ROLE OF THE PVN IN TNB ANOREXIA

ROLE OF THE PARAVENTRICULAR NUCLEUS IN TNB-INDUCED ANOREXIA

By

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ABSTRACT

Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract, often associated with reduced food intake (anorexia) and weight loss. The anorexia manifest following gastrointestinal inflammation can only be expressed if appropriate signals are communicated from the inflamed segment to the brain. Yet, the nature of these signals, and the identity of the brain sites processing these anorexigenic signals, are unknown. The present experiment evaluates the contribution of the paraventricular nucleus (PVN), a brain site rich in corticotropin releasing factor (CRF) receptors and known to be involved in the control of food intake, in the anorexia associated with experimental colitis. Colitis was induced, by trinitrobenzenesulfonic acid (TNB) treatment, in animals in which the PVN was ablated or in rats with sham brain surgeries. Results indicated clearly that the expression of the anorexia following TNB treatment is fully expressed even in the absence of the PVN. This result indicates that the integrity of the PVN is not necessary for the reduction of eating associated with intestinal inflammation, thus suggesting that CRF is also not critical to colitis-induced anorexia.

inflammatory bowel disease (IBD); feeding; anorexia; gut-brain communication; paraventricular nucleus (PVN); interleukin-1 (IL-1); corticotropin-releasing-factor (CRF); neuroimmunology

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INTRODUCTION

Inflammatory Bowel Diseases (IBD) are a host of different clinical entities that involve inflammation of the gastrointestinal tract. IBD is a serious problem that is increasing in incidence (Barton, Gillon & Ferguson, 1989), and, as a consequence, an increased cost to our health care system (Barton et al., 1989). There are many different forms of IBD, but the most prevalent ones are ulcerative colitis (UC) and Crohn's disease (CD) (Langman, 1990), two similar but pathologically distinct disorders. UC is characterized by mucosal inflammation involving only the colon, while CD is characterized by transmural inflammation that may affect the gastrointestinal tract anywhere from the esophagus to the anus (Sutherland, 1987). Although these disorders have been discussed in the medical literature since the 19th century, the underlying causes of both forms of IBD have not yet been identified. A number of theories of pathogenesis have been put forward, including ones of a genetic contribution, altered permeability of the mucosa, vasculitis, and mycobacterial infection (Andrews & Goulston, 1994). In general, though, the end result of the defining event (ie., the inflammatory response), is viewed either as an appropriate response to an unidentified antigen, or an inappropriate inflammation which results from a hyper-reactive immune system (Silk & Payne-James, 1989).

Inflammation of the gastrointestinal tract in humans is accompanied by a reduction in body weight which could reflect reduced food intake (anorexia), increased metabolic rate due to effects of chronic disease, and/or impaired nutrient absorption (Anderson, Sutherland, & Hassall, 1989). Weight loss is observed in as many as 70%-80% of Crohn's patients (Silk et al.,1989) and can be so dramatic that this population has been used as a positive control in studies of anorexia nervosa (McCallum, Grill, Lange, Planky, Glass, & Greenfield, 1985). Due to reduced food intake, IBD patients often do not receive the appropriate amount of vitamins and nutrients and may become malnourished (Imes,

Pinchbeck, & Thompson, 1987). These nutritional deficits appear to contribute to increased mortality and morbidity for many IBD patients (Kirschner, 1988; Barton et al.,1989; Heatley, 1986), but are particularly severe for children and adolescent IBD patients. Up to 40 percent of IBD cases occur before the age of 18 years, and many in this patient population experience growth deficiencies and delayed puberty (Anderson et al., 1989). In addition to the physical consequences in the younger IBD population, the psychosocial ones are also serious; growth failure, pubertal delay, and side effects from steroid therapy may lead to body image problems and low self-esteem (Anderson et al., 1989).

Correction of malnutrition is an important component of the treatment of IBD. The ability to do so serves also to reduce the need for medication and surgery (Greenberg, Flemming, Jeejeebhoy, Rosenberg, Sales, & Tremaine, 1988; Gassull, Abad, Cabre, Gonzalez-Huix, Gine, & Dolz, 1986; Silk et al., 1989). Uncovering and understanding the mechanisms mediating this anorexia may allow for easier management of nutrition for IBD patients, the opportunity for increased quality of life, and prevention of growth problems in children with IBD.

To understand what underlies the perturbation of food intake in IBD patients, it is reasonable to think, initially, of the mechanisms that control normal food intake, and which of these may be disrupted in the case of IBD. The adaptive control of food intake requires communication of signals between the periphery and brain. The gastrointestinal tract, especially, is the source of many gut signals that provide stimuli to the brain regarding the control of eating (Campfield & Smith, 1990). In many cases, the exact mechanism by which these signals are transmitted to the brain, as well as the identity of brain sites where these signals are received , remain unknown. However, disruptions in eating behaviour may reflect disturbances in gut-brain communication. Obviously, in the

case of IBD, the inflamed segment elaborates an abnormal anorexigenic signal that is communicated to the brain, or the inflammation alters mechanisms that normally regulate food intake. It is difficult to study this communication, at the biological level, between gut and brain in human patients with IBD because of the invasive nature of the techniques necessary. Therefore, an animal model of IBD is required. Ideally, this model should resemble IBD in as many pathological features as possible, including the nature and time course of the inflammation and its mediators, response to drugs, and ability to show relaspses. Also, an ideal model should be economical, easy to induce and maintain, and be reproducible and predictable. There is no one model that meets all of these criteria, so it becomes necessary to determine which particular components one needs represented in order to choose the appropriate model. For the purposes of the present experiment, an important feature is the ability to observe reliable and reproducible reductions of food intake in an accessible species in response to intestinal inflammation. Also, the most relevant models may be those involving colon inflammation, a colitis, since these are the clinical conditions most associated with anorexia in human IBD, and the colon is far removed from gut areas, such as the stomach and proximal small intestine, involved directly in the control of eating.

There are several models of colitis currently available. A specific sub-group of New World marosets often develop a mild chronic colitis that is spontaneously relapsing in captive animals (Lushbaugh, Humason, & Clapp, 1985). Unfortunately, there is limited access to this species, and so it is unsuitable for most researchers. There also exists several inducible colitis models, such as the peptidoglycan-polysaccaride model (Sartor, 1992), the carrageenin and dextran sulphate sodium model (Marzio, Blennerhassett, Chiverton, Vermillion, Langer, & Collins, 1990), and the immune complex colitis model (Hodgson, Potter, Skinner, & Jewell, 1978). Although these models are promising in

some aspects, there are associated problems with each, especially costliness and variable results.

