The gut reaction to traumatic brain injury

Rebeccah J. Katzenberger, Barry Ganetzky, and David A. Wassarman

Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI 53706

Corresponding author: David A. Wassarman; E-mail: dawassarman@wisc.edu

Key words: bacteria, Drosophila, glucose, innate immune response, intestine, mortality, septate junction, TBI, tight junction, traumatic brain injury

Citation: Katzenberger RJ, Chtarbanova S, Rimkus SA, Fischer JA, Kaur G, Seppala JM, Swanson LC, Zajac JE, Ganetzky B, Wassarman DA. Death following traumatic brain injury in Drosophila is associated with intestinal barrier dysfunction. eLife 2015; 10.7554/eLife.04790.

Abstract

Traumatic brain injury (TBI) is a complex disorder that affects millions of people worldwide. The complexity of TBI partly stems from the fact that injuries to the brain instigate non-neurological injuries to other organs such as the intestine. Additionally, genetic variation is thought to play a large role in determining the nature and severity of non-neurological injuries. We recently reported that TBI in flies, as in humans, increases permeability of the intestinal epithelial barrier resulting in hyperglycemia and a

higher risk of death. Furthermore, we demonstrated that genetic variation in flies is also pertinent to the complexity of non-neurological injuries following TBI. The goals of this review are to place our findings in the context of what is known about TBI-induced intestinal permeability from studies of TBI patients and rodent TBI models and to draw attention to how studies of the fly TBI model can provide unique insights that may facilitate diagnosis and treatment of TBI.

Traumatic brain injury (TBI) is a major health issue (1, 2). According to the U.S. Centers for Disease Control and Prevention, TBIs result in more than 2 million emergency room visits, 280,000 hospitalizations, and 50,000 deaths each year (3). In addition, 2.5-6.5 million people are living with TBI-caused disabilities. Despite the name, injuries that are characteristic of TBI are not limited to the brain (4-7). Non-neurological injuries such as systemic inflammation as well as organ dysfunction involving cardiovascular, respiratory, and gastrointestinal systems often occur minutes to months after initial mechanical injury to the brain and substantially contribute to morbidity and mortality. Therefore, with the eventual goal of developing therapeutic interventions for TBI, it is necessary to understand the metabolic, molecular, and cellular events that underlie non-neurological injuries. Here, we focus on gastrointestinal injuries and highlight how studies using a *Drosophila melanogaster* TBI model can provide unique insights into causal mechanisms and therapies.

TBI increases intestinal permeability in mammals

Dysfunction of the gastrointestinal tract is a common occurrence in TBI (4-7). In the first few weeks after injury, most patients with moderate to severe TBI have reduced intestinal contractile activity and absorption, which is manifested by vomiting and abdominal distension. In addition, evidence from humans, rodents, and now from our work in flies indicates that TBI can disrupt the intestinal barrier that normally functions to block the flow of certain ions, solutes, proteins, bacteria, and bacterial products between the inside and outside of the intestine. Severe consequences can result from disruption of the intestinal barrier. For instance, increased intestinal permeability plays a key role

in the pathogenesis of Crohn's disease, Celiac disease, and diabetes (8), and death may result from translocated bacteria that induce a systemic inflammatory response and sepsis with subsequent multiple organ failure (9, 10).

Defective tight junctions underlie increased intestinal permeability following TBI in mammals

A primary determinant of intestinal permeability is the extent of opening of intercellular tight junctions (Figure 1). The intestine is lined with a single layer of epithelial cells that separates the intestinal lumen from extra-intestinal sites (11). Close contacts formed by tight junctions between adjacent epithelial cells restrict passive paracellular permeability to small molecules such as solvents and solutes with radii up to ~$3.5X10^{-4}$ μm (12). Restricted permeability is mediated by transmembrane proteins Claudins and Occludin that form charge- and size-selective paracellular channels controlled by intracellular scaffolding proteins such as PDZ (PSD-95, Discs-large, ZO-1) domain proteins as well as by myosin light chain kinase (MLCK) regulation of actomyosin contraction and Occludin endocytosis (12, 13). In contrast, active transcellular mechanisms typically regulate permeability to macromolecules such as nutrients (*e.g.*, glucose), proteins, and bacteria and their products (also known as Pathogen-associated molecular patterns (PAMPs)) (14). However, tight junctions are not static structures. Altered expression, post-translational modification, localization, or activity of tight junction proteins or their regulators can change the degree of permeability to macromolecules. For example, increased Occludin endocytosis, which occurs in response to PAMP-triggered

expression of the pro-inflammatory cytokine tumor-necrosis factor-α (TNF-α), can increase permeability of tight junctions to macromolecules (15).

