Inflammation-induced anorexia and fever are elicited by distinct prostaglandin dependent mechanisms, whereas conditioned taste aversion is prostaglandin independent

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A B S T R A C T
Systemic inflammation evokes an array of brain-mediated responses including fever, anorexia and taste aversion. Both fever and anorexia are prostaglandin dependent but it has been unclear if the cell-type that synthesizes the critical prostaglandins is the same. Here we show that pharmacological inhibition or genetic deletion of cyclooxygenase (COX)-2, but not of COX-1, attenuates inflammation-induced anorexia. Mice with deletions of COX-2 selectively in brain endothelial cells displayed attenuated fever, as demonstrated previously, but intact anorexia in response to peripherally injected lipopolysaccharide (10 μg/kg). Whereas intracerebroventricular injection of a cyclooxygenase inhibitor markedly reduced anorexia, deletion of COX-2 selectively in neural cells, in myeloid cells or in both brain endothelial and neural cells had no effect on LPS-induced anorexia. In addition, COX-2 in myeloid and neural cells was dispensable for the fever response. Inflammation-induced conditioned taste aversion did not involve prostaglandin signaling at all. These findings collectively show that anorexia, fever and taste aversion are triggered by distinct routes of immune-to-brain signaling.

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1. Introduction

Anorexia, i.e. loss of appetite, and taste aversion are common and debilitating symptoms that occur as consequence of acute and chronic inflammatory disease (Saper et al., 2012). While it is well established that inflammation-induced anorexia is prostaglandin (PG) dependent, as demonstrated by the amelioration of the food intake by cyclooxygenase (COX) inhibitors (Baile et al., 1981; Langhans et al., 1989; Lugarini et al., 2002; Swiergiel and Dunn, 2002), the cellular source of the prostaglandins that evoke anorexia has not been identified. This is in contrast to fever, another cardinal symptom of the inflammatory response. As demonstrated in genetically modified mice, fever is dependent on PGE2 synthesized in brain endothelial cells (Engstrom et al., 2012; Wilhelms et al., 2014), and its subsequent binding to dedicated receptors on thermosensory neurons in the preoptic hypothalamus (Lazarus et al., 2007). However, prostaglandins are induced in several other cell-types upon inflammation and the induction in different cell-types may mediate distinct symptoms and has even been reported to elicit opposing effects (An et al., 2014; Serrats et al., 2010). In the same vein, neural prostaglandin synthesis is critical for the hyperalgesia induced by a local inflammation but dispensable for fever (Vardeh et al., 2009), whereas brain endothelial prostaglandins synthesis is critical for immune-induced fever but dispensable for the reduction in locomotor activity triggered by the same stimulus (Wilhelms et al., 2014).

We here examined the cellular source for prostaglandins eliciting inflammation-induced anorexia by using mice with cell-type specific deletions of COX-2, the COX isoform that is responsible for eliciting both fever (Cao et al., 1997; Li et al., 1999) and anorexia (Lugarini et al., 2002; Swiergiel and Dunn, 2002). To directly test if anorexia and fever are driven by COX-2 in the same cell-types we also monitored fever induced by LPS of the same type and dose (10 μg/kg) as used in the anorexia experiments. Furthermore, we included analysis of inflammation-induced conditioned taste aversion (CTA) since this symptom is closely related to anorexia but has been shown, at least partly, to involve distinct mechanisms (Bauer et al., 1995; Kopf et al., 2011).
2. Materials and methods

2.1. Animals

All experiments followed international and national guidelines, and were approved by the animal care and use committee in Linköping. Mice were kept on a 12–12 h light-dark cycle. Food and water were provided ad libitum if not stated otherwise. Adult mice (older than 8 weeks), of both sexes were used. During the experimental period, all mice were housed individually. For fever experiments, mice were kept at near-thermoneutral ambient temperature of 28 °C ± 1 °C (humidity 42–49%). All other experiments were performed at room temperature (21 °C ± 1 °C, humidity 40–43%).