The model that has been most exploited to study the food intake disturbances associated with IBD is a rat model of experimental colitis with a similar symptomology to IBD (Allgayer, Deschryver & Stenson, 1989; Morris, Beck, Herridge, Depew, Szewczuk & Wallace, 1989). In this model, colitis is induced in the distal colon of rats by intrarectal (IR) infusions of 2,4,6-trinitrobenzenesulfonic acid (TNB) dissolved in ethanol (EtOH). This treatment causes an acute inflammation, followed by a more chronic inflammation characterized by ulcers, luminal narrowing and smooth muscle hypertrophy (Allgayer et al., 1989). Most importantly for the present purposes, TNB treatment results in an immediate suppression of food intake that is greatest on the first day following treatment, and then returns to normal by the fourth day (McHugh, Weingarten, Keenan, Wallace & Collins, 1993a). The model is attractive because it is accessible and convenient because the inflammation is induced by a single IR infusion. The food intake changes are robust and reprodicible. One limitation of the TNB model is that it cannot reproduce the chronic, relapsing nature of human IBD. However, the TNB model remains the most promising tool to examine the relationship between colonic inflammation and anorexia.

The experimental colitis induced by TNB produces a robust suppression of food intake, approximately a 75% reduction of daily caloric intake compared to pretreatment baseline, during the first 72 hours following treatment. An important issue that needed to be resolved for this model, however, is whether the anorexia results simply from malaise or visceral illness due to the harshness of the procedures used to induce the model. Generalized malaise, though, is not an adequate explanation for the large suppression of food intake following TNB treatment. This conclusion is based on a number of demonstrations. First, TNB-treated rats demonstrate normal levels of water intake and

sham feeding even when they are severely anorexic (McHugh, Castonguay, Collins, Weingarten, 1993b). This demonstrates that they can perform the necessary ingestive behaviours and are not generally suppressing all appetitive behaviour, a profile indicative of malaise. Also, McHugh et al. (1993b) showed through meal pattern analysis that the reduction of eating during experimental colitis results specifically from a suppression of meal size, not a reduction in number of meals initiated. These findings suggest that the reduced food intake results from an exaggerated satiety signal. A reduction in the number of meals initiated is regarded, typically, as evidence of malaise. It appears, then, that the anorexia depends on an inflammation-related signal that is communicated to the brain. However, the mechanism by which this message is transmitted to the brain and where this message is received in the brain, remain unclear. Elucidating these gut-brain communication issues in the context of the TNB model would be beneficial to treatments to attempt to understand and correct malnutrition in IBD, as well as expanding our understanding in general about gut-brain communication.

Research on inflammation-related signals that may be relevant to anorexia have focused on cytokines, that are produced in abundance in the tissues of patients with IBD (Ligumsky, Simon, Karmeli, & Rachemilewitz, 1990; Stevens, Walz, Singaram, Lipman, Zanker, Muggia, Antonioli, Peppercorn, Strom, 1992). Some of these cytokines have been demonstrated to cause perturbations in areas of the gastrointestinal tract as well as other systems involved in the control of feeding (Stevens et al.,1992). Specifically, the cytokine interleukin-1 (IL-1), appears to correlate well with IBD activity, (Brynskov, Nielson, Ahnfelt-Ronne, & Bendtzen,1992) and has been shown to decrease gastric acid secretion, delay gastric emptying, and stimulate the release of cholecystokinin (CCK) (Robert, Olafsson, Lancaster, & Zhang, 1991; Uehara, Sekiya, Takasugi, Namiki & Arimura, 1989). IL-1 has also been implicated in TNB-induced anorexia in rats. For

example, it has been shown that IL-1 levels are elevated within the first 24 hours following TNB treatment, and that acute administration of IL-1, peripherally or centrally, suppresses feeding (Plata-Salaman, French-Mullen, 1992; Chance & Fischer, 1991). Chronic administration of IL-1 peripherally results in a pattern of anorexia similar to that seen following TNB treatment (Mrosovsky, Molony, Conn, & Kluger, 1989). Also, administration of the endogenous human IL-1 receptor antagonist (IL-1ra), has been shown to attenuate, at least partially, TNB-induced anorexia (McHugh, Collins & Weingarten, 1994).

It would appear, then, that the proinflammatory cytokine IL-1 may be involved in mediating the anorexia following TNB treatment. However, there remains some question as to how IL-1 released in the periphery in response to inflammation, is related to IL-1 mediated effects in the brain, since a large molecule substance like IL-1 is not presumed to pass the blood-brain barrier (BBB) (Blatteis, 1990; Dinarello, 1991). It may be that IL-1 in the periphery is actively transported through the BBB into the central nervous system (CNS); there is evidence that radiolabelled IL-1 is transported from the blood to the brain by a saturable active transport mechanism (Banks, Ortiz, Plotkin, & Kastin, 1991). There is also speculation that IL-1 may enter the CNS where the blood brain barrier is leaky, such as in the circumventricular organ, the organum vasculosum lateral terminalis (OVLT), especially since the integrity of the OVLT is critical for the production of IL-1 induced fever and other effects (Blatteis, 1990). Others speculate peripheral IL-1 may stimulate prostaglandin synthesis in the brain periventricular areas or in the ventricular endothelium; then, the increased prostaglandin stimulates IL-1 production in the brain (Blatteis, 1990).

The discussion above indicates that it is still unclear how peripheral pools of IL-1 communicate with IL-1 in the brain. However, studies using IL-1 receptor antagonists

show that IL-1 receptors in both periphery and central nervous system contribute to the suppression of food intake during acute experimental colitis (McHugh et al., 1994; Plata-Salaman et al., 1992). It appears, then, that central IL-1 is mediating the suppression of food intake, however it is unknown where in the brain this is taking place, and what other central mediators may be implicated in the expression of anorexia as well. An important consideration in how central IL-1 mediates food intake is the potential role of the hypothalamic neuropeptide, corticotropin releasing factor (CRF). One of the most consistently observed effects of CRF on behaviour is a suppression food intake (Britton, Koob, River, & Vale, 1982; Morley & Levine, 1982; Glowa, Barrett, Russell, & Gold, 1991). Intracerebroventricular (icv) administration of CRF in the rat results in dose-dependent decreases in food intake that can be observed within 15 minutes following administration (Glowa et al., 1991). Also, CRF has the ability to attenuate the increases in food intake that are produced by such agents as muscimol, norepinephrine, insulin, and dynorphin (Gosnell, Morley, & Levine, 1983).

