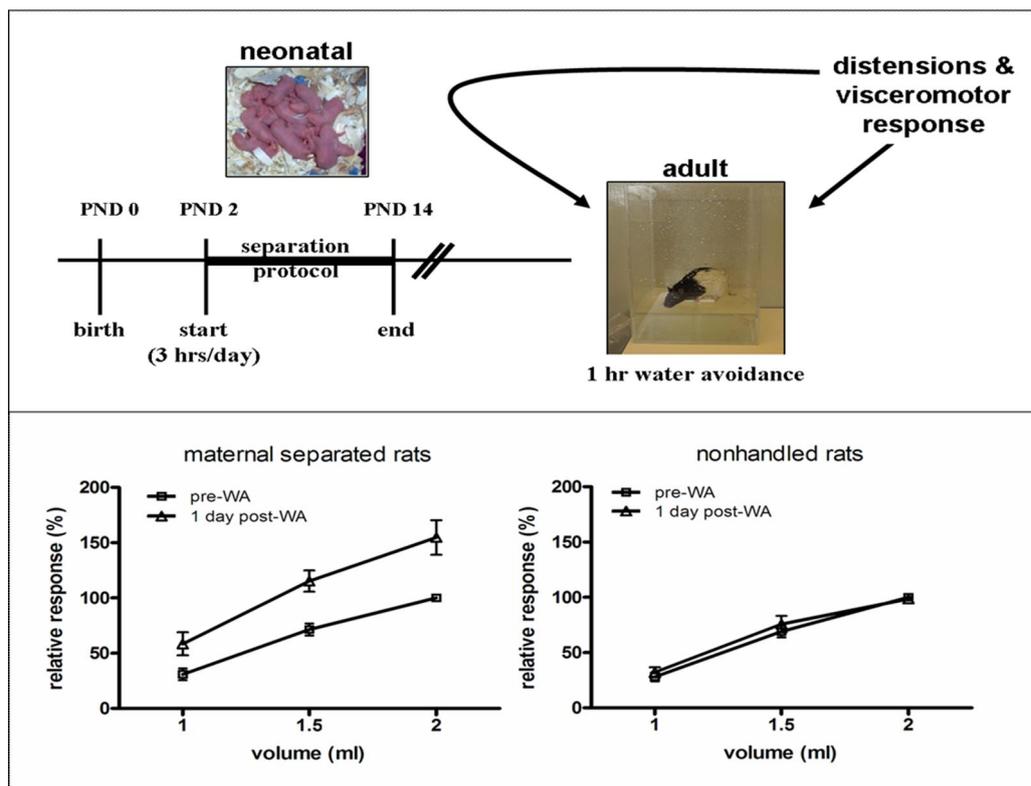


## Irritable Bowel Syndrome (IBS) model

### The maternal separation model for IBS

Early life stressors are known to contribute to IBS in adults and the maternal separation model in rat is often used to mimic such predisposing factors. In this model pups are separated from their mothers for 3 hours daily from postnatal days 2 to 14. Upon acute stress at adult age maternal separated Long Evans rats display enhanced sensitivity to colorectal distension whereas non-handled rats remain normo-sensitive.<sup>1</sup> This so called visceral hypersensitivity is considered a pathophysiological mechanism in IBS. In rats it is usually quantified by measuring the visceromotor response; the quantification of reproducible abdominal contractions (by EMG) that occur due to a spinal reflex.