**C57B/6** wild type (WT) mice used for the pharmacological studies were purchased from Scanbur (Karlslund, Denmark), COX-1 KO (Langenbach et al., 1995) and COX-2 KO (Morham et al., 1995) were purchased from Taconic Biosciences Inc (Ejby, Denmark), and were on a mixed B6;129P2 background. Mice that had the gene encoding COX-2 flanked by loxP sites (floxed) (Ishikawa and Herschman, 1995) were purchased from Scanbur (Karlslunde, Denmark). COX-1 KO (Langenbach et al., 1995) and COX-2 KO (Morham et al., 1995) were inserted into the abdominal cavity of the mice under general anesthesia (isoﬂurane, 1%), and the weight of the food was measured again at 4, 7 and/or 13 h after injection. Visible food spillage in the cage was measured and accounted for. If food spillage was detected after experimental endpoint, the data from that particular animal was excluded. Pretreatment with indomethacin or SC-560 was done once, 1 h before LPS or saline injection. Parecoxib was given 30 min before LPS or saline and an additional dose was given after 4.5 h. The timing for administration of pretreatment was chosen in concordance with studies in which the drugs have been demonstrated to be effective in inhibiting a LPS response (Fritz et al., 2016; Nilsson et al., 2009).

For experiments using i.c.v. administration of indomethacin, mice were single housed 5 d before experimental onset. On experimental day, food was withdrawn 2 h prior to dark period onset. Four hours after food withdrawal, 3 μl indomethacin (15 μg) was injected with a Hamilton syringe into the i.c.v. cannula under brief gas anesthesia (isoﬂurane, 1%), and LPS was administered i.p. simultaneously. Ten minutes after injections, pre-weighed food was reintroduced and food intake was measured after 1 h by weighing the food tray.

2.2. Drugs

To induce anorexia, 100 μl LPS (O55:B5, Sigma Aldrich, St. Louis, MO; 10 μg/kg) was given i.p. The unspecific COX-inhibitor indomethacin (Conforti; Alpharma, Langenfeld, Germany; 5 mg/kg) and the COX-2 specific inhibitor parecoxib (Dynastat; Pfizer, New York, NY; 10 mg/kg) were given i.p. at a volume of 100 μl. These doses were selected from previous studies (Nilsson et al., 2009; Ruud et al., 2013a) and tested by titration to robustly inhibit anorexia induced by the given dose of LPS. Indomethacin (Conforti) was also administered intracerebroventricularly (i.c.v.; 15 μg in 3 μl). The COX-1 specific inhibitor SC-560 (Cayman Chemicals, Ann Arbor, MI; 30 mg/kg in 300 μl) was given by gavage. The selected dose has previously been shown to robustly inhibit COX-1 in a food intake paradigm (Ruud et al., 2013a). Indomethacin, parecoxib and LPS were diluted in saline, whereas SC-560 was diluted in a mixture of methylcellulose and tween 80. Due to the problem with tolerance against LPS, each animal was only given LPS once.

2.3. Fever experiments

One week prior to the recordings, temperature transmitters (model TA11TA10, Data Sciences International, New Brighton, MN) were inserted into the abdominal cavity of the mouse under brief gas anesthesia (isoﬂurane, 1%). Temgesic was given peri-operatively. Basal core temperature was measured 24 h prior to experimental onset. About 2–3 h after lights on, mice were given a single i.p. injection of LPS or saline and core body temperature was measured for 12 h. Experiments were performed during daylight in order to minimize activity-related changes in body temperature. Fever was defined as any prolonged LPS-induced increase in body temperature (i.e. when LPS-treated mice had a body temperature that was statistically significantly higher than that of NaCl-treated mice).

2.4. Food intake experiments

Mice were single housed for a minimum of 5 d prior to food intake measurements. All food intake measurements were performed during the active period of the mice, i.e. during the dark period. On the test day, food was withdrawn and mice were given a single injection of either LPS or saline 1 h before onset of the dark period (7 p.m.). At dark period onset, pre-weighed food was given, and the weight of the food was measured again at 4, 7 and/or 13 h after injection. Visible food spillage in the cage was measured and accounted for. If food spillage was detected after experimental endpoint, the data from that particular animal was excluded. Pretreatment with indomethacin or SC-560 was done once, 1 h before LPS or saline injection. Parecoxib was given 30 min before LPS or saline and an additional dose was given after 4.5 h. The timing for administration of pretreatment was chosen in concordance with studies in which the drugs have been demonstrated to be efficient in inhibiting a LPS response (Fritz et al., 2016; Nilsson et al., 2009).