The suppressive effects of both IL-1 and CRF on food intake allow some speculation about whether these effects are linked in some way. This speculation is fueled by the observation that IL-1 activates the hypothalamic-pituitary-adrenal (H-P-A) axis by stimulating the release of CRF. Also, it is known that CRF is the main stimulus of adrenocorticotropic hormone (ACTH), and IL-1-stimulated ACTH secretion acts via a mechanism that involves endogenous CRF (Uehara et al., 1989). Based on the observation that IL-1 stimulates the secretion of hypothalamic CRF, Uehara et al. (1989) examined whether stimulation of IL-1 production would result in decreased food intake, and whether this anorexia was mediated by increased endogenous CRF production in the brain. They found that exogenously administered IL-1, as well as endogenously released IL-1 (using lipopolysaccharide, a potent stimulant of endogenous production and release

of IL-1) suppressed food intake, and it did so by a mechanism that involved increased release of endogenous CRF in the brain. Based on a number of other studies as well, findings allow the conclusion that the main site of action of IL-1 and tumor necrosis factor (TNF) is in the hypothalamus, through the production of CRF (Elenkov, Kiss, Stark & Bertok, 1992; Sapolsky, Rivier, Yamanoto, Plotsky, & Vale, 1987).

When looking for an area of the brain that may be receiving the message from the gut about inflammation, one then might look for an area that is rich in CRF receptors, in an area already associated with feeding as potential area.

The paraventricular nucleus (PVN) of the hypothalamus contains numerous CRF-like immunoreactive neurons and fibers as well as CRF receptors (DeSouza, Insel, Perrin, Rivier, Vale & Kuhar, 1985; Silverman, Hou-Yu, & Chen, 1989). In fact, the PVN is the major source of neuropeptides (CRF-41, vasopressin, etc.) involved in regulation of ACTH synthesis/ release from the pituitary gland (Elenkov et al., 1992). Also, research on stress-induced alterations of gastric and colonic motor activity indicate that endogenous CRF in the PVN mediates gastric emptying and colonic transit, and that the PVN is a specific responsive site for gastric alterations induced by CRF (Monnikes, Schmidt, Raybould, and Tache, 1992). The PVN is also a strong mediator of feeding; lesions of this hypothalamic nucleus have long been recognized to result in overeating and obesity (Leibowitz, Hammer & Chang, 1981; Aravich & Sclafani, 1983).

The hypothesis guiding the present experiment is that the PVN is involved in processing the anorexigenic signal from the inflamed gut. This hypothesis is based on several considerations. First, IL-1 suppresses food intake and is clearly involved in the anorexia expressed during inflammation. Second, IL-1 acts centrally to release CRF, a neuropeptide that is a strong mediator of food intake. The PVN is an area rich in CRF receptors, and also involved in the control of food intake.

In the present experiment, I determined if the PVN mediates the anorexia expressed during acute experimental colitis induced by TNB. To do so, the PVN was removed surgically. Colitis was induced by intrarectal infusions of TNB. Sham brain surgeries and intrarectal infusions of EtOH (vehicle) served as controls. The expectation was that if the PVN mediated the peripheral anorexigenic signals, ablation of the PVN should attenuate the anorexia observed following TNB compared to sham-operated controls.

METHODS

Subjects. Thirty-three Sprague Dawley albino rats, weighing approximately 300 - 350 g at time of surgery, were housed in individual stainless steel hanging cages, under controlled (12:12) light/dark conditions. They were fed mash (65% H_2O / 35% powdered rat chow) and ad libitum water.

Surgery. To ablate the PVN, a knife was used (Makara, Stark, Kapocs, & Antoni, 1986) made from 0.5-mm OD stainless steel tubing, that, when extended, assumed the shape of the PVN. This knife was positioned, using standard sterotaxic procedures, to the PVN, and when rotated, effectively cut an island around the PVN.

For this surgery, animals were anaesthetized with Ketamine (0.9 ml/kg) and Xylazine (0.5 ml/kg), and mounted in the stereotaxic apparatus (nose bar set at -6mm from horizontal). The knife was positioned 2.0 mm behind bregma, in the midline, and touching the base of the skull. The knife was then rotated 360 degrees to the right and to the left, making an inverted pyramidal-shaped lesion centered on the PVN. For sham operations, the knife was simply lowered to the base of the skull but was not rotated. The PVN was cut in 21 rats (Cut group); another 12 rats served as controls (Sham group).

Measurement of Food Intake (FI) and Body Weight (BW). The 24 hour FI and BW of each animal were recorded daily for five days prior to surgery. Two weeks

following stereotaxic surgery, the FI and BW were again recorded for four days prior to TNB or EtOH treatment.

TNB/EtOH Treatment. Half of the rats in both the Cut and the Sham groups were randomly assigned to receive intrarectal (IR) infusions of 30 mg trinitrobenzenesulfonic acid (TNB) in 0.25 mls of 50% ethanol or equivolumes of 0.25 ml of the 50% EtOH vehicle only. Then FI and BW were recorded daily for the next five days.

Sacrifice. On the sixth day following TNB or EtOH treatment, animals were sacrificed by a lethal dose of chloral hydrate (65 mg/rat). Then, approximately 150 mg of colon adjacent to the site of maximum inflammation was removed from each rat, flash-frozen in liquid nitrogen, and stored at -70°C. The degree of colon inflammation was indexed by assay of myeloperoxidase (MPO) activity, and is reported as units of MPO activity per gram of wet tissue, where one unit of MPO is the amount able to convert 1 umol of hydrogen peroxide to water in 1 minute (at 22°C).