In mammals, increased paracellular permeability appears to be the basis of increased intestinal permeability following TBI. Studies of TBI patients show an increase in the ratio of orally ingested lactulose (a marker of paracellular permeability) to mannitol (a marker of transcellular permeability) in urine (16, 17). The lactulose-mannitol test as well as a dye permeability test also shows that injury inflicted exclusively to the brain of rodents by methods such as controlled cortical impact (CCI) is sufficient to increase paracellular permeability (18-20). Furthermore, disruption of the intestinal epithelial barrier following TBI in rodents is accompanied by decreased expression of tight junction proteins Occludin and ZO-1 as well increased expression of MLCK in the intestine, providing additional support for a paracellular mechanism (20, 21). TBI-induced gaps in intestinal tight junctions must be at least 0.25 μm (*i.e.*, the minimum dimension of *E. coli*, which is ~700 times larger than the normal gap in tight junctions) to account for the observed translocation of commensal bacteria to blood plasma and organs such as lungs (22). Thus, rodent studies establish a direct cause-effect relationship between brain injury and increased intestinal permeability. The intermediary between brain injury and intestinal injury is yet to be resolved, but current evidence points to the enteric nervous system and neuroendocrine signals, both of which control the function of the gastrointestinal system (23, 24).

TBI activates a feedback loop that enhances intestinal permeability in mammals

TNF-α is a central player in the tight junction-mediated mechanism that increases intestinal permeability following TBI in mammals (Figure 1). Treatment of mice with the hormone ghrelin following TBI blocks the increase in TNF-α and MLCK expression along with the increase in intestinal permeability (21). Similarly, electrical stimulation of the vagus nerve before TBI in mice (25, 26), increasing glutamine levels in the diet following TBI in rats (27, 28), or injection of the antioxidant-sedative propofol following TBI in rats (29) blocks the increase in TNF-α expression as well as the increase in intestinal permeability following TBI. Conversely, mice deficient for nuclear factor erythroid 2-related factor 2 (Nrf2) have higher TNF-α expression and higher intestinal permeability than wild-type mice following TBI (30). Ghrelin, vagus nerve stimulation, glutamine, propofol, and Nrf2 use different mechanisms to regulate TNF-α. For example, ghrelin binds the growth hormone secretagogue receptor (GHS-R) on immune cells and inhibits PAMP-induced release of TNF-α (31), and vagus nerve stimulation promotes the release of acetylcholine (ACh) by efferent vagus nerves to inhibit the production of TNF-α by acetylcholine receptor (AChR)-expressing immune cells (32).

TNF-α expression in mammals is induced by Toll-like receptor (TLR) signaling pathways in macrophages and other cytokine-producing cells that are part of the defense mechanism provided by the innate immune response (33). Activation of TLRs by PAMPs turns on the NF-κB transcription factor, which promotes TNF-α transcription. Secreted TNF-α then binds TNF receptors (TNFRs) on intestinal epithelial cells and activates several pathways, including an NF-κB pathway that upregulates genes encoding pro-inflammatory cytokines such as TNF-α, interleukin 1β (IL-1β), and IL-6,

which is sufficient to enhance tight junction permeability in the intestine (33, 34). So, a positive feedback loop, anchored by PAMPs and TNF-α, disrupts intestinal barrier function following TBI (Figure 1). Alternatively, the positive feedback loop might be anchored by damage-associated molecular patterns (DAMPs), molecules such as nucleic acids that are released from apoptotic and necrotic cells following TBI (35-37). DAMPs function similarly to PAMPs. They are recognized by TLRs, turn on NF-κB transcription factors, and promote TNF-α transcription. However, the relative contribution of PAMPs and DAMPs to disruption of the intestinal barrier following TBI in mammals is yet to be determined.

TBI increases intestinal permeability in flies

Our recent publication implicates increased intestinal permeability in the non-neurological injury cascade that causes flies to die from TBI (38). This work builds upon our prior publications that describe the development and initial characterization of a fly TBI model that uses a spring-loaded device to inflict closed-head TBI in adult flies (39, 40). We found that one of the consequences of TBI is death within 24 hrs of the initial injury and that genotype plays a major role in determining the percentage of flies that die within 24 hrs, which we call the mortality index at 24 hrs (MI_{24}) (38). Through Genome-wide association study (GWAS) analysis of many wild-type fly lines, we identified single-nucleotide polymorphisms (SNPs) in three epithelial barrier-related genes, *big bang* (*bbg*), *scribble* (*scrib*), and *grainyhead* (*grh*), as being significantly associated with the MI_{24}. *bbg* encodes a PDZ domain protein that regulates the barrier function of septate junctions (41), structures in invertebrates that are functional