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2.5. Conditioned taste aversion experiments

All behavioral testing was conducted during the dark phase. Mice were separated and housed one and one a minimum of 5 d prior to experimental onset. Days 1–7, mice were habituated to water deprivation for 4 h a day. Day 8 (conditioning day), mice were water deprived for 4 h, then given access to a saccharin solution (0.15%) for 1 h, and immediately thereafter given an injection of either LPS or saline. Days 9–10 mice were only exposed to water deprivation. At the test day (Day 11) mice were water deprived for 4 h and then given saccharin solution for 1 h after which saccharin intake was measured. Pretreatment with SC-560 and parecoxib was given 1 h before LPS injection on the conditioning day (Day 8), i.e. at the same time mice got access to saccharin. The timing for administration of these inhibitors is in concordance with the timing of administration in the food intake studies.

2.6. Statistics

Results are presented as mean ± SEM. Anorexia and fever were analyzed with a 2-way repeated measures ANOVA, followed by a post hoc analysis using Tukey’s (anorexia) or Holm-Sidak’s (fever) multiple comparisons test. Mean fever and saccharin intake were analyzed with a 2-way ANOVA, followed by a post hoc analysis using Tukey’s multiple comparisons test. P < 0.05 was considered statistically significant.
3. Results

3.1. LPS-induced anorexia is COX-2 dependent

In order to induce anorexia, we used a low dose (10 µg/kg) of LPS. The dose was kept low in order to mimic natural disease conditions. As expected, LPS induced a robust anorexia that was evident both 3 and 6 h after injection (Fig. 1A) and to some extent also after 12 h (Supplementary Fig. 1A). For the subsequent analysis of anorexia, we restricted our primary analysis to the first 6 h and show data from 12 h in Supplementary Fig. 1A–G. To confirm that the LPS-induced anorexia was prostaglandin dependent, we pretreated mice with the non-selective COX-inhibitor indomethacin. Whereas LPS induced a robust anorexia in vehicle-treated mice, indomethacin-treated animals displayed normal food intake (Fig. 1A). To examine if the anorexia in the present paradigm was driven by COX-1 or COX-2, we next used selective inhibitors of the two enzymes. Mice given the COX-1 selective inhibitor SC-560 showed anorexic responses comparable to those of vehicle-treated mice when both groups were injected with LPS (Fig. 1B). In contrast, mice given the COX-2 selective inhibitor parecoxib showed a strongly attenuated inflammation-induced anorexia (Fig. 1B). We next validated these findings using mice with genetic deletions of COX-1 or COX-2. As expected, both WT mice and mice lacking COX-1 displayed normal anorexic responses to LPS (Fig. 1C). Mice without COX-2 displayed a blunted anorexic response (Fig. 1D) but the effect of the COX-2 deletion was less strong compared to that obtained by pharmacological COX-2 inhibition (Fig. 1D). Mice with COX-2 deletion are anorectic to the same degree as littermates with intact COX-2 expression (WT; Fig. 2A). We next investigated the febrile response in Cox2bEnd mice to the same low dose of LPS as was used in the food intake experiments and found that it was significantly attenuated (Fig. 2B, C). These findings indicate that the prostaglandins that trigger immune-induced fever and anorexia, respectively, are synthesized in distinct cell types. Next, we generated mice lacking COX-2 in neural cells (i.e. neurons, astrocytes and oligodendrocytes; Cox2ANes) (Braun et al., 2012). To this end we used a mouse line in which Cre expression was driven by the nestin promoter. Similar to the mice with COX-2 deletion in the brain endothelium, the mice lacking COX-2 in neural cells reduced their food intake in response to LPS to the same degree as did WT mice (Fig. 2D). The mutant mice also showed a normal febrile response after injection.

