Systemic Inflammation Impairs Attention and Cognitive Flexibility but Not Associative Learning in Aged Rats: Possible Implications for Delirium

Citation

Published Version
doi:10.3389/fnagi.2014.00107

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Accessibility
Systemic inflammation impairs attention and cognitive flexibility but not associative learning in aged rats: possible implications for delirium

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INTRODUCTION

Delirium, an acute neuropsychiatric syndrome, occurs commonly in hospitalized older medical and surgical patients. It is often precipitated by peripheral infection or inflammation induced by surgical trauma (Young et al., 1990; Wofford et al., 1996; Klausen et al., 1997; Broadhurst and Wilson, 2001; Buvanendran et al., 2006; Inouye, 2006; Nelson et al., 2006) and is important clinically because it is associated with poor functional outcome, a high rate of discharge to an extended care facility, a steeper trajectory of subsequent cognitive decline, and increased 1-year mortality (Greene et al., 2009; Smith et al., 2009; Szczypka et al., 2012). The pathogenesis of delirium is poorly understood, in part because there is no widely accepted animal model for examining mechanisms of the disorder. There are a multitude of putative causes of delirium but inflammation is a prime suspect based on clinical studies that reveal it is often precipitated in elderly patients by proinflammatory events such as infectious illness or surgery and laboratory evidence that cytokines and chemokines impair memory and learning directly (Marcantonio et al., 2006; Rudolph et al., 2008; van Gool et al., 2010; Murray et al., 2012). The difficulty, however, is that although there is little evidence for hippocampal dysfunction in delirium, most preclinical work on inflammation and cognition examines hippocampally mediated fear, reference, or working memory. Several cognitive domains can be affected in delirium but its pathognomonic feature is inattention. Efficient regulation of attention is thought to require close interaction between the prefrontal and parietal cortex, as well as neuromodulation from cholinergic basal forebrain neurons (Corbetta and Shulman, 2002; Sarter and Paolone, 2011). Hence, the inattention that occurs during delirium implies dysfunction of these cortical circuits. Recent functional imaging studies substantiate this view; in patients imaged during active delirium, the...
prefrontal cortex was hyper-active relative to the resting state. As such, behavioral tests mediated by the relevant cortical networks may be better suited than hippocampus-dependent behaviors to studying relationships between inflammation and a delirium-like phenotype in animals. The attentional set-shifting task (AST) is such a behavioral test. Developed for rats, the AST includes a set of discrimination problems based on stimulus dimensions such as odor and intra- and extra-dimensional cues and is analogous to the Wisconsin Card Sorting Test (WCST) in humans, which is used clinically to detect impairments in attention and executive function due to prefrontal cortex damage or dysfunction (Milner, 1963; Roberts et al., 1988). Here we used the AST to test the hypothesis that by promoting sustained neuroinflammation, systemic inflammation impairs attention and executive function in aged animals. We chose this task primarily because it can be rapidly acquired by aged rodents in contrast to other available tests of attention and executive function, for example, the 5-choice serial reaction time task, which require months of training and therefore pose a challenge for the study of aged animals.

**MATERIALS AND METHODS**

This protocol was approved by the Harvard Medical Area Standing Committee on Animals. A total of 31 24-month-old Fischer-344 rats were included; 11 were used for the behavioral part of the study and 20 were used for measurement of a representative cytokine and chemokine in plasma and brain. Systemic inflammation was induced by intraperitoneal (i.p.) administration of lipopolysaccharide (LPS; 50 µg/kg) while controls received an equal volume of saline i.p. LPS is a proinflammatory protein from the cell wall of bacteria that is used widely to model systemic infection/inflammation. This dose was selected on the basis of published reports and preliminary studies showing that aged rats became ill but recovered within a few days.

