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Use of an immunocapture device to detect cytokine release in discrete brain regions

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Production of proinflammatory cytokines in the central nervous system is a key process in the neuroinflammatory response to trauma, infection, and neurodegenerative diseases (Kumar, 2019). These intercellular signaling molecules play multiple roles in the central nervous system immune response including the orchestration of the sickness response to innate immune perturbations in the brain (Dantzer et al., 2008). Brain innate immune cells such as microglia and other macrophages (perivascular, meningeal) are considered a significant source of cytokines (Ransohoff and Cardona, 2010) during neuroinflammatory conditions. Thus, quantification of cytokines in the central nervous system is essential to understanding the neuroimmune mechanisms underpinning neuroinflammatory conditions and to monitor the effects of treatment. However, quantification of brain cytokines has largely been limited to end-point measures of tissue protein levels of cytokines using techniques such as enzyme-linked immunosorbent assay (ELISA), western blot assay, or immunohistochemistry, which fail to discriminate between intracellular and extracellular levels of cytokines. In other words, an experimental change in total tissue levels of cytokines does not necessarily mean that the protein was secreted into the interstitial space within a brain region. Proinflammatory cytokine receptor antagonists as well as germ-line knockouts have been employed to block the behavioral, physiological, and neuroinflammatory response to stress (Goshen and Yirmiya, 2009) and immune challenge (McCusker and Kelley, 2013), which implicates, but does not directly demonstrate cytokine release in the brain. A further limitation of measuring cytokines in whole tissue is that measurements are restricted to a single time point post-mortem. This limitation necessitates using a between-subjects experimental design to conduct time course measurements of cytokines, which introduces error variance due to between-subject variability in biological responses. Notably, inflammatory cytokines such as interleukin (IL)-1β have very short half-lives (Liu et al., 2021). Thus, methods that are limited to measuring single time points post-immune challenge lack the temporal resolution to capture the rapid kinetic changes in inflammatory cytokines. In this Perspective piece, we explore a recent technological advance that allowed us to serially quantify cytokines within the interstitial space of discrete brain regions of freely behaving rodents. This approach not only permits quantification of cytokine release into the extracellular space, but also provides increased spatial and temporal resolution of cytokine release in the brain under neuroinflammatory conditions.

An *in vivo* immunocapture device: As a general overview, the immunosensing device consists of a modified optical fiber, which is labeled with a capture antibody specific to a cytokine of interest (**Figure 1**). The device is then inserted into a perforated guide cannula, which is implanted in the brain of rodents to target a region of interest. The perforated guide cannula permits cytokines to passively diffuse from the extracellular space into the lumen of the cannula and bind the capture antibody on the optical fiber. The fiber remains in the guide cannula for a period to capture cumulative cytokine release within the region of interest. The optical fiber is then removed from the guide cannula at some time point during the neuroinflammatory process and ELISA is conducted to measure the cumulative amount of cytokine bound to the fiber. Protein standards comprised of optical fibers exposed to known concentrations of cytokine are used to quantify the amount of cytokine bound to the test fiber. A new optical fiber can then be inserted into the cannula to capture cytokine release over a subsequent time interval. Thus, this process can be repeated to provide serial measurements of cytokine release over an extended time interval in the same animal, thereby capturing the rapid kinetics of cytokine secretion in the brain of living subjects.

In our initial studies, we labeled an optical fiber with an anti-rat IL-1β capture antibody (Zhang et al., 2018). We chose to measure the proinflammatory cytokine IL-1β because it is considered a "gatekeeper" of neuroinflammation that initiates a cascade of neuroinflammatory events (Dinarello, 1997). To determine the linear range of detection, the optical fiber was exposed to increasing concentrations of rat IL-1β *in vitro* and ELISA was conducted. The fluorescence intensity of the fiber was imaged using a confocal microscope. We found that a linear relationship (r = 0.99) between fluorescent intensity and log IL-1β concentration was obtained from 3.9 pg/mL to 500 pg/mL IL-1β with a lower detection limit of 1.2 pg/mL. A subsequent study replicated this linear relationship (r = 0.99) and found a lower detection limit of 1.07 pg/mL (Frank et al., 2020). Preincubation of the fiber with excess (200 pg/mL) alternate cytokines (tumor necrosis factor, IL-6, interferon-gamma, IL-2 or IL-4) failed to significantly alter the signal intensity of a known concentration of IL-1β (10 pg/mL) suggesting that the fiber sensor selectively binds to IL-1β (Zhang et al., 2018; Frank et al., 2020).