3.2. LPS-induced anorexia occurs independently of COX-2 expression in neural-, myeloid- or brain endothelial cells

To determine which cell type that generates the prostaglandins responsible for LPS-induced anorexia, we used cell-type specific deletions of COX-2. Since brain endothelial COX-2 has been shown to be involved in immune-induced fever (Engstrom et al., 2012; Wilhelms et al., 2014), we first generated mice lacking COX-2 in brain endothelial cells (Cox2bEnd). This was done by crossing mice in which critical parts of the gene encoding COX-2 was floxed (Ishikawa and Herschman, 2006), with a mouse line expressing an inducible Cre-recombinase under the control of the Sloc1c1-promoter. This promoter drives Cre expression selectively in the brain endothelium (Ridder et al., 2011) and has previously been used to dissect the role of prostaglandin synthesis in those cells for the febrile response (Eskilsson et al., 2014; Wilhelms et al., 2014). When these mice were tested for LPS-induced anorexia, we found that Cox2bEnd mice became anorectic to the same extent as littermates with intact COX-2 expression (WT; Fig. 2A). We next investigated the febrile response in Cox2bEnd mice to the same low dose of LPS as was used in the food intake experiments and found that it was significantly attenuated (Fig. 2B, C). These findings indicate that the prostaglandins that trigger immune-induced fever and anorexia, respectively, are synthesized in distinct cell types. Next, we generated mice lacking COX-2 in neural cells (i.e. neurons, astrocytes and oligodendrocytes; Cox2ANes) (Braun et al., 2012). To this end we used a mouse line in which Cre expression was driven by the nestin promoter. Similar to the mice with COX-2 deletion in the brain endothelium, the mice lacking COX-2 in neural cells reduced their food intake in response to LPS to the same degree as did WT mice (Fig. 2D). The mutant mice also showed a normal febrile response after injection.
of the same dose of LPS (Fig. 2E, F). Finally, we deleted COX-2 in myeloid cell using the LysM-Cre line. Mice without COX-2 in myeloid cells (Cox2−/−) showed intact anorexia (A; n = 6, Cox2−/−-LPS; n = 6, Cox2−/−-NaCl) and fever (A; n = 7, WT-NaCl; 2-way ANOVA, group × time F (6, 42) = 123.7, P < 0.001) but attenuated fever in response to LPS (B; n = 11, Cox2−/−-LPS; n = 10, Cox2−/−-NaCl; n = 13, WT-LPS, n = 12, WT-NaCl; 2-way ANOVA, group × time F (900, 12,900) = 5.589, P < 0.001). Mice lacking COX-2 in neural cells (Cox2−/−) showed intact anorexia (D; n = 6, Cox2−/−-LPS; n = 6, Cox2−/−-NaCl; n = 6, WT-LPS; n = 6, WT-NaCl; 2-way ANOVA, group × time F (6, 88) = 48.49, P < 0.001) and fever (E; n = 16, Cox2−/−-LPS; n = 15, Cox2−/−-NaCl; n = 14, WT-LPS; n = 14, WT-NaCl; 2-way ANOVA, group × time F (729, 13,122) = 2.891, P < 0.001) after injection of LPS. The same was the case for mice lacking COX-2 in myeloid cells (C; n = 9, Cox2−/−-LPS-m; n = 6, Cox2−/−-M-LysM-NaCl; n = 9, WT-LPS; n = 9, WT-NaCl; and H; n = 12, Cox2−/−-M-LysM-NPS; n = 12, Cox2−/−-M-LysM-NaCl; n = 11, WT-LPS, n = 11, WT-NaCl; Anorexia: 2-way ANOVA, group × time F (6, 58) = 20.89, P < 0.001; Fever: group × time F (849, 11,886) = 4.497, P < 0.001). P < 0.05, **P < 0.01, ***P < 0.001 repeated measurement ANOVA (A, D, G) or ANOVA (B, C, E, F, H, I) followed by Tukey’s (anorexia) or Holm-Sidak’s (fever curves) post hoc tests. Only differences between genotypes are indicated.

3.3. LPS-induced conditioned taste aversion is prostaglandin-independent

We established a protocol for conditioned taste aversion with the same dose of LPS as was used in the anorexia and fever experiments (Fig. 4A). Intraperitoneal injection of 10 μg/kg of LPS given after saccharine drinking robustly reduced saccharin drinking three days later (Fig. 4B). This effect was a specific reaction to the taste, since fluid intake was normal in mice subjected to the same protocol but without access to saccharine (Fig. 4C). One further conditioning session made the effect more pronounced (Fig. 4B), but since the difference was only incremental we continued with
a protocol including only one conditioning session (4D). Since unselective COX inhibition induced taste aversion by itself (Supplementary Fig. 1H–I), we moved directly to inhibition of COX-1 and COX-2. Neither COX-1 inhibition (Fig. 4E, F) nor COX-2 inhibition (Fig. 4G, H) had any effect on the conditioned taste aversion induced by LPS. These observations indicate that the anorexia and the taste aversion induced by inflammation are triggered by distinct mechanisms and that only the mechanism behind anorexia requires prostaglandins.