Immediately after colon removal, each animal was perfused transcardially with 0.15 M saline followed by 10% buffered formalin. The brain was removed and soaked in 4% paraformaldehyde with 25% (w/v) sucrose. Brains were subsequently frozen and sectioned in 40 µm sections in a cryostat. Sections were then mounted, stained with cresyl violet, and evaluated for degree of PVN damage.

Inclusion Criteria. Damage to the PVN was assessed by an observer blind to experimental results. An animal was included in the PVN group if the neural damage removed at least 80% of the PVN bilaterally. Rats with unilateral PVN lesions, or bilateral damage to less than 80% of the PVN, were not included in the successful PVN group. Figure 1 provides examples of successful and unsuccessful lesions.

Insert Figure 1 about here

Statistical Analysis. FI and BW data following TNB or EtOH treatment were analyzed with a 3-way mixed ANOVA. The three independent variables were: 1)intrarectal infusion (**IR**), with levels TNB and EtOH (between subject); 2)Brain **Lesion**, either PVN cut or sham (between subject); and 3)posttreatment **Day**, including 5 days (within subject).

RESULTS

Based on the inclusion criteria, the final size of the four groups were: TNB-Sham (n=6), EtOH-Sham (n=6), TNB-Cut (n=7), and EtOH-Cut (n=4).

Food Intake. Prior to TNB/EtOH treatment, the 24-hour FI of the four groups were as follows: TNB-Sham: 102+4 g; EtOH-Sham: 101+4 g; TNB-Cut: 120+11 g; and EtOH-Cut: 123+11 g. A two-way ANOVA revealed that rats with PVN lesions rats ate significantly more mash in 24 hours than their sham-operated controls prior to IR treatment [$F(1,19) = 6.004$, $p < 0.024$].

After IR treatment, ANOVA revealed that rats treated with TNB ate significantly less than EtOH controls over the 5 posttreatment days [$F(1,19) = 10.31$, $p < 0.005$]. The significant IR x Day interaction [$F(4,76) = 12.64$, $p < 0.000$] and inspection of Figure 2 indicated that TNB-treated rats were initially anorexic, but then recovered food intake by about Day 4.

Insert Figure 2 about here

Most importantly, the absence of a significant interaction of TNB/EtOH treatment and

PVN lesion (IR x Lesion) [$F(4,76) = 0.54$], indicated that rats with PVN lesions and treated with TNB showed the same pattern of anorexia as their sham-operated controls. (See Figure 2).

Because the presence of PVN lesions induced different levels of food intake prior to IR treatment, the post-IR treatment data were also expressed as an absolute change from baseline. Analysis of the data confirmed the results obtained with the absolute levels of food intake. Specifically, rats treated with TNB ate significantly less than EtOH controls [$F(1,19) = 9.76$, $p < 0.006$]. The significant IR x Day interaction [$F(4,76) = 11.002$, $p < 0.000$], and inspection of Figure 3 show the same pattern as with absolute food intakes; TNB treated rats were initially anorexia but recovered by about Day 4.

Insert Figure 3 about here

But, inspection of Figure 3 and ANOVA indicated that PVN lesions did not affect the pattern of food intake for either TNB or EtOH treated rats over the post-treatment days (IR x Lesion); [$F(4,76) = 0.61$].

Body weight. The baseline body weights of the groups prior to IR treatment were: TNB-Sham: 410+17 g; EtOH-Sham: 428+15 g; TNB-Cut: 438+12 g; and EtOH-Cut: 470+32 g. A two-way ANOVA revealed that rats with PVN lesions weighed significantly more than their sham-operated controls [$F(1,19) = 4.84$, $p < 0.049$].

Changes in body weight following TNB/EtOH treatment paralleled the FI changes. Rats treated with TNB weighed significantly less over the posttreatment days than controls [$F(1,19) = 6.76$, $p < 0.018$]. Again, the significant IR x Day interaction [$F(4,76) = 7.41$, $p < 0.000$], and inspection of Figure 4, indicate that body weights of TNB-treated rats initially dropped, but eventually recovered.

Insert Figure 4 about here

Since baseline pre-IR treatment baseline body weights were different, a clearer presentation of BW data is shown in Figure 5, where BW's over the five posttreatment days are expressed as absolute change from each group's respective baseline weight.

Insert Figure 5 about here

Inspection of Figure 5, and the significant IR x Day interaction [$F(4,76) = 7.41$, $p < 0.000$], indicate the body weight reduction of TNB-treated rats compared to their EtOH controls over the 5 posttreatment days. However, there was no effect of PVN lesion on BW profiles post-treatment.

MPO Values. Group mean MPO values are shown in Figure 6. Results from a two-way ANOVA (IR x Lesion), show that TNB treatment significantly elevated MPO levels compared to EtOH [$F(1,19) = 18.68$, $p < 0.0004$].

Insert Figure 6 about here

There was no significant interaction of IR x Lesion [$F(1,19) = 1.39$] indicating that MPO levels achieved in TNB-treated rats were similar in rats with ablated or intact PVN's.

Misplaced Lesions. The most important results of this thesis are derived from rats with accurate PVN lesions, I also report results from those rats excluded from analysis because of misplaced lesions. Food intake measures confirmed what was determined by brain histology; specifically, those rats with misplaced PVN lesions did not

eat significantly more than sham operated controls [F(1,17) = 0.36] during the baseline period. Except for this distinction in baseline data, rats with misplaced lesions showed the same post-treatment trend in food intake as other rats treated with TNB or EtOH (see Figure 7).

Insert Figure 7 about here

The body weights of rats with misplaced lesions were also not significantly greater than their sham controls [F(1,17) = 0.02]. Also, inspection of Figure 8 (actual body weights) and Figure 9 (body weight as change from baseline) revealed that the rats with misplaced lesions manifest the same trends in body weight as rats with accurate lesions.

Insert Figures 8 and 9 about here

DISCUSSION

Inflammatory bowel disease involves chronic inflammation of the gastrointestinal tract that frequently results in severe weight loss and anorexia. Because there is no known cause nor cure for IBD, there is a need to understand more about this disease, especially about the associated anorexia that complicates medical treatment and contributes to morbidity.

This particular study was designed to determine if a particular area in the brain, the PVN, was involved in the receipt of the anorexigenic signal or its processing. Identification of the brain sites mediating such a signal, could allow for the development of pharmacological agents that could ameliorate the anorexia in human patients.