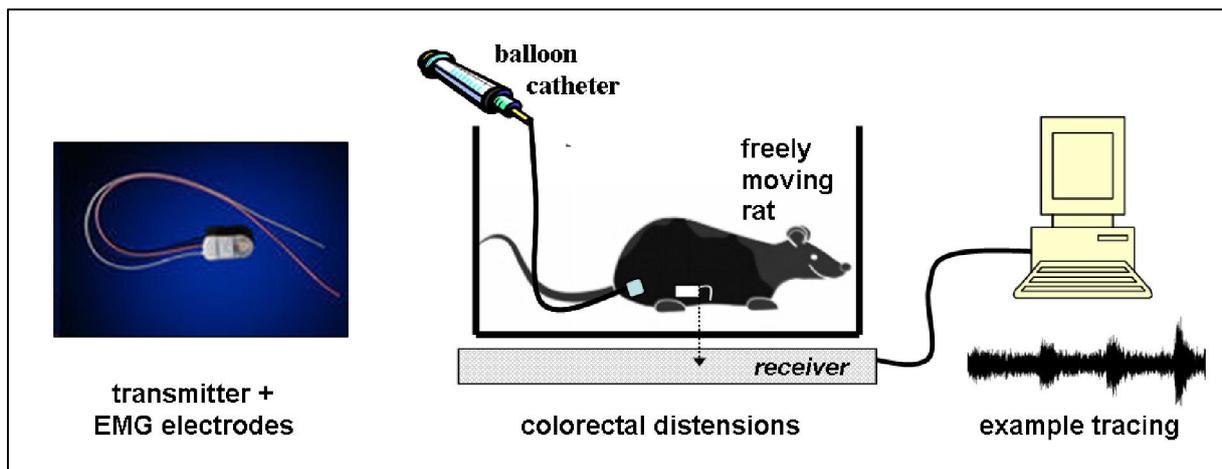


**Figure 1.** Maternal separation combined with acute stress at adult age. Only maternal separated rats develop post-water avoidance visceral hypersensitivity.

Importantly, onset of visceral hypersensitivity depends on rat strain; Wistar rats do not require the additional acute stress trigger at adult age. However, at Gut Research we prefer to work with Long Evans rats because also in IBS-patients acute stress is an important trigger for visceral hypersensitivity and symptom generation.

### Measurement of visceral sensitivity

When stress is used as a trigger for visceral hypersensitivity it is crucial to ensure that baseline sensitivity data are gathered in a stress free setting. Most laboratories assessing visceral sensitivity (i.e. the visceromotor response) in IBS models tunnel the abdominal EMG-electrodes to the neck and subsequently attach them to a recording device. In these experiments damage to electrodes is usually prevented by putting animals in Bollman restrainers during distension protocols. Since restraint is a stressor in itself this methodology will obscure baseline measurements. Therefore Gut Research has chosen to work with a radio-telemetry system that enables measurements in non-restrained rats. In our set up a radio-telemetric transmitter is stitched into the abdominal cavity. During distension protocols the transmitter is switched on by a magnet, the EMG signal is then detected by a receiver located directly under the cage in which the rats can freely move.<sup>1</sup>

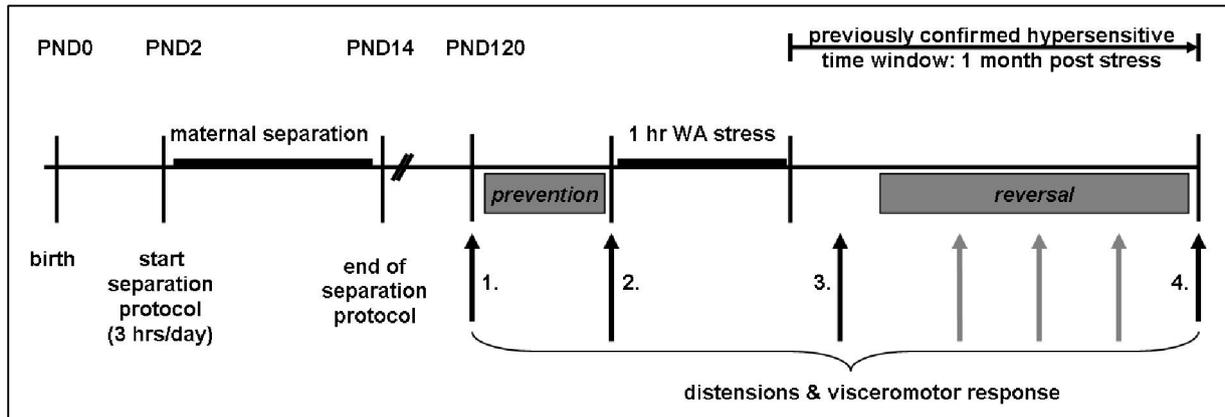


**Figure 2.** Radio-telemetry system used to evaluate visceral sensitivity during colorectal distensions in freely moving animals.

### Prevention vs reversal of stress-induced visceral hypersensitivity

When considering the most suitable intervention strategy (i.e. the choice between pre-stress vs post-stress treatment of adult maternal separated rats) it is important to notice that the experimental outcome may differ between strategies. This becomes clear from the following example.