Given these validation studies, we examined whether this technique could detect cytokine secretion *in vivo* under neuroinflammatory conditions. Our initial study utilized a bacterial mimetic to produce a proinflammatory cytokine response in the brain of rodents (Zhang et al., 2018). For this purpose, we utilized lipopolysaccharide (LPS), which is a component of the cell wall of gram-negative bacteria. LPS is a pathogen-associated molecular pattern that produces a robust inflammatory response via signaling through the pattern recognition receptor Toll-like receptor 4, which is expressed by multiple innate immune cell types (Kawai and Akira, 2010). To elicit a cytokine response in the brain, we administered LPS into the peritoneal cavity, which on the face of it is counter intuitive. However, it is well established that generating a cytokine response in the periphery produces a cytokine response in the brain via immune-to-

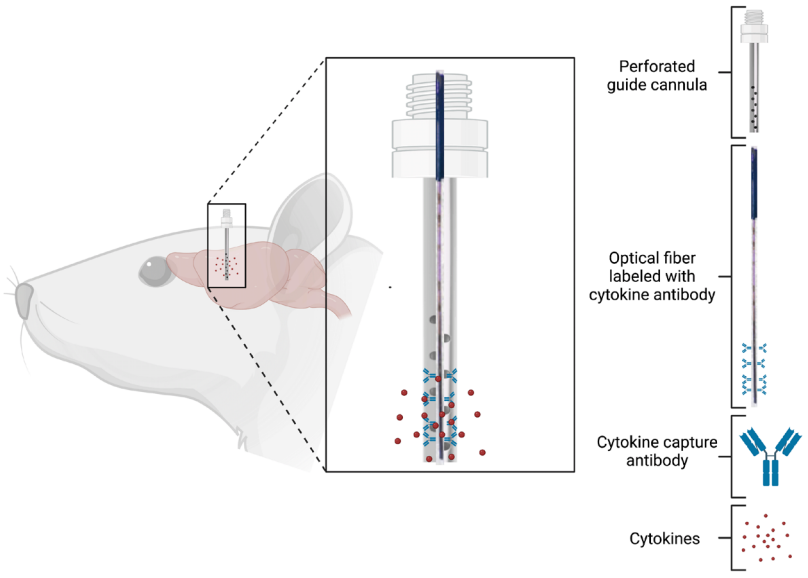

Figure 1 | **Components of an *in vivo* immunosensing device.**
A perforated guide cannula is implanted in the brain of rodents targeting a region of interest. The cannula is perforated only at the distal tip, which permits cytokines to passively diffuse into the lumen of the cannula from the interstitial space within a discrete region of interest. An optical fiber is labeled with a capture antibody targeting a cytokine of interest. The optical fiber is inserted into the guide cannula prior to the onset of a neuroinflammatory event. Baseline measures of cytokine secretion are then measured. The rodent undergoes a proinflammatory challenge that elicits the release of cytokines within the brain. Cytokines enter the guide cannula and bind to the capture antibody. After a period, the optical fiber with bound cytokine is removed and enzyme-linked immunosorbent assay is conducted *ex vivo* to quantify the amount of bound cytokine. Additional fibers may then be inserted over subsequent time intervals to provide serial measurements of cytokine release post-immune challenge. Created with BioRender.com.

brain signaling pathways (McCusker and Kelley, 2013). Prior to administering LPS, rodents were implanted with a perforated guide cannula targeting the dorsal hippocampus and allowed to recover for 4 weeks after surgery. Of note, the dorsal hippocampus was also cannulated in vehicle-treated animals to control for the potential neuroinflammatory effects of the surgical procedure as well as chronic cannulation. The procedure and timing of fiber insertion and removal were identical for both the LPS and vehicle-treated animals. To capture baseline IL-1β secretion, extracellular IL-1β was measured over 2 consecutive 20-minute time intervals prior to LPS treatment. At 1 and 4 hours after LPS treatment, separate fibers were inserted and remained in place for 20 minutes to capture cumulative IL-1β secretion. At 1 hour post-injection, we found that LPS produced a robust IL-1β increase in the interstitial space of the dorsal hippocampus compared to vehicle control as well as baseline values. Interestingly, this effect of LPS on IL-1β secretion was no longer evident at 4 hours post-LPS, which supports prior studies demonstrating the stringent regulation of IL-1β release and short half-life (Dinarello, 1997).