analogues of tight junctions in vertebrates (42-44). Intestines of *bbg* mutants have wider paracellular gaps and greater permeability to pathogenic bacteria than wild-type flies (41). *scrib* also encodes a PDZ domain protein that functions in the formation of septate junctions (45, 46). Lastly, *grh* and its mammalian orthologs encode transcription factors that regulate expression of septate junction and tight junction genes, respectively (47-49). Our data suggest that flies carrying SNPs in *bbg*, *scrib*, or *grh*, produce septate junctions with altered sensitivity to disruption by mechanisms induced by TBI.

Moreover, we found that TBI disrupts the intestinal barrier. Our data show that, following TBI, previously ingested blue dye leaks from the intestine into the hemolymph (the circulatory fluid of flies) and travels throughout the fly producing a 'Smurf' phenotype (38, 50). In all fly lines tested, including those with extremely low or extremely high MI_{24}'s, there is an almost perfect correlation between flies that Smurf and flies that die within 24 hrs following TBI, indicating that genetic variation plays a critical role in determining the magnitude of intestinal permeability following TBI. Additionally, these data suggest that death within 24 hrs is caused by factors that leak from the intestine. This conclusion is consistent with the finding that increased intestinal permeability, as assayed by Smurfing, serves as a marker of impending death caused by normal aging processes (51).

Bacteria do not affect death-causing intestinal permeability following TBI in flies

Surprisingly, we found that while commensal bacteria translocate from the intestine to the hemolymph within 1 hr following TBI, they do not appear to contribute to mortality within 24 hrs (38). Bacteria-free flies that we generated by feeding flies a cocktail of antibiotics have the same MI_{24} and SI_{24} (Smurfing index at 24 hrs) as bacteria-containing flies. These findings appear to contradict the findings from rodent TBI models, which indicate that PAMPs are integral to the positive feedback mechanism that enhances intestinal permeability (Figure 1). However, the apparently different role for PAMPs in fly and rodent TBI models may be explained by differences in the nature of the initial injury: in the fly TBI model, TBI occurs in the context of polytrauma and is severe enough to cause death, whereas in rodent TBI models, the initial injury is restricted to the brain and is not severe enough to cause death. So, DAMPs may play a larger role in the fly TBI model than the rodent TBI models. Alternatively, in both fly and rodent TBI models, PAMPs play a negligible role relative to DAMPs in enhancing intestinal permeability immediately following TBI, and the relative contributions of these mechanisms is only noticeable when PAMPs are eliminated (Figure 2).

A negligible role for PAMPs in death following TBI does not mean that they are irrelevant to the pathophysiology of TBI. In fact, we found that following TBI in some fly lines, commensal bacteria significantly contribute to activation of the innate immune response (38), most likely through the Toll and Immune deficiency (Imd) pathways, which are analogous to the TLR and TNF-α pathways, respectively, in mammals (52). The Toll and Imd pathways use NF-κB proteins to activate the expression of antimicrobial peptide (AMP) genes (53), which encode proteins that are cytotoxic to

bacteria as well as host cells through their ability to cause membrane permeabilization (54). Work by us and others has shown that AMP expression is associated with progressive neurodegeneration in fly models of human neurodegenerative diseases (55-58) and that brain-specific expression of AMPs is sufficient to cause neurodegeneration in flies (59). Excessive expression of AMPs as well as TNF-α in mammals is also associated with neurodegenerative diseases (60, 61). Thus, following TBI, PAMPs that leak from the intestine activate similar molecular pathways in flies and mammals and induce the expression of proteins that may produce long-term deleterious consequences.

Glucose ingested after TBI increases death-causing intestinal permeability in flies

Remarkably, we found that feeding flies water rather than standard molasses food immediately after TBI significantly reduces the percent death within 24 hrs, with some fly lines having a greater than 50% reduction in percent death within 24 hrs (38). Thus, indigestion of a component of molasses food immediately after TBI substantially enhances the death-causing mechanism. Our data indicate that glucose is the component. Glucose levels in the hemolymph are significantly increased during the 24 hr period following TBI, indicating that TBI causes glucose to leak from the intestine (Figure 2). Furthermore, feeding flies glucose rather than water immediately after TBI is sufficient to significantly increase the percent death within 24 hrs, indicating that glucose stimulates the mechanism that increases intestinal permeability and death following TBI. Thus, glucose may be a key component of an uncharacterized positive feedback loop that increases intestinal permeability following TBI.

