Journal Club

Editor's Note: These short, critical reviews of recent papers in the Journal, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Bradykinin: A Microglia Attractant *In Vivo*?

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Review of Ifuku et al. (<http://www.jneurosci.org/cgi/content/full/27/48/13065>)

From several studies over the last decades, it has become clear that under pathological conditions microglia are involved in tissue repair, inflammatory effects, and neurodegeneration. In order for microglia to engage in neuroprotection or neurodestruction, they need to be attracted to the site of injury by means of signals that induce migration.

A number of signaling molecules are known to influence microglial activity, and recently new signaling pathways for inducing microglial migration toward the site of injury have been suggested. For example, Davalos et al. (2005) have shown that ATP released from damaged tissue after traumatic brain injury seems to be an important signal for inducing rapid microglia migration toward injury. Even more recently, the neuropeptide bradykinin has been discovered as a trigger of microglial activity (Noda et al., 2007).

Bradykinin is an endogenous peptide that is rapidly released after brain injury or stroke (Gröger et al., 2005). Binding of bradykinin to its receptors influences a variety of cellular events, depending on the pathological condition. These events can be neurotoxic by promoting inflammation, but Noda et al. (2007) found that the actions of bradykinin can also be neuro-

protective by interacting with bradykinin receptors on microglia, which induces an anti-inflammatory cascade. The way in which bradykinin interacts with microglia, however, remains uncertain.

A recently published study by Ifuku et al. (2007) in *The Journal of Neuroscience* investigated whether bradykinin can attract microglia to a lesion site. Ifuku et al. (2007) first conducted an *in vitro* study, wherein rat microglial cells were isolated from mixed cultures of cerebrocortical cells in postnatal day 3 Wistar rats. They analyzed the motility of microglia by monitoring individual cells by time-lapse video microscopy at different times after the addition of bradykinin. The authors found a significant increase in microglial motility in the presence of bradykinin [Ifuku et al. (2007), their Fig. 1B (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F1>)]. In the presence of a B₁ receptor antagonist, the motility of microglia was strongly reduced [Ifuku et al. (2007), their Fig. 1C (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F1>)], whereas B₁ receptor agonists mimicked the response of bradykinin [Ifuku et al. (2007), their Fig. 1E,F (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F1>)]. In contrast, a B₂ receptor antagonist did not affect the motility [Ifuku et al. (2007), their Fig. 1D (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F1>)], suggesting that B₁ receptors and not B₂ receptors are essential in microglial motility. These findings were confirmed by studying bradykinin-in-

duced chemotaxis of microglia from B₁ and B₂ receptor-knock-out mice, using a microchemotaxis (Boyden) chamber. Bradykinin did not affect microglial migration in B₁ receptor-knock-out mice [Ifuku et al. (2007), their Fig. 2B (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F2>)], but increased migration in B₂ receptor-knock-out mice [Ifuku et al. (2007), their Fig. 2C (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F2>)].

The authors next studied several steps in the signaling cascade mediating microglial migration after B₁ receptor activation. The results indicate that bradykinin is metabolized into a B₁ agonist, which binds to B₁ receptors coupled to G_{q/11}-proteins and thereby activates several protein kinases, including PKC (protein kinase C) and PI3K (phosphoinositide 3-kinase). These kinases phosphorylate and activate NCXs (Na⁺–Ca²⁺ exchangers), inducing an influx of extracellular Ca²⁺. Increased Ca²⁺ activates Ca²⁺-dependent K⁺ channels, and this leads to microglial migration by a mechanism that remains unclear [Ifuku et al. (2007), their Fig. 7 (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F7>)].

To be certain that bradykinin attracts microglia not only *in vitro* but also *in vivo*, the authors compared microglial density 18 h after a focal stab wound in normal and B₁- and B₂-deficient mice. Only in the B₁ receptor-knock-out mice was microglial accumulation significantly decreased compared with wild-type mice [Ifuku et

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al. (2007), their Fig. 9 (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F9>)). The *in vivo* study also found some evidence for the suggested signaling pathway: injection of a Ca^{2+} -dependent K^{+} current blocker significantly reduced the number of microglia around the lesion [Ifuku et al. (2007), their Fig. 9 (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F9>))].

These results clearly show that bradykinin, which is released after brain injury or stroke, induces migration of microglia to a lesion site *in vitro*. The authors identified several steps in the underlying cellular mechanisms by which bradykinin attracts the microglia, and this pathway seems to be distinct from pathways underlying migration induced by ATP. This is significant because it has been found that the amount of ATP released during ischemic stroke is strongly reduced (Sims and Anderson, 2002). In contrast, bradykinin is upregulated during ischemic stroke, as are bradykinin receptors (Gröger et al., 2005), suggesting that bradykinin-induced migration of microglia may be more important in the ischemic brain. It would be interesting to investigate which role the different signaling pathways induced by ATP and bradykinin play in other pathological conditions. This could be of clinical relevance for the development of treatments for specific pathological conditions.

The *in vitro* experiments by Ifuku et al. (2007) were well performed and controlled and clearly demonstrated bradykinin-induced microglial migra-

tion. On the other hand, the results of the *in vivo* experiment [Ifuku et al. (2007), their Fig. 9 (<http://www.jneurosci.org/cgi/content/full/27/48/13065/F9>))], which show an increase in microglia at the lesion site, could have resulted from microglial proliferation as well as migration. In response to tissue injury, microglia both migrate to and proliferate at the lesion site. Although the *in vitro* results combined with the relatively low microglia count in B_1 knock-out mice *in vivo* suggest bradykinin-induced migration, additional measurements would clarify whether migration, proliferation, or both cause the increase in microglia. To do this, one could inject the proliferation marker bromodeoxyuridine (BrdU). If a high percentage of the microglia at the lesion site incorporate BrdU, this would indicate that the heightened number of microglia is largely attributable to proliferation. It would also be interesting to study *in vitro* whether bradykinin induces proliferation of microglia.

At present, there is no marker that exclusively stains microglia. The immunohistochemical staining with anti-IBA-1, which was used in the *in vivo* experiment by Ifuku et al. (2007), not only stains microglia, but also stains macrophages. Riva-Depaty et al. (1994) showed that in traumatically lesioned rats, macrophages were “maximally recruited” 1–3 d after lesion. This indicates that macrophages are also recruited in traumatic (e.g., stab) lesions. For that reason, the contribution of macrophages to the positive cell population should be taken into consideration in

future *in vivo* research that studies the role of bradykinin in traumatic injury. These additional measures could strengthen the conclusion that bradykinin induces microglial migration *in vivo*, as was convincingly demonstrated by Ifuku et al. (2007) *in vitro*.

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