4. Discussion

Anorexia and fever are hallmarks of systemic inflammation. Both these symptoms are dependent on prostaglandins synthesized by COX-2 (Johnson et al., 2002; Li et al., 1999; Lugarini et al., 2002; Swiergiel and Dunn, 2002), but it has been unclear if the cellular source of the critical prostaglandins is identical. Using cell-type specific gene deletions, we here studied the role of prostaglandin synthesis in anorexia, fever and conditioned taste aversion induced by the same dose of LPS.

We recently demonstrated that mice with selective deletion of COX-2 in the endothelial cells of the blood-brain barrier displayed attenuated LPS-induced fever (Wilhelms et al., 2014). Here we extended this finding by showing that fever in response to an LPS dose ten times lower than the one used in our previous study also is attenuated in these mice, although the attenuation is less pronounced. Possible reasons for the only partial effect by the gene deletion on fever include incomplete recombination by the Cre-line and contribution of COX-2 from other cell-types. In contrast to the attenuated fever, mice lacking COX-2 expression in the endothelial cells of the blood-brain barrier displayed an intact LPS-induced anorexia. Taken together with the finding that deletion of Tak1 in the brain endothelium dampens fever but not anorexia in response to IL-1β (Ridder et al., 2011), the present data indicates that the brain endothelium is not involved in the immune-to-brain signaling underpinning anorexia and, thus, that the prostaglandin-dependent mechanism triggering anorexia is distinct from the one eliciting fever. These findings are also supported by studies showing that LPS-induced fever is dependent on microsomal prostaglandin E synthase-1 (mPGES-1) production (Engblom et al., 2003) but that mPGES-1 knock-out mice still develop anorexia after LPS (Elander et al., 2007), emphasizing the discrepancy between the mechanisms generating anorexia and fever.

Activation of myeloid cells is critical for LPS-induced anorexia. Thus, deletion in myeloid cells of myeloid differentiation primary response gene 88 (MyD88), which is an adapter protein for Toll like and interleukin-1 receptor signaling, largely inhibits LPS-induced anorexia (Ruud et al., 2013b). Our present findings strongly indicate that the critical MyD88 dependent event in these cells is not the induction of COX-2. Further, our present results show that COX-2 production in neural cells, such as neurons, astrocytes and oligodendrocytes, is not critical for LPS-induce anorexia. Collectively, these data suggests that prostaglandin synthesis in brain endothelial cells, neural cells and myeloid cells is not critical for anorexia. However it should be noted that the role of myeloid cells could vary with the dose of LPS and the time point studied (Chakravarty and Herkenham, 2005).

There are several possible explanations for the somewhat surprising finding that none of the cell type-specific deletions of COX-2 attenuated the inflammation-induced anorexia. One possibility is that COX-2 expressed in a cell-type not targeted in this study is critical for anorexia. Since indomethacin injected i.c.v. (this study) and a COX-2 inhibitor injected in the dorsal raphe nucleus (Kopf et al., 2011) have been shown to attenuate LPS-induced anorexia, it is likely that a cell-type residing in the brain is responsible for the critical prostaglandin synthesis. Since microglia and perivascular cells are not targeted by the Cre-lines used here, they are possible candidates; however, they express no or little COX-2 after immune challenge (Engstrom et al., 2012; but cf. Schiltz and Sawchenko, 2002). Another possibility is that, in contrast to what is case for fever, there are redundant COX-2 dependent signaling pathways for anorexia and that COX-2 in one or more of the cell-types investigated is sufficient but not necessary for eliciting anorexia. We found no attenuation of the anorexic response in mice lacking COX-2 in both neural cells and brain endothelial cells, but there are of course several additional combinations that could be tested. Finally, it is possible that COX-2 in one of the cell-types investigated is indeed critical for anorexia but that the Cre-line used to target the deletion to those cells is not efficient enough. However, this is unlikely since the endothelium specific deletion affected fever, and COX-2 deletion in neural cells, using the same Cre-line as used here, has been shown to affect mechanical hyperalgesia (Vardeh et al., 2009). Furthermore, deletion of MyD88 in myeloid cells with LysM-Cre, inhibited LPS-induced anorexia in a previous study from this laboratory (Ruud et al., 2013b).