Studies of mammalian systems shed some light on the mechanism by which glucose may increase intestinal permeability. Under high glucose (*i.e*., hyperglycemic) conditions, transcellular Na^+-glucose cotransport causes an increase in paracellular permeability of tight junctions through phosphorylation of MLC by MLCK and reduced expression of Occludin and ZO-1 (62, 63). A transcellular mechanism may also be involved in flies, since we found that a SNP in a gene *CG7882* predicted to carry out transcellular glucose transport is significantly associated with the MI_{24} (38). *CG7882* encodes a fly ortholog of human solute carrier transporter 2 (SLC2) family proteins that are required for glucose transport in the intestine (64). Furthermore, hyperglycemia is characteristic of TBI in humans. Patients with severe TBI have higher blood glucose levels than patients with moderate or mild TBI (65-69). Moreover, hyperglycemia is predictive of death following TBI (70-72), and patients with diabetes are at increased risk of death following TBI (73, 74). It remains to be determined whether hyperglycemia causes death following TBI and if so how. Glucose could directly feed into a toxic metabolic pathway or glucose could indirectly cause toxicity by facilitating paracellular leakage of a toxic molecule from the intestine (Figure 2).

Future directions

Our study indicates that cellular and molecular mechanisms underlying TBI-induced disruption of the intestinal epithelial barrier are conserved from humans to flies. This conserved mechanism provides the opportunity to use the fly experimental toolbox to make discoveries that could improve the diagnosis and treatment of TBI in humans.

Our GWAS analysis has identified several potential players in the mechanisms, but much remains to be learned. By screening for mutations that modify the percentage of flies that die within 24 hrs following TBI, we should be able to address key questions such as what are the signals from the injured brain that trigger increased intestinal permeability, what role does glucose play in the TBI-induced intestinal permeability mechanism, why does increased intestinal permeability following TBI cause death, and does bacteria-dependent activation of the innate immune response following TBI cause neurodegeneration.

Acknowledgements

We thank Stanislava Chtarbanova and an anonymous reviewer for their thoughtful comments on the manuscript and members of the Ganetzky and Wassarman labs for their contributions to research on the fly TBI model.

References

1. Blennow K, Hardy J, Zetterberg H. The neuropathology and neurobiology of traumatic brain injury. Neuron 2012; 76:886-899.
2. Kabadi SV, Faden AI. Neuroprotective strategies for traumatic brain injury: improving clinical translation. Int J Mol Sci 2014; 15:1216-1236.
3. http://www.cdc.gov/traumaticbraininjury/get_the_facts.html
4. Pilitsis JG, Rengachary SS. Complications of head injury. Neurol Res 2001; 23:227-236.
5. Zygun DA, Kortbeek JB, Fick GH, Laupland KB, Doig CJ. Non-neurologic organ dysfunction in severe traumatic brain injury. Crit Care Med 2005; 33:654-660.
6. Kemp CD, Johnson JC, Riordan WP, Cotton BA. How we die: the impact of nonneurologic organ dysfunction after severe traumatic brain injury. Am Surg 2008; 74:866-872.
7. Masel B, DeWitt DS. Traumatic brain injury: A disease process, not an event. J Neurotrauma 2010; 27:1529-1540.
8. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, Tilg H, Watson A, Wells JM. Intestinal permeability—new target for disease prevention and therapy. BMC Gastroenterol 2014; 14:19.
9. Doig CJ, Sutherland LR, Sandham JD, Fick GH, Verhoef M, Meddings JB. Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. Am J Respir Crit Care Med 1998; 158:444-451.

10. De-Souza DA, Greene LJ. Intestinal permeability and systemic infections in critically ill patients: effect of glutamine. Crit Care Med 2005; 33:1125-1135.
11. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014; 14:141-153.
12. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci 2013; 70:631-659.
13. Cunningham KE, Turner JR. Myosin light chain kinase: pulling the springs of epithelial tight junction function. Ann N Y Acad Sci 2012; 1258:34-42.
14. Barreau F, Hugot JP. Intestinal barrier dysfunction triggered by invasive bacteria. Curr Opin Microbiol 2014; 17:91-98.
15. Marchiando AM, Shen L, Graham WV, Weber CR, Schwarz BT, Austin JR, Raleigh DR, Guan Y, Watson AJ, Montrose MH, Turner JR. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation *in vivo*. J Cell Biol 2010; 189:111-126.
16. Hernandez G, Hasbun P, Velasco N, Wainstein C, Bugedo G, Bruhn A, Klaassen J, Castillo L. Splanchnic ischemia and gut permeability after acute brain injury secondary to intracranial hemorrhage. Neurocrit Care 2007; 7:40-44.
17. Sequeira IR, Lentle RG, Kruger MC, Hurst RD. Standardizing the lactulose mannitol test of gut permeability to minimize error and promote comparability. PLoS One 2014; 9:e99256.
18. Hang CH, Shi JX, Li JS, Wu W, Yin HX. Alternations of intestinal mucosa structure and barrier function following traumatic brain injury in rats. World J Gastroenterol 2003; 9:2776-2781.