In our rat model as well as in patients there is ample evidence for an important role of mast cells.<sup>2-4</sup> Based on pre-stress treatment protocols in a variety of animal models carried out by other labs, the current dogma states that corticotrophin releasing hormone (CRH) is responsible for peripheral mast cell activation. However, two recent clinical trials with CRH-receptor antagonists in IBS patients failed. These contradicting findings may be explained by our recent observations in the maternal separation model. We showed mast cell dependent visceral hypersensitivity for up to one month post-water avoidance stress. Pre-stress treatment with a CRH-receptor antagonist prevented long term visceral hypersensitivity but post-stress treatment with the same antagonist could not reverse it (whereas post stress treatment with a mast cell stabilizer was successful).<sup>5</sup> Clearly, these results indicate that successful prevention does not guarantee successful reversal of the post stress phenotype by the same compound. Since reversal is more relevant in patients it is now clear why the CRH-receptor antagonist clinical trials failed and why Gut Research prefers post stress intervention protocols when treatment is the ultimate goal.

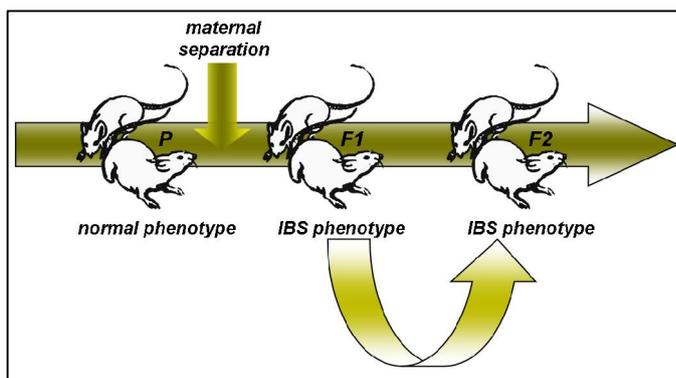


**Figure 3.** General scheme for intervention protocols.

- When choosing a **prevention** protocol, 3 distension sessions are needed per animal: #1 & #2 to ensure that ‘the compound’ or its vehicle does not change baseline sensitivity and #3 to establish whether the compound prevented post stress visceral hypersensitivity. In this type of experiment the pre-stress treatment period is flexible as long as we start with full grown animals (explaining the 4 month starting age).
- When choosing a **reversal** protocol, baseline sensitivity is only measured at point #2 followed by a post stress session (#3: usually 24 hours post WA) to confirm the IBS-like phenotype. Treatment can start directly after this measurement. Our previous research showed that stress-induced visceral hypersensitivity is still present 1 month post stress,<sup>5</sup> suggesting that treatment may take up to one month or shorter. Measurement #4 will show possible success. If preferred, additional measurements can be carried out between #3 & #4.

### Prevention of transfer across generations

We recently expanded the possibilities of our rat model by demonstrating that enhanced susceptibility to stress-induced hypersensitivity, as observed in maternally separated (F1) rats, can be transferred to next generation (F2) animals without further separation protocols.<sup>6</sup> This is highly relevant because IBS was shown to cluster in families in the absence of clear genetic components, suggesting possible ‘nongenomic’ transfer across generations. Our data open up possibilities to investigate treatment strategies that may interfere with such transfer (e.g. treatment of lactating F1-mothers and/or F2-pups with dietary approaches).



**Figure 4.** Susceptibility to stress induced visceral hypersensitivity in maternally separated F1 rats can be transferred to the next (F2) generation without further separation protocols. Our cross fostering experiments showed that transfer depends on maternal care.

### **Relevance for human IBS**

We recently translated our successful mast cell interventions in the maternal separation model into a clinical trial where we showed effectiveness of the mast cell stabilizer and histamine-1-receptor (H1R) antagonist ketotifen in IBS patients.<sup>7</sup> From tissue samples obtained in this trial we next concluded that H1R antagonism was the most probable mechanism of action of ketotifen. We subsequently tested two highly selective and peripherally restricted H1R antagonists in our rat model; both showed reversal of post stress visceral hypersensitivity.<sup>2</sup> Based on these results one of these antagonists was recently evaluated in a new clinical trial (conducted at Catholic University of Leuven) with positive outcome.<sup>8</sup> These data indicate that the Gut Research IBS model of maternal separation combined with acute stress at adult age is predicative of successful interventions in the human setting.

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