± the standard
error of the mean. A Student’s t-test was performed where
P < 0.05 was considered statistically significant.

Gastrocaecal time for 10-day-old pups with their dam and
without their dam was 4–5 and 2.5–5 h, respectively
(Table 1). For 15-day-old rats, the gastrocaecal times with
and without the dam was 4–6 and 3.5–5 h, respectively.
In the older Sprague Dawley pups, the GItt increased in
both the maintenance (P < 0.05) and separation groups
(P < 0.05) in comparison with those for their 10-day-old
counterparts (Figure 1). Observable developmental
changes in 15-day-old rats in comparison to their
10-day-old counterparts included additional body fur and
increased palpebral fissuring. The effect of maternal pres-
ence was observed in 10-day-old pups that demon-
strated a significant difference in their GItt (P < 0.001).

Due to limitations of the study, the frequency of radio-
graphs and animal feeding times could not be performed
simultaneously on all 21 animals. For this reason, the gastro-
caecal data were expressed as ranges (Table 1). However,
statistical analysis could be performed with GItt, since it
was possible to measure barium sulphate defecation using
several stopwatches.

Maternal separation may alter the response to stressors
and therefore GItt in rat pups through factors such as a
lack of maternal stimuli, new diet and physical stress. The
mother provided on-demand feeding for the pups and
performed anogenital licking, which is thought to stimulate
urine and faeces elimination.9 The effect of infant formula
and stress induced from subcutaneous saline injections
must also be considered when examining GItt differences.
Considering that rats wean at 20–22 days, pups may start
tolerating maternal absence as early as 15 days of age.
Whereas, age-related differences in GItt may be considered
in light of recent data. Older rats have lengthened colons,
increased colonic mucosa, increased spontaneous contractile
activity and show development of cholinergic neuromuscu-
lar transmission.8

Knowing that maternal separation induces stress in
neonate rats10 and that stress influences GItt in the Wistar
strains,3 one may infer that decreased GItt was a result of
stress caused by maternal separation. The present data
suggest that future experiments monitoring absorption,
digestion, metabolism and excretion or pharmacokinetic
studies using neonatal rats may be performed while main-
taining them in the presence of their mothers. This may
benefit the experimental outcome by reducing variation in
transit time and lessening the impact on digestion and
metabolism.

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**Table 1** Gastrocaecal and gastrointestinal transit time (GItt) in 10-
and 15-day-old rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animals</th>
<th>Rat age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal presence</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Transit (h)</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Gastrocaecal</td>
<td>M⁺ (n = 5)</td>
<td>M⁺ (n = 5)</td>
</tr>
<tr>
<td>4–5</td>
<td>2.5–5</td>
<td>4–6</td>
</tr>
<tr>
<td>Glitt</td>
<td>9.5–17</td>
<td>7.8–12.8</td>
</tr>
</tbody>
</table>

*Gastrocaecal and Glitt are defined as duration for leading edge of orally
gavaged barium sulphate to enter the caecum and exit the anus*

![Figure 1](image-url)
8 de Vries P, Soryl E, Heloury Y, Neunlist M. Postnatal development of myenteric neurochemical phenotype and impact on neuromuscular transmission in the rat colon. *Am J Physiol Gastrointest Liver Physiol* 2010;299:G539–47

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