Behavioral testing consisted of the AST. This procedure includes a sequence of discrimination problems in which rats dig for a food reward buried in one of two pots filled with digging medium (Birrell and Brown, 2000; Barense et al., 2002). The pots can be distinguished on several dimensions, including odor, type of digging medium, and texture applied to the outside of the pot. A sequence of problems is given in which only the relevant dimension is present (the simple discrimination, or SD), the irrelevant dimension is introduced alongside the same exemplars of the relevant dimension (the compound discrimination, or CD), new exemplars of all dimensions are introduced but the relevant dimension stays the same (the intradimensional shift, or IDS), and new exemplars of all dimensions are introduced but the relevant dimension changes (the extradimensional shift, or EDS). Problems are also reversed, such that the same stimuli are presented as the previous problem but the correct exemplar changes. An example sequence of problems is given in Table 1. In the present study, the two dimensions used were the digging medium and shape of a white plastic object placed on the wall immediately adjacent to the pot. Testing procedures were similar to those published previously (Barense et al., 2002; Fox et al., 2003; Chen et al., 2004) except that the stimulus dimensions, rather than digging medium and odor, were digging medium and shape of a white plastic object placed on the wall immediately adjacent to the pot. Rats encountered the sequence of discrimination problems (Table 1) over four test sessions.

Once the animals were trained to dig reliably (defined as vigorous digging in six sequential trials in a row), the two SD tests were performed to establish a baseline (day 0; Figure 1). Then the rats were randomized to receive either LPS ($N = 6$) or saline ($N = 5$). The next day, each rat was tested on the CD and CD-R components of the AST; on day 2, rats were tested on the IDS and IDS-R components, and on day 3 they were each tested on the EDS. Each discrimination problem was presented until the rat reached performance criteria, defined as six consecutive correct trials. A “dig” was scored when the rat vigorously displaced the

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**Table 1 | Relevant dimension and positive discriminators used for each stage of the AST.**

<table>
<thead>
<tr>
<th>Relevant dimension</th>
<th>S+</th>
<th>S−</th>
</tr>
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<tbody>
<tr>
<td>SD1 Paper vs.</td>
<td>Aspen</td>
<td>Medium</td>
</tr>
<tr>
<td>SD2 Heart vs.</td>
<td>Square</td>
<td>Shape</td>
</tr>
<tr>
<td>CD Paper/flower vs. or paper/triangle</td>
<td>Aspen/triangle</td>
<td>Aspen/flower</td>
</tr>
<tr>
<td>CD-R Aspen/flower vs. or Aspen/triangle</td>
<td>Paper/triangle</td>
<td>Paper/flower</td>
</tr>
<tr>
<td>IDS Straw/diamond vs. or straw/cross</td>
<td>Cardboard/cross</td>
<td>Cardboard/diamond</td>
</tr>
<tr>
<td>IDS-R Cardboard/diamond vs. or cardboard/cross</td>
<td>Straw/cross</td>
<td>Straw/diamond</td>
</tr>
<tr>
<td>EDS Wax paper/star vs. or cotton/star</td>
<td>Cottar/circle</td>
<td>Wax paper/circle</td>
</tr>
</tbody>
</table>

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Inflammation impairs attention
digging medium, because the reward was buried deeply within the pot. Thus, rats could investigate the digging medium with paws or snout before executing a “dig” response, and these choices were not scored. All media contained a small amount of powdered Cheerios reward, to mask the scent of the hidden reward. The order of relevant dimensions for the discrimination problems, and the assignment of specific stimuli to particular problems were the same between rats, to minimize intersubject variability. We recorded the number of trials to criterion for each discrimination problem (minimum of six) and this was the dependent variable for statistical analysis.

To assess the inflammatory response, we measured TNFα and CCL2 in the plasma and frontal cortex of a separate group of identically treated, age-matched rats (N = 5 LPS treated rats per group at 2, 24, or 48 h after injection and five controls) by commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA). Both TNFα, a cytokine, and CCL2, a chemokine, are well-established mediators of inflammation and have been implicated in the pathogenesis of delirium (Rudolph et al., 2008; van Gool et al., 2010; Murray et al., 2012). Core blood was collected at the time of sacrifice and samples placed in EDTA-coated microcentrifuge tubes and centrifuged for 20 min at approximately 1000 × g within 30 min of collection. The plasma was then removed and stored at −20°C until used for measurement. Likewise, the brain was removed rapidly and frontal cortex frozen at −20°C in isopentane until assay. Assays were performed according to the manufacturer’s instructions, with the optical density of each sample determined using a microplate reader (SpectroMax2, Molecular Devices, Sunnyvale, CA, USA) at 450 nm with wavelength correction at 550.

Behavioral data were analyzed using a two-way ANOVA with treatment and trial number as the independent variables. ELISA data were analyzed with a one-way ANOVA comparing each time point to the control. Data are expressed as mean ± SEM and P < 0.05 was considered statistically significant.