The results from this study demonstrate clearly that the integrity of the PVN is not necessary for the expression of TNB-induced anorexia. In fact there were no differences in the magnitude or time course of TNB-induced anorexia between those with completely ablated PVN lesions and sham-operated controls.

As outlined in the introduction, the PVN was chosen as the site of study due to its richness in CRF fibers and receptors, as well as its role in mediating food intake. It appears from many studies that CRF is an important neuropeptide for stimulating thermogenesis during chronic inflammation (Cooper & Rothwell, 1991), as well as mediating food intake (Glowa et al., 1992). Therefore, it seemed plausible that interrupting CRF functioning by lesioning the PVN, might disrupt the inflammation-induced signal from the periphery. The fact that this was not found, does not necessarily mean that CRF is not mediating the anorexigenic signal, as there are CRF producing neurons and receptors present in many brain sites (Rothwell, 1992). Perhaps another approach would be to examine CRF levels, both peripherally and centrally, following induction of colitis. Alternatively, examination of the role of CRF might be attempted by antagonizing CRF in the CNS during colon inflammation.

Although the mechanisms by which anorexia is expressed following TNB treatment are yet to be revealed, the results from this study show that the PVN does not mediate the anorexigenic signal from the periphery. This allows one to rule out at least one brain area that may have been necessary for receiving and/or mediating the anorexigenic signal. This study encourages further examination of other brain areas to determine, or rule out, their role in inflammation-induced signals.

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FIGURE CAPTIONS

Figure 1. Photographs of brain sections: **A**: sham operated control (PVN fully intact); **B**: successful lesion (fully bilateral, centered on PVN), **C** & **D**: incomplete PVN lesions.

Figure 2. 24 hr food intakes (35% powdered chow / 65% water) in the baseline period (*bsln*) and for 5 days following TNB / EtOH treatment. TNB-Sham (n=6); TNB-Cut (n=7); EtOH-Sham (n=6): and EtOH-Cut (n=4). Data shown are group means ± SE.

Figure 3. Food intake (expressed as absolute change from baseline intake) for 5 days following TNB / EtOH treatment. Baseline intake = 0 gms for all groups: TNB-Sham (n=6); TNB-Cut (n=7); EtOH-Sham (n=6): and EtOH-Cut (n=4). Data shown are group means ± SE.

Figure 4. Body weights in the baseline period (*bsln*)and for 5 days following TNB / EtOH treatment. TNB-Sham (n=6); TNB-Cut (n=7); EtOH-Sham (n=6): and EtOH-Cut (n=4). Data shown are group means ± SE.

Figure 5. Body weights (expressed as absolute change from baseline weight) for 5 days following TNB / EtOH treatment. Baseline weight = 0 gms for all groups: TNB-Sham (n=6); TNB-Cut (n=7); EtOH-Sham (n=6): and EtOH-Cut (n=4). Data shown are group means ± SE.

Figure 6. Myeloperoxidase (MPO) activity in colon on *day 6* after treatment with TNB or EtOH. TNB-Sham (n=6); TNB-Cut (n=7); EtOH-Sham (n=6): and EtOH-Cut (n=4). Values are group means ± 1SE.

Figure 7. Levels of 24 hr food intakes of animals with misplaced lesions compared to sham operated controls. Data shown are group means ± 1SE in the baseline period (*bsln*) and for 5 days following TNB / EtOH treatment. TNB-Sham (n=6); TNB-Cut (misplaced) (n=4); EtOH-Sham (n=6): and EtOH-Cut (n=5).

Figure 8. Body weights (expressed as absolute change from baseline weight) of animals with misplaced lesions, compared to sham operated controls. Data shown are group means ± 1SE for 5 days following TNB / EtOH treatment (baseline weight = 0 for all groups). TNB-Sham (n=6); TNB-Cut (n=4); EtOH-Sham (n=6): and EtOH-Cut (n=5).

Figure 9. Body weights of animals with misplaced lesions, compared to sham operated controls. Data shown are group means ± 1SE for baseline period (*bsln*) and for 5 days following TNB / EtOH treatment. TNB-Sham (n=6); TNB-Cut (n=4); EtOH-Sham (n=6): and EtOH-Cut (n=5).

Figure 1.