19. Feighery L, Smyth A, Keely S, Baird AW, O'Connor WT, Callanan JJ, Brayden DJ. Increased intestinal permeability in rats subjected to traumatic frontal lobe percussion brain injury. J Trauma 2008; 64:131-138.
20. Bansal V, Constantini T, Kroll L, Peterson C, Loomis W, Eliceiri B, Baird A, Wolf P, Coimbra R. Traumatic brain injury and intestinal dysfunction: Uncovering the neuro-enteric axis. J Neurotrauma 2009; 26:1353-1359.
21. Bansal V, Ryu SY, Blow C, Constantini T, Loomis W, Eliceiri B, Baird A, Wolf P, Coimbra R. The hormone ghrelin prevents traumatic brain injury induced intestinal dysfunction. J Neurotrauma 2010; 27:2255-2260.
22. Zhang X, Jiang X. Effects of enteral nutrition on the barrier function of the intestinal mucosa and dopamine receptor expression in rats with traumatic brain injury. J Parenter Enteral Nutr 2015; 39:114-123.
23. Ringel Y, Maharshak N. Intestinal microbiota and immune function in the pathogenesis of irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2013; 305:G529-G541.
24. Furness JB, Callaghan BP, Rivera LR, Cho HJ. The enteric nervous system and gastrointestinal innervation: integrated local and central control. Adv Exp Med Biol 2014; 817:39-71.
25. Bansal V, Constantini T, Ryu SY, Petersen C, Loomis W, Putnam J, Elicieri B, Baird A, Coimbra R. Stimulating the central nervous system to prevent intestinal dysfunction after traumatic brain injury. J Trauma 2010; 68:1059-1064.

26. Pruitt D, Schmid A, Kim L, Abe C, Trieu J, Choua C, Hays S, Kilgard M, Rennaker Li RL. Vagus nerve stimulation delivered with motor training enhances recovery of function after traumatic brain injury. J Neurotrauma 2015; (in press).
27. Feng D, Xu W, Chen G, Hang C, Gao H, Yin H. Influence of glutamine on intestinal inflammatory response, mucosa structure alternations and apoptosis following traumatic brain injury in rats. J Int Med Res 2007; 35:644-656.
28. Chen G, Shi J, Qi, M, Yin H, Hang C. Glutamine decreases intestinal nuclear factor kappa B activity and pro-inflammatory cytokines expression after traumatic brain injury in rats. Inflamm Res 2008; 57:57-64.
29. Sun J, Wang L, Shen J, Wang Z, Qian Y. Effect of propofol on mucous permeability and inflammatory mediators expression in the intestine following traumatic brain injury in rats. Cytokine 2007; 40:151-156.
30. Jin W, Wang H, Ji Y, Hu Q, Yan W, Chen G, Yin H. Increased intestinal inflammatory response and gut barrier dysfunction in Nrf2-deficient mice after traumatic brain injury. Cytokine 2008; 44:135-140.
31. Beynon AL, Brown MR, Wright R, Rees MI, Sheldon IM, Davies JS. Ghrelin inhibits LPS-induced release of IL-6 from mouse dopaminergic neurons. J Neuroinflammation 2013; 10:40.
32. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000; 405:458-462.

33. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF-κB: variations on a common theme. Cell Mol Life Sci 2008; 65:2964-2978.
34. Al-Sadi R, Ye D, Boivin M, Guo S, Hashimi M, Ereifej L, Ma TY. Interleukin-6 modulation of intestinal epithelial tight junction permeability is mediated by JNK pathway activation of claudin-2 gene. PLoS One 2014; 9:e85345.
35. Stoecklein VM, Osuka A, Lederer. Trauma equals danger-damage control by the immune system. J Leukoc Biol 2012; 92:539-551.
36. Land WG, Messmer K. The danger theory in view of the injury hypothesis: 20 years later. Front Immunol 2012; 3:349.
37. Pradeu T, Cooper EL. The danger theory: 20 years later. Front Immunol 2012; 3:287.
38. Katzenberger RJ, Chtarbanova S, Rimkus SA, Fischer JA, Kaur G, Seppala JM, Swanson LC, Zajac JE, Ganetzky B, Wassarman DA. Death following traumatic brain injury in Drosophila is associated with intestinal barrier dysfunction. eLife 2015; 10.7554/eLife.04790.
39. Katzenberger RJ, Loewen CA, Wassarman DR, Petersen AJ, Ganetzky B, Wassarman DA. A Drosophila model of closed head traumatic brain injury. Proc Natl Acad Sci USA 2013; 110:E4152-E4159.
40. Katzenberger RJ, Loewen CA, Bockstruck RT, Woods MA, Ganetzky B, Wassarman DA. A method to inflict closed head traumatic brain injury in Drosophila. J Vis Exp 2015; 100:e52905.

41. Bonnay F, Cohen-Berros E, Hoffmann M, Kim SY, Boulianne GL, Hoffmann JA, Matt N, Reichhart JM. *big bang* gene modulates gut immune tolerance in Drosophila. Proc Natl Acad Sci USA 2013; 110:2957-2962.
42. Furuse M, Tsukita S. Claudins in occluding junctions of humans and flies. Trends Cell Biol 2006; 16:181-188.
43. Hindle SJ, Bainton RJ. Barrier mechanisms in the Drosophila blood-brain barrier. Front Neurosci 2014; 8:414.
44. Izumi Y, Furuse M. Molecular organization and function of invertebrate occluding junctions. Semin Cell Dev Biol 2014; 36:186-193.
45. Bilder D, Perrimon N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature 2000; 403:676-680.
46. Bahri S, Wang S, Conder R, Choy J, Vlachos S, Dong K, Merino C, Sigrist S, Molnar C, Yang X, Manser E, Harden N. The leading edge during dorsal closure as a model for epithelial plasticity: Pak is required for recruitment of the Scribble complex and septate junction formation. Development 2010; 137:2023-2032.
47. Narasimha M, Uv A, Krejci A, Brown NH, Bray SJ. Grainy head promotes expression of septate junction proteins and influences epithelial morphogenesis. J Cell Sci 2008; 121:747-752.
48. Yu Z, Bhandari A, Mannik J, Pham T, Xu X, Andersen B. Grainyhead-like factor Get1/Grhl3 regulates formation of the epidermal leading edge during eyelid closure. Dev Biol 2008; 319:56-67.
49. Senga K, Mostov KE, Mitaka T, Miyajima A, Tanimizu N. Grainyhead-like 2 regulates epithelial morphogenesis by establishing functional tight junctions through the

organization of a molecular network among claudin3, claudin4, and Rab25. Mol Biol Cell 2012; 23:2845-2855.

50. Rera M, Bahadorani S, Cho J, Koehler CL, Ulgherait M, Hur JH, Ansari WS, Lo T, Jones DL, Walker DW. Modulation of longevity and tissue homeostasis by the Drosophila PGC-1 homolog. Cell Metab 2011; 14:623-634.
51. Rera M, Clark RI, Walker DW. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. Proc Natl Acad Sci USA 2012; 109:21528-21533.
52. Hoffmann JA, Reichhart JM. Drosophila innate immunity: an evolutionary perspective. Nat Immunol 2002; 3:121-126.
53. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol 2007; 25:697-743.
54. Guilhelmelli F, Vilela N, Albuquerque P, Derengowski LS, Silva-Pereira I, Kyaw CM. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. Front Microbiol 2013; 4:353.
55. Tan L, Schedl P, Song HJ, Garza D, Konsolaki M. The Toll-NFkappaB signaling pathway mediated the neuropathological effects of the human Alzheimer's Abeta42 polypeptide in Drosophila. PLoS One 2008; 3:e3966.
56. Chinchore Y, Gerber GF, Dolph PJ. Alternative pathway of cell death in Drosophila mediated by NF-□B transcription factor Relish. Proc Natl Acad Sci USA 2012; 109:E605-E612.