RESULTS
As expected, there was a significant increase in plasma TNFα and CCL2 2 h after LPS (P < 0.001, Figures 1A,B), indicating a robust peripheral inflammatory response. This was transient, however, as plasma TNFα returned to baseline by the following day and CCL2 recovered within 48 h. Despite the changes in plasma, there was no increase in TNFα in the frontal cortex at any time (Figure 2A). In contrast, CCL2 was elevated nearly threefold in the frontal cortex 2 h after LPS but recovered to control levels within

![FIGURE 1](lipopolysaccharide.png) **FIGURE 1** Lipopolysaccharide (LPS) produces a robust but transient increase in plasma TNFα and CCL2. Plasma was sampled at the time of sacrifice from aged Fisher 344 rats 2, 24, and 48 h after administration of 50 µg/kg LPS (N = 5 per group) or a control group (N = 5) that received an equal volume of saline intraperitoneally. There was a marked increase in both TNFα (A) and CCL2 (B) 2 h after LPS but the effect resolved completely by 24 or 48 h, respectively. Data are mean ± SEM. ***P < 0.001 by one-way ANOVA.

![FIGURE 2](frontal_cortex.png) **FIGURE 2** Lipopolysaccharide (LPS) produces a robust but transient increase in TNFα and CCL2 in the frontal cortex. Rats (N = 5 per group) were treated as described in Figure 1 and frontal cortex harvested at the time of sacrifice. There was no change in TNFα in the frontal cortex at any time compared to control animals (A). CCL2 was elevated nearly threefold above control 2 h after LPS but this resolved by 24 h after treatment (B). Data are mean ± SEM. ***P < 0.001 by one-way ANOVA.
ANOV A revealed a main effect of LPS treatment, specifically for the IDS-R, indicating that an attentional set was formed, because although the previous correct stimulus is correct, a strategy that has also been used to model delirium (Trepa et al., 1992). As such, much like the WCST in humans, this task allows testing of the impact of cognitive inflexibility and impaired attention/executive function and shows the behavioral disability cannot be explained by lack of motivation, malaise due to "sickness behavior," or a global, non-specific impairment in CNS function. Moreover, while LPS induced a robust inflammatory response, the attentional deficit was present even after the systemic and frontal cortical proinflammatory changes resolved. As such, it appears that systemic inflammation triggers impairment of fronto-cortically-mediated aspects of cognition in aged rats but is not required to sustain it. To the extent that inattention is a defining characteristic of delirium, our results support a role for inflammation in the pathophysiology of this condition and suggest attentional set-shifting is a promising behavioral paradigm for investigating this relationship more closely (Inouye, 2006; Greene et al., 2009).

The AST challenges attention and executive function in several ways. Rats must adjust to changes in stimulus–reward contingencies in reversal problems, in which the previously correct stimulus is now incorrect, and vice versa, thus taxing behavioral flexibility. A different aspect of behavioral flexibility is tapped in the EDS, because the previously correct strategy for solving the discrimination problems (pay attention to digging medium, but not shape) must now be adjusted. These two kinds of behavioral shifts require the integrity of different regions of prefrontal cortex (Dias et al., 1996; Birrell and Brown, 2000; McAlonan and Brown, 2003). Moreover, both are sensitive to brain-wide blockade of muscarinic cholinergic receptors (Chen et al., 2004), a strategy that has also been used to model delirium (Trepa et al., 1992). As such, much like the WCST in humans, this task allows testing of the impact of an insult such as systemic inflammation or surgery on attention and executive function, as distinct from learning per se. In fact, our data show that deficits in attention and learning are dissociable during systemic inflammation. Consequently, the AST assesses performance on the EDS reflects a more difficult discrimination problem rather than a shift in attention. Note, however, that all rats had solved SDs with each dimension in the first phase of training. Although SD2 (shape) is more difficult than SD1 (medium) when performance is analyzed in the entire group of rats (SD1 mean 6.36, SD mean 10.73, t(10) = 2.52, P = 0.031), the EDS is more difficult than SD2 in both control (t(4) = 4.65, P = 0.010) and LPS (t(5) = 5.75, P = 0.002) groups. Thus, performance in the EDS reflects a cost of shifting attention between medium and shape, even though the two dimensions are not necessarily matched for difficulty.