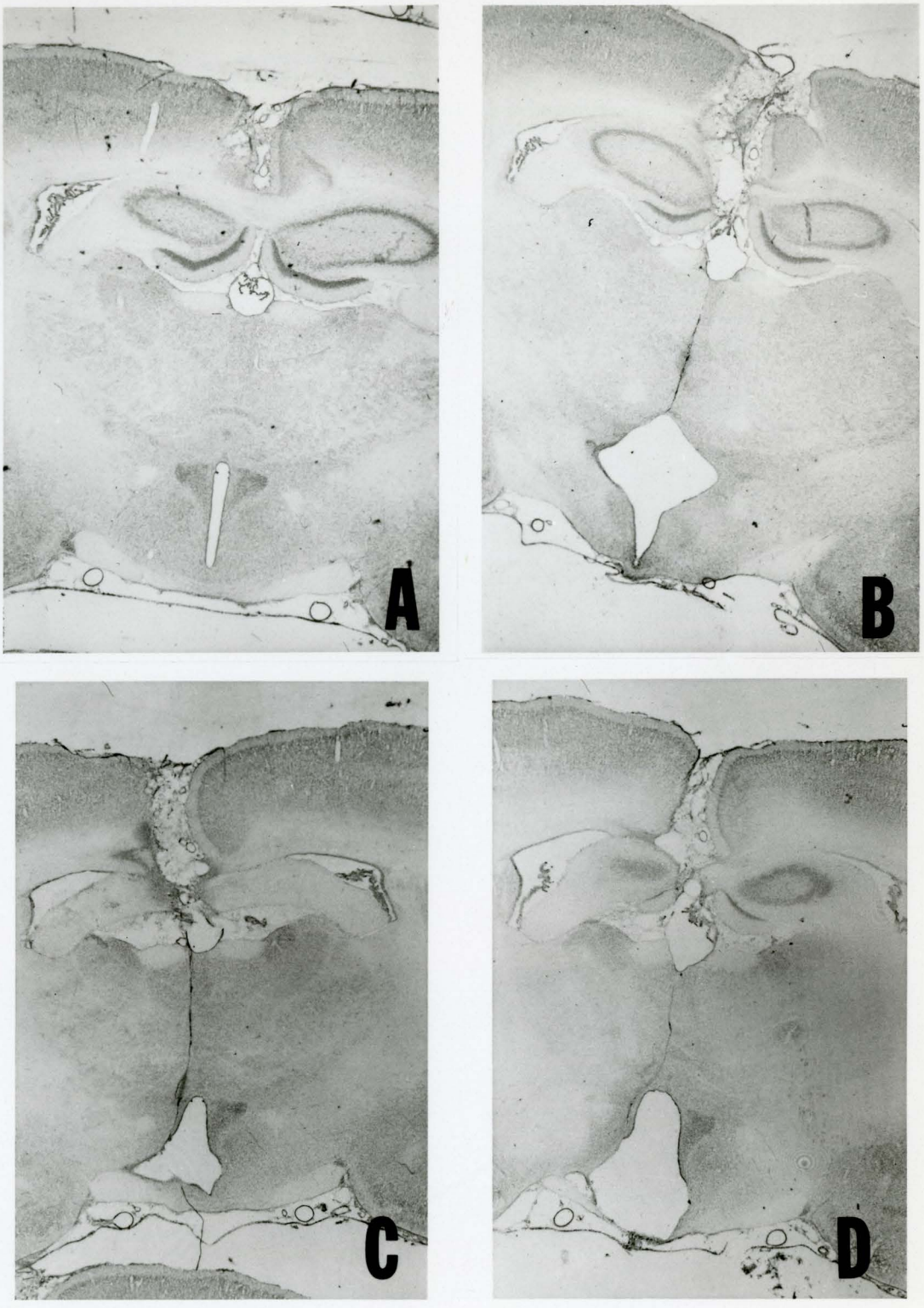

Figure 2: Food intake

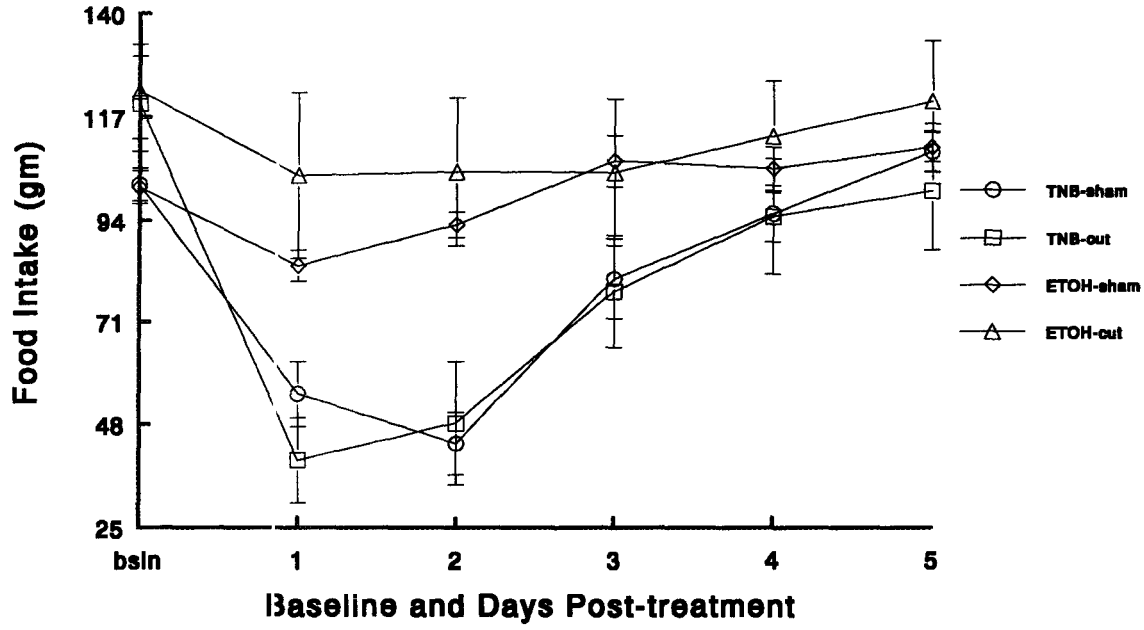

Figure 3: Food Intake
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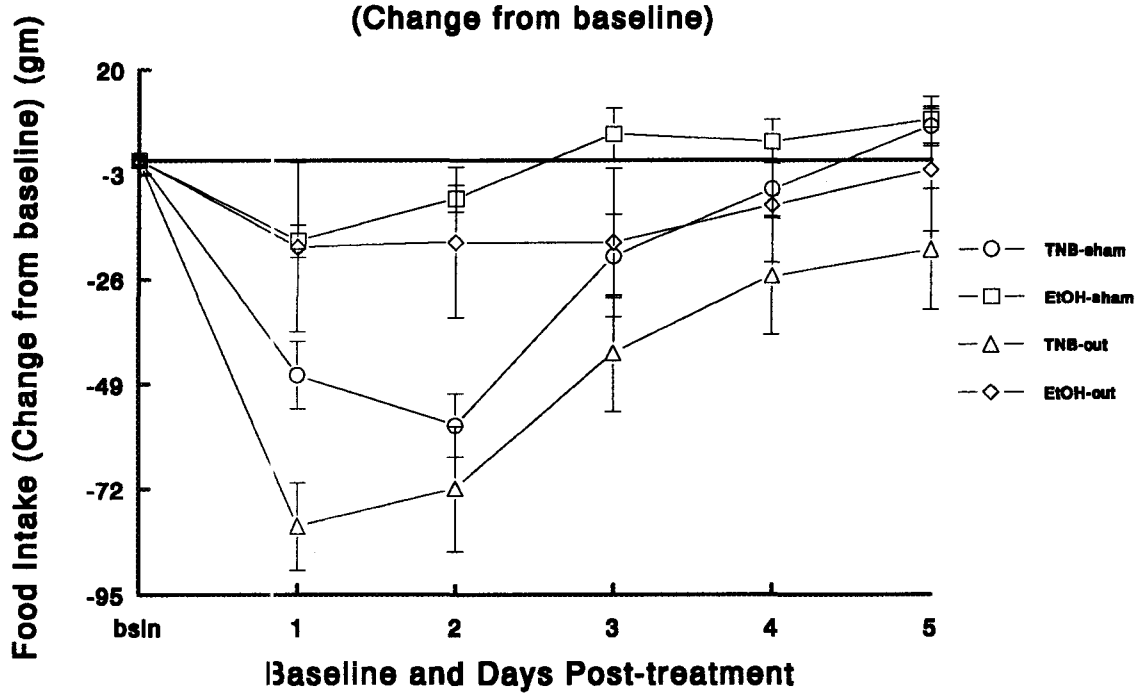