To further assess the utility of this approach to measure cytokine secretion in the brain, we exposed rats to a robust acute stressor and measured IL-1β secretion in the dorsal hippocampus (Frank et al., 2020). As noted, the behavioral effects of stress are mediated, in part, by IL-1β (Goshen and Yirmiya, 2009). However, stress-induced IL-1β secretion had not been directly demonstrated. Like the LPS experiment described previously, the dorsal hippocampus was cannulated, and animals were allowed to recover from surgery for 4 weeks. Baseline IL-1β secretion was measured over two consecutive 20-minute intervals prior to stress exposure. Non-stressed home cage controls also underwent cannulation and fiber insertion/removal procedures in parallel with stress animals. Immediately after termination of the stressor, a fiber was inserted and IL-1β secretion was measured over a 20-minute interval. After removal of the fiber, a subsequent fiber was inserted 4 hours after termination of the stressor and IL-1β secretion was measured over another 20-minute interval. We found that stress increased IL-1β secretion immediately after stress exposure compared to home cage controls as well as baseline levels. However, the effect of stress on IL-1β secretion was no longer apparent at 4 hours post-stress. These findings are consistent with the effects of LPS on IL-1β extracellular release, which demonstrates that IL-1β secretion is highly transient under these neuroinflammatory conditions.

There are several limitations to this technique that should be noted. Under some experimental conditions, cytokines are not released from cells, thus this immunocapture device would fail to detect the accumulation of intracellular cytokines, which might be a critical immune outcome. A major consideration is that the cannulation procedure per se might produce a neuroinflammatory response and/or prime this response to subsequent immune challenges. The placement of an indwelling cannula in the brain is akin to a stab wound, which produces tissue damage and the release of proinflammatory damage-associated molecular patterns. Of note, the cannula used in the present approach has a diameter of 511 mm and the detection fiber has a diameter of 125 mm, although these parameters are scalable depending on the application. We examined this issue of tissue damage in a prior study (Holguin et al., 2007) using a cannula with a diameter of 460 mm. We found that cannulation of the hippocampus failed to produce a proinflammatory cytokine response at 1, 2 and 4 weeks post-cannulation. However, cannulation potentiated the cytokine response to a subsequent immune challenge (LPS) at 1 and 2 weeks, but not 4 weeks after cannulation. Consistent with these findings, we found that cannulation also failed to potentiate the cytokine effects of a severe acute stressor administered 4 weeks after surgery suggesting that cannulation fails to prime the neuroinflammatory response to stress (Frank et al., 2020). Further, cannulation did not increase total cytokine levels compared to sham surgery in the hippocampus. Another possibility that should be considered is that cells might adhere to the cannula, which could function as a cytokine "sink" if the cells express receptors for the cytokine of interest. This consequence of cannulation might reduce the detection of cytokine release. Similarly, if a portion of the cannula abuts against cells and is not exposed to the interstitial fluid, cytokine detection might be reduced resulting in an underestimation of cytokine release. Finally, a concern is that the capture antibodies on the detection fiber compete with endogenous receptors for extracellular cytokines, which is a likely possibility. In doing so, cytokine binding to endogenous receptors might be reduced resulting in alterations of downstream immune signaling and thus the biological and behavioral consequences of that signaling.

Taken together, the findings discussed here illustrate that this *in vivo* immunocapture device effectively measures cytokine secretion into the interstitial space of discrete brain regions. Further, this approach allows investigators to perform within-subjects serial measurements of cytokine secretion, which greatly increases the temporal resolution of cytokine measurement in brain tissue. Thus, the increased spatial and temporal resolution of this *in vivo* approach to measuring cytokine release in the brain allows investigators to capture the rapid kinetic nature of cytokine release under neuroinflammatory conditions. Future efforts should extend this strategy for multiplex cytokine detection given that cytokines act in concert to impart their function. Measurement of multiple cytokines in a single immunoassay offers the advantage of minimizing sample and reagent volumes, while potentially uncovering distinct disease neuroimmune profiles.

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