57. Petersen AJ, Rimkus SA, Wassarman DA. ATM kinase inhibition in glial cells activates the innate immune response and causes neurodegeneration in Drosophila. Proc Natl Acad Sci USA 2012; 109:E656-E664.
58. Petersen AJ, Katzenberger RJ, Wassarman DA. The innate immune response transcription factor relish is necessary for neurodegeneration in a Drosophila model of ataxia-telangiectasia. Genetics 2013; 194:133-142.
59. Cao Y, Chtarbanova S, Petersen AJ, Ganetzky B. Dnr1 mutations cause neurodegeneration in Drosophila by activating the innate immune response in the brain. Proc Natl Acad Sci USA 2013; 110:E1752-E1760.
60. Socia SJ, Kirby JE, Washincosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, Mior RD. The Alzheimer's disease-associated amyloid β-protein is an antimicrobial peptide. PLoS One 2010; 5:e9505.
61. Sriram K, O'Callaghan JP. Divergent roles for tumor necrosis factor-alpha in the brain. J Neuroimmune Pharmacol 2007; 2:140-153.
62. Berglund JJ, Riegler M, Zolotarevsky Y, Wenzel E, Turner JR. Regulation of human jejunel transmucosal resistance and MLC phosphorylation by Na(+)-glucose cotransport. J Physiol Gastrointest Liver Physiol 2001; 281:G1487-G1493.
63. Qing Q, Zhang S, Chen Y, Li R, Mao H, Chen Q. High glucose-induced intestinal epithelial barrier damage is aggravated by syndecan-1 destruction and heparanase overexpression. J Cell Mol Med 2015; 19:1366-1374.
64. Lin L, Yee SW, Kim RB, Giacomini KM. SLC transporters as therapeutic targets: emerging opportunities. Nat Rev Drug Discov 2015; (in press).

65. Rovlias A, Kotsou S. The influence of hyperglycemia on neurological outcome in patients with severe head injury. Neurosurgery 2000; 46:335-342.

66. Salim A, Hadjizacharia P, Dubose J, Brown C, Inaba K, Chan LS, Margulies D. Persistent hyperglycemia in severe traumatic brain injury: an independent predictor of outcome. Am Surg 2009; 75:25-29.

67. Harun Haron R, Imran MK, Haspani MS. An observational study of blood glucose levels during admission and 24 hours post-operation in a sample of patients with traumatic injury in a hospital in Kuala Lumpur. Malays. J Med Sci 2011; 18:69-77.

68. Yuan Q, Liu H, Xu Y, Wu X, Sun Y, Hu J. Continuous measurement of the cumulative amplitude and duration of hyperglycemia best predicts outcome after traumatic brain injury. Neurocrit Care 2014; 20:69-76.

69. Alexiou GA, Lianos G, Fotakopoulos G, Michos E, Pachatouridis D, Voulgaris S. Admission glucose and coagulopathy occurrence in patients with traumatic brain injury. Brain Inj 2014; 28:438-441.

70. Prisco L, Iscra F, Ganau M, Berlot G. Early predictive factors on mortality in head injured patients: a retrospective analysis of 112 traumatic brain injured patients. J Neurosurg Sci 2012; 56:131-136.

71. Elkon B, Cambrin JR, Hirshberg E, Bratton SL. Hyperglycemia: an independent risk factor for poor outcome in children with traumatic brain injury. Pediatr Crit Care Med 2014; 15:623-631.

72. Chong SL, Haranto S, Testoni D, Ng ZM, Low CY, Lee KP, Lee JH. Early hyperglycemia in pediatric traumatic brain injury predicts for mortality, prolonged

duration of mechanical ventilation, and intensive care stay. Int J Endocrinol 2015; 2015:719476.

73. Ley EJ, Srour MK, Clond MA, Barnajian M, Tillou A, Mirocha J, Salim A. Diabetic patients with traumatic brain injury: insulin deficiency is associated with increased mortality. J Trauma 2011; 70:1141-1144.

74. Lustenberger T, Talving P, Lam L, Inaba K, Bass M, Plurad D, Demetriades D. Effect of diabetes mellitus on outcome in patients with traumatic brain injury: a national traumatic databank analysis. Brain Inj 2013; 27:281-285.

75. Nelson KS, Furuse M, Beitel GJ. The Drosophila Claudin Kune-kune is required for septate junction organization and tracheal tube size control. Genetics 2010; 185:831-839.