Figure 4: Body Weight

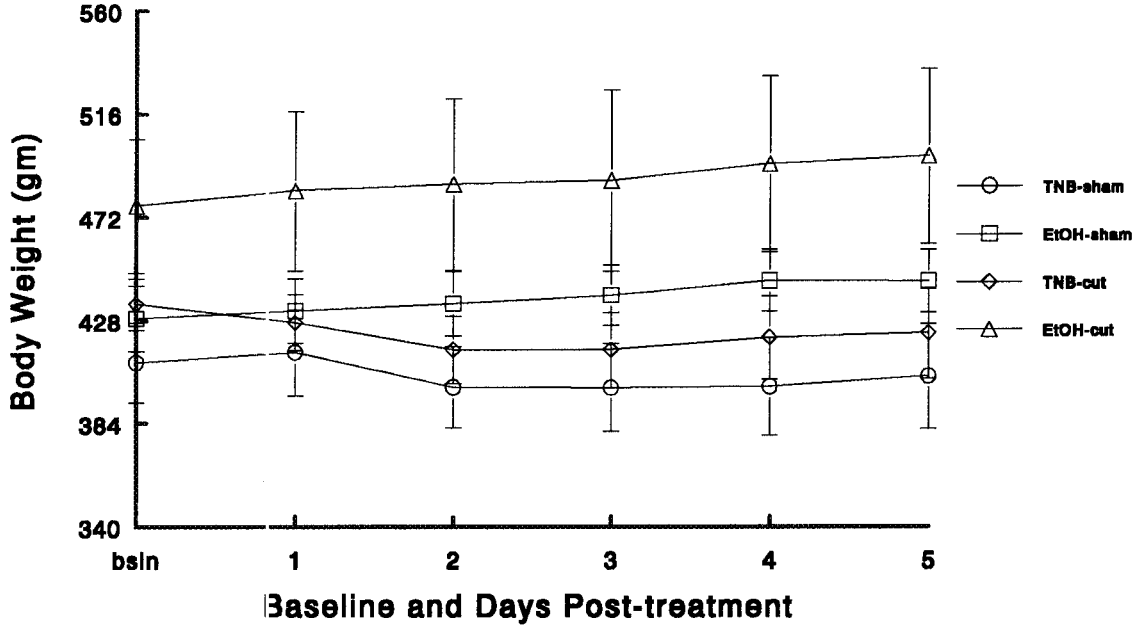

Figure 5: BW (Change from baseline)