Based on a previous study (Mormede et al., 2004), we developed a protocol for inflammation-induced conditioned taste aversion. In this protocol, mice learn to associate the taste of saccharine with the effect of LPS. We found that one injection of a low-dose of LPS after saccharine drinking was sufficient to reduce saccharine drinking three days later, and we demonstrate that conditioned taste aversion to a dose of LPS that elicits a prostaglandin-dependent anorexia is independent of prostaglandins. This finding is in line with the results from a previous study using the non-selective COX inhibitor indomethacin and a protocol for condi-

![Fig. 3](image-url) LPS-induced anorexia is attenuated in mice given indomethacin i.c.v. but remain unaffected in mice lacking COX-2 in both brain endothelial and neural cells. A. Food intake in response to i.p. injection of LPS and i.c.v. injection of indomethacin (n = 3, vehicle + NaCl, vehicle + LPS, indomethacin + NaCl; n = 4, indomethacin + LPS, 2-way ANOVA, pre-treatment × treatment F (1, 9) = 10.28, P = 0.0107). B. Mice lacking COX-2 in brain endothelial and neural cells (Cox2AEnd Nes) showed intact anorexia (D; n = 6, Cox2AEnd + Nes-LPS; n = 8, Cox2AEnd + Nes-NaCl; n = 11, WT-LPS; n = 9, WT-NaCl; 2-way ANOVA, treatment F (3, 33) = 13.94, P < 0.001).
**Fig. 4.** LPS-induced conditioned taste aversion is neither dependent on COX-1 nor on COX-2. Conditioned taste aversion was initially tested by a protocol with two conditioning sessions (A). Saccharine drinking was reduced already after one conditioning session and further reduced after a second conditioning session (B, CTA-test 1; \(n = 6\), LPS; \(n = 6\), NaCl; 2-way ANOVA, session x treatment \(F(2, 20) = 12.87, P < 0.001\)). The same protocol but without saccharine did not result in reduced fluid intake at the test session (C; \(n = 6\), LPS; \(n = 5\), NaCl), showing that the aversion is specifically related to the taste of saccharine. Since one conditioning session was enough to induce taste aversion we used only one conditioning session for the subsequent experiments (D). COX-1 inhibition with SC-560 did not affect saccharine intake before the conditioning (E; \(n = 22\), LPS; \(n = 21\), NaCl), and did not interfere with the reduction in saccharine intake induced by conditioning (F; 2-way ANOVA, treatment x pre-treatment \(F(1, 82) = 34.62, P < 0.001\). The same was the case for COX-2 inhibition with parecoxib (G, H; \(n = 8\), LPS; \(n = 8\), NaCl; 2-way ANOVA, treatment x pre-treatment \(F(1, 28) = 51.08, P < 0.001\)). * \(P < 0.05\), ** \(P < 0.01\), *** \(P < 0.001\); ANOVA followed by Tukey’s post hoc test.
tioned taste aversion in which rats were given LPS prior to exposure to the taste but in which a sweetened diet was used instead of water with saccharin (Weingarten et al., 1993). We recently demonstrated a pathway mediating inflammation-induced conditioned place aversion, using LPS at the dose used in this study. We found that conditioned place aversion induced by LPS is dependent on PGE2 production by COX-1 and subsequent binding of PGE2 to EP3 receptors on dopamine D1 receptor expressing neurons in the striatum (Fritz et al., 2016). Thus, LPS-induced place aversion and taste aversion are elicited by very different mechanisms. On a more general level, most brain-mediated responses to systemic LPS injection are completely or partially prostaglandin dependent. In addition to fever, anorexia and conditioned place aversion, also cortisol release (Elander et al., 2009; Gadek-Michalska and Bugajski, 2004), hyperalgesia (Schmelzer et al., 2006) and lethargy (Harden et al., 2011) in response to LPS are dependent on prostat glandin synthesis. Thus, the immune-to-brain signaling mechanism behind conditioned taste aversion differs in a fundamental way from those behind most other brain-mediated illness symptoms by not being prostaglandin dependent.

5. Conclusion

In conclusion, we show that the anorexia and fever induced by a low dose of LPS are triggered by prostaglandin synthesis in distinct cell-types. Fever is dependent on COX-2 in brain endothelial cells, and whereas anorexia also is COX-2 dependent, it is not inhibited by deletion of COX-2 neither in the brain endothelium, nor in neural cells or myeloid cells. Furthermore, inflammation-induced taste aversion is independent of prostaglandins. These findings illustrate the complexity of the inflammatory induced sickness syndrome, and open up avenues for the selective amelioration of each distinct symptom.

Conflict of interest statement

All authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2016.12.007.

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