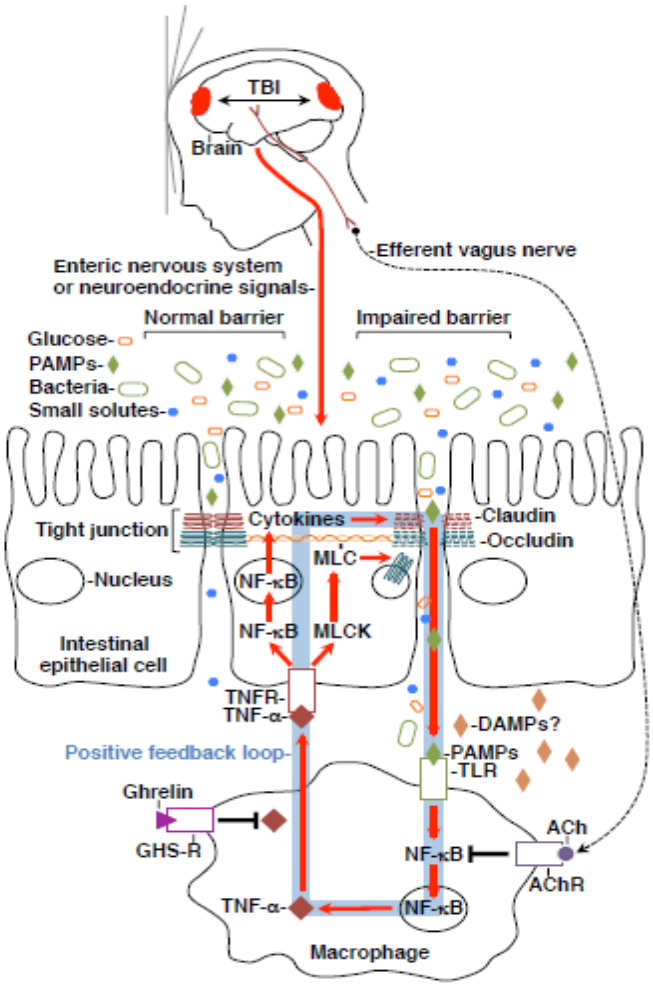

Figure 1. A model describing how TBI might increase intestinal permeability in humans. A detailed description of the model is presented in the text. Discontinuous lines for Claudin and Occludin indicate a molecular change that results in increased tight junction permeability. An Occludin-containing endosome is indicated by the cytoplasmic circle. Red spots in the brain indicate injuries, and the associated double-headed arrow indicates movement of the brain due to impact of the head with a solid object. The question mark after "DAMPs" indicates that the relative roles of PAMPs and DAMPs in this mechanism is not known.

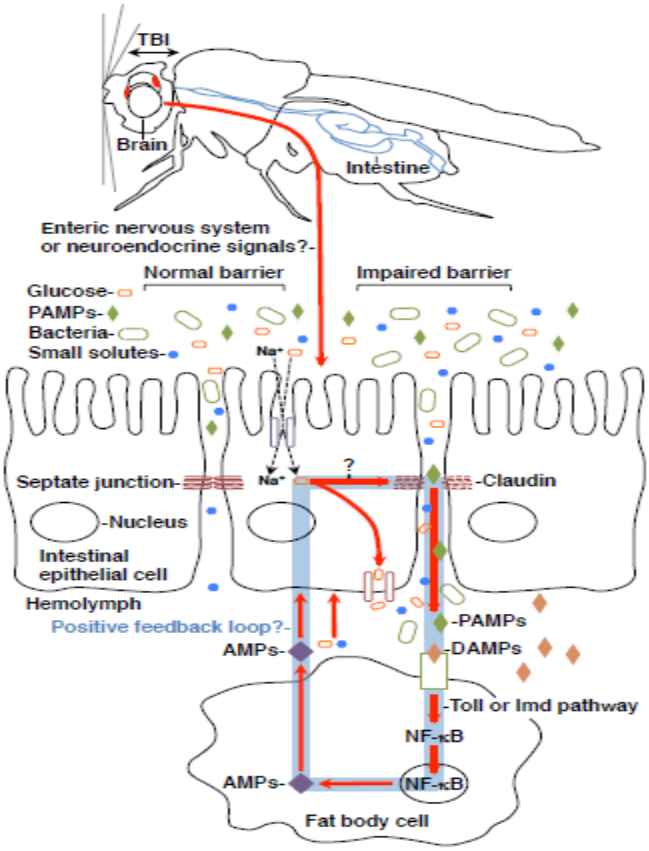

Figure 2. A model describing how TBI might increase intestinal permeability and cause death in flies. A detailed description of the model is presented in the text. "Claudin" refers to Claudin-like Drosophila proteins Kune-kune, Megatrachea, and Sinuous (75). Red spots in the brain indicate injuries, and the associated double-headed arrow indicates movement of the brain due to impact of the head with a solid object. The foregut is not included in the diagram of the fly. AMPs are not only expressed by fat body cells but also by other cells, including intestinal epithelial cells. Question marks indicate events that are presumed to occur but the mechanisms for which are unknown. Major differences between the fly model and the human model (Figure 1) are roles for glucose and DAMPs (rather than PAMPs) in the positive feedback loop that increases intestinal permeability following TBI.