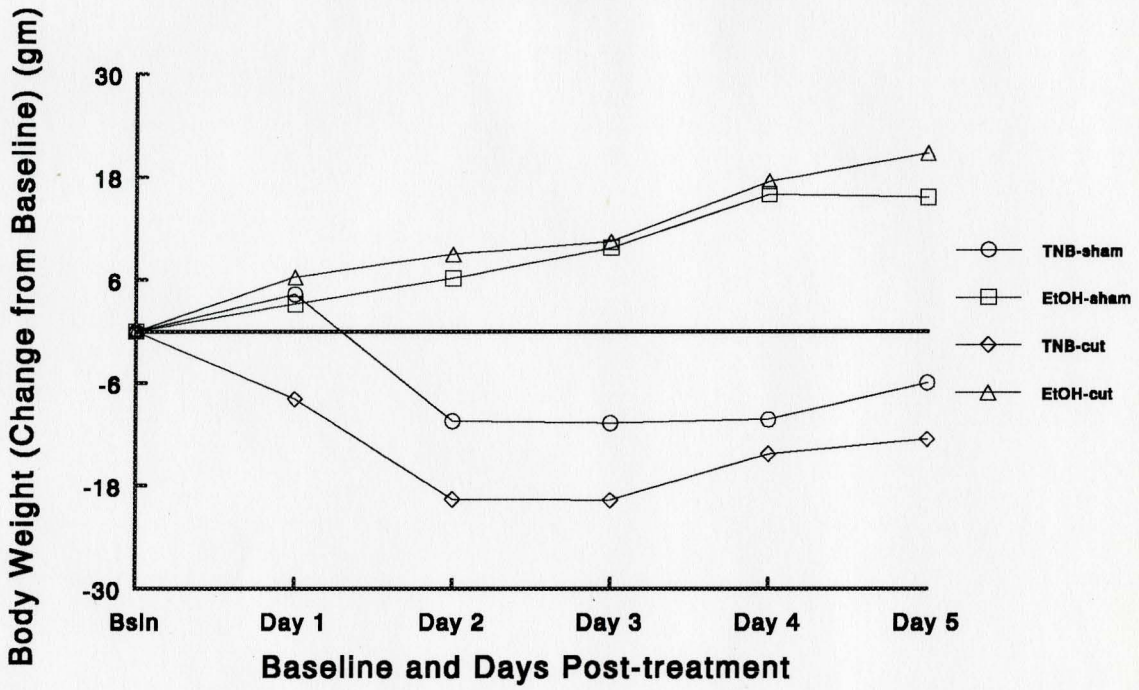

Figure 6: MPO Values

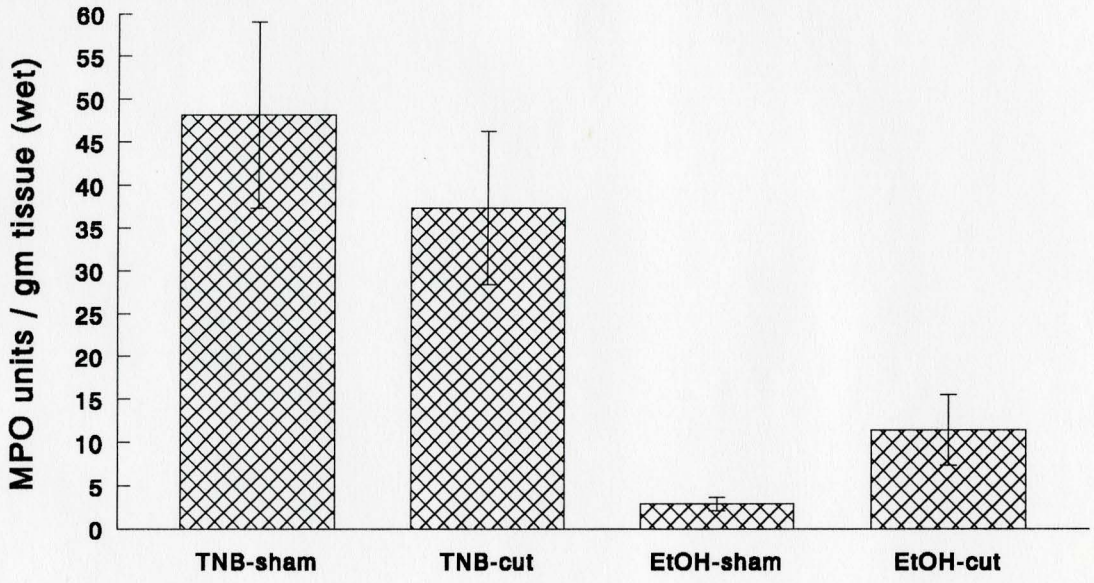

Figure 7: Food Intake
(misplaced lesions)

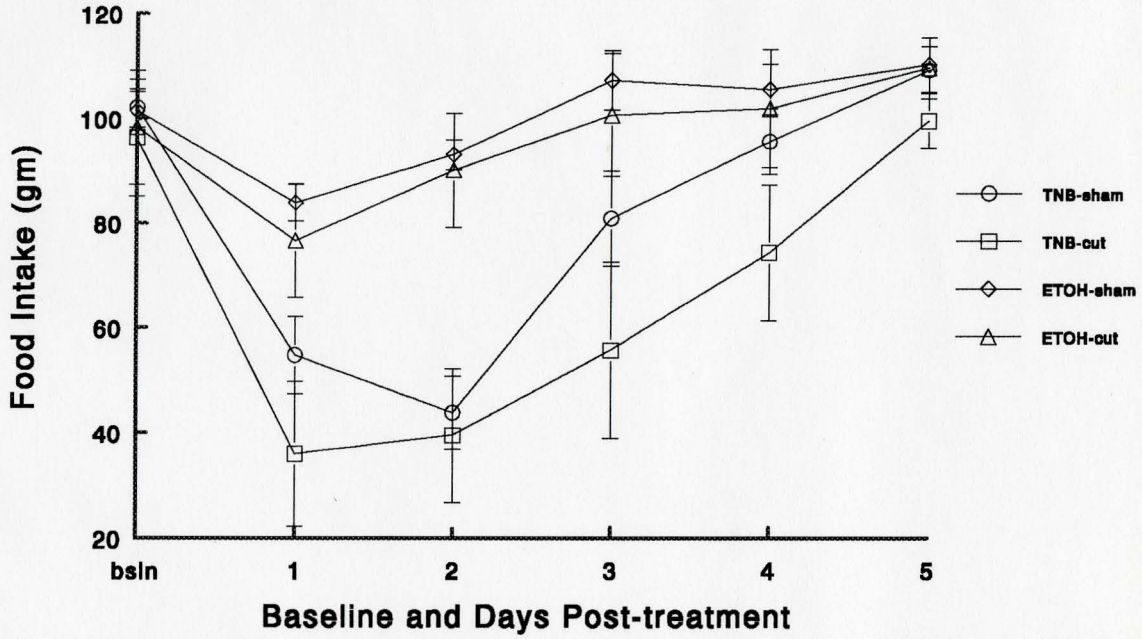

Figure 8: Body Weight
(misplaced lesions)

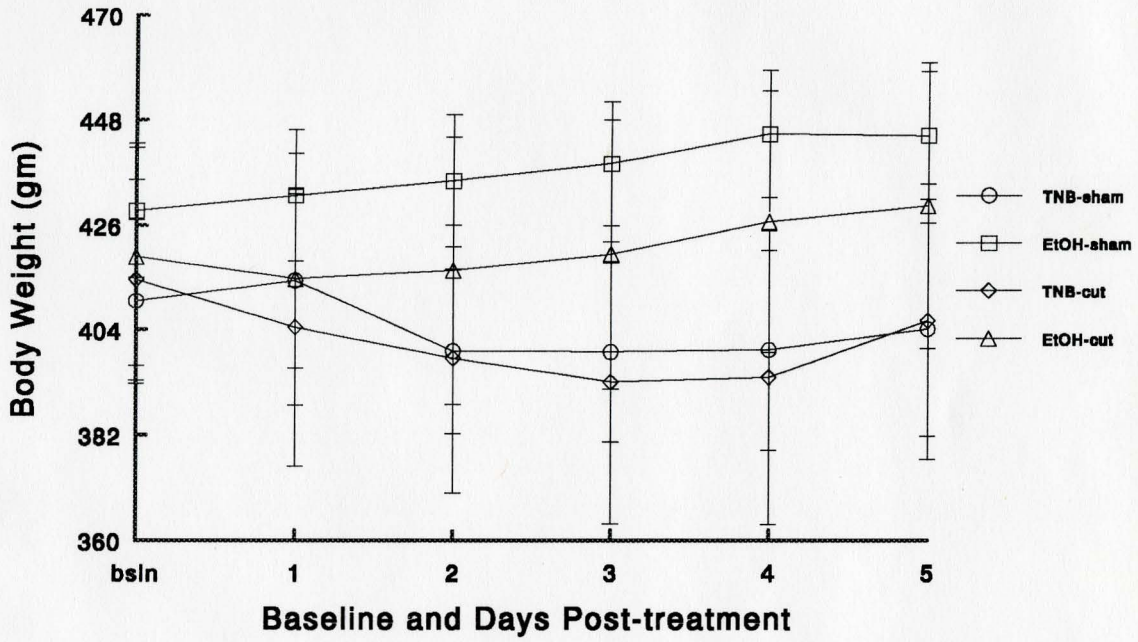

Figure 9: BW (Change from baseline)
(misplaced lesions)