DISCUSSION
To our knowledge, this is the first study to document that systemic inflammation impairs attention and executive function specifically. This impairment was selective to the extent that associative learning was not affected, as aged rats challenged with LPS were able to learn discrimination problems presented in the AST as well as those treated only with saline. However, when the task became more complex and demanded more cognitive flexibility and greater attention/executive function — reversing stimulus–reward associations or shifting the focus of attention to a different perceptual quality of the stimuli — the aged rats challenged with LPS were impaired. This is consistent with a phenotype of cognitive inflexibility and impaired attention/executive function and shows the behavioral disability cannot be explained by lack of motivation, malaise due to “sickness behavior,” or a global, non-specific impairment in CNS function. Moreover, while LPS induced a robust inflammatory response, the attentional deficit was present even after the systemic and frontal cortical proinflammatory changes resolved. As such, it appears that systemic inflammation triggers impairment of fronto-cortically-mediated aspects of cognition in aged rats but is not required to sustain it. To the extent that inattention is a defining characteristic of delirium, our results support a role for inflammation in the pathophysiology of this condition and suggest attentional set-shifting is a promising behavioral paradigm for investigating this relationship more closely (Inouye, 2006; Greene et al., 2009).

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DISCUS...
To the extent that the ability to solve discrimination problems that required a shift of attention between sensory dimensions or stimulus–reward contingencies (the reversals) was impaired but associative learning was intact, our data show that in aged rodents systemic inflammation selectively impairs attention and, by inference, fronto-parietal circuits without affecting hippocampal networks. This differential effect is interesting in light of recent evidence that crystallized cognition is preserved in human delirium whereas fluid processing is impaired (Brown et al., 2011). Third, in contrast to the concurrent normalization of the inflammatory and behavioral responses observed in most models using hippocampal-mediated tasks, the behavioral deficit on AST testing persisted after the inflammatory response resolved, suggesting that frontal–cortical and hippocampal circuits are differentially sensitive to neuroinflammation. Together, these data add strong support for the hypothesis that systemic inflammation causes a delirium-like phenotype in aged rodents.

Nonetheless, this study is limited in a few important respects. Impaired performance in the AST could be the result of inattention (moment to moment deficiencies in orienting to discrimination problems) and/or impaired executive function (higher-order deficits in changing behavioral strategies) so our results could reflect deficits in both cognitive domains. The task does, however, test the prefrontal cortex, which is a region implicated in the pathogenesis of delirium, and does not involve the hippocampus (Brooks et al., 2012). Delirium can occur in hospitalized young adults but it is rare; therefore, we did not include young adult rats in this study. Extrapolation of results in rodents to a behaviorally complex and heterogeneous clinical condition such as delirium, a pathophysiologically poorly characterized syndrome, must also be made cautiously. Delirium affects cognitive domains beyond attention and executive function, including memory, arousal, and affect, so a deficit in attention or executive function yields only an incomplete picture of the clinical syndrome. In addition, we examined just two representative proinflammatory mediators of the several that have been implicated in delirium pathogenesis (Rudolph et al., 2008; van Gool et al., 2010; Murray et al., 2012; Cunningham and MacLullich, 2013). As such, although TNFα and CCL2 returned to normal levels by the time of behavioral testing, we cannot exclude a role for ongoing neuroinflammation in the behavioral deficits we observed because abnormalities could be signaled by other mediators of inflammation. Finally, we did not assess cognitive recovery and therefore cannot exclude the possibility that the behavioral features we observed reflect the early stages of an inflammation-induced chronic neurodegenerative process, as opposed to a transient or fluctuating disruption of neural circuitry and function by inflammatory mediators.

In conclusion, our data show that LPS-induced systemic inflammation selectively impairs the ability of aged rodents to attend to and process environmentally salient stimuli and does so without global cognitive dysfunction or a persistent increase in TNFα or CCL2 expression in the frontal cortex. Inasmuch as inattention is a key diagnostic feature of clinical delirium, our data support a role for inflammation in the pathogenesis of this clinical syndrome and suggest this animal model could be useful for studying that relationship further.
ACKNOWLEDGMENTS

This study was supported by NIH R01 GM088817.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 January 2014; accepted: 15 May 2014; published online: 10 June 2014.


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