Central Blockade of IL-1 Does Not Impair Taste-LPS Associative Learning

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Key Words
IL-1 receptor antagonist · Lipopolysaccharide · Associative learning

Abstract
After saccharin intake is associated with the consequences of peripheral lipopolysaccharide (LPS) administration, rats develop a strong conditioned avoidance behavior against this gustatory stimulus. To investigate the role of central interleukin-1 (IL-1) as a key signal during taste-LPS engram formation, rats were chronically infused with IL-1 receptor antagonist into the lateral ventricle of the brain before, during and after a single association trial. The results indicate that a stable taste-LPS engram can be formed even under the chronic blockade of central IL-1 signaling during engram formation and consolidation. More importantly, our data show that animals which did not experience a fever response during association phase (due to the LPS encounter) were unable to elicit hyperthermia as part of the conditioned response. These data indicate that pairing a relevant taste stimulus with an immune challenge, such as LPS, might result in the formation of multiple engrams, specifically codifying independent information.

Introduction
It is well documented that the immune, neuroendocrine and central nervous systems are functionally interconnected, and extensively exchange information [1–3]. The activation of the innate immune system by infectious living microorganisms or its components, such as the Gram-negative bacteria lipopolysaccharide (LPS), results in the synthesis and release of pro-inflammatory cytokines, including interleukin-1β (IL-1β) [4]. Pro-inflammatory cytokines are responsible for the development of the peripheral inflammatory response, but also for many of the central components of the acute-phase responses, including fever, neuroendocrine activation and behavioral changes [5, 6].

Taste-immune associative learning is based on the naturalistic relation of the immuno-toxicological postprandial consequences of food consumption, which may induce neurobehavioral, endocrine and immune modifications after evoking specific memories of this experience [7, 8]. So far, ample data indicate that the trace memory of a relevant and novel taste, such as saccharin, can be effectively and strongly associated with the effects of peripheral LPS administration [9, 10].
such taste-LPS engram results in conditioned reduced consummatory feeding behavior, and more specifically, in changes in the palatability value of the conditioned stimulus [11–14]. In addition to these behavioral conditioned responses, it has been reported that evoking a taste-LPS engram results in conditioned activation of the hypothalamic-pituitary-adrenal axis, a reduction of splenic noradrenaline content, and IL-2 production [15], as well as changes in body temperature [9]. In contrast, the conditioned response does not activate cytokine gene expression in the spleen and hypothalamus of mice [16].

Peripheral and central cytokines, in particular pro-inflammatory cytokines (such as IL-1 and TNF-α), seem to play an important role during immune-to-brain communication [17–19]. On the other hand, several experimental data indicate a relevant role for such molecules during the initial formation and consolidation of hippocampal-dependent memories [20–23]. However, the role of central IL-1 on the taste-LPS engram formation has not been assessed yet. Thus, to further contribute to the understanding of the principles of taste-LPS associative learning, we performed experiments in order to test the role of central IL-1, by intracerebroventricularly (i.c.v.) infusing the IL-1 receptor antagonist (IL-1ra). Moreover, body temperature was constantly monitored during association and evocation phases via an intraperitoneal (i.p.) implanted temperature data logger.

**Methods**

**Animals**

Twenty-eight male Dark Agouti rats (Harlan Laboratories, Netherlands), weighing 220–250 g at the beginning of the experiment, were employed. The animals were individually housed on an inverted light-dark schedule (light on at 7:00) with food available ad libitum. Water was available ad libitum except during the water deprivation regimen. All animal procedures were carried out as approved by the ethics committee of the Medical Faculty, University of Duisburg-Essen, Germany.

**Experiment 1**

The aim of this experiment was to establish a single association/single evocation conditioning protocol, associating the taste of saccharin as the conditioned stimulus with an i.p. injection of LPS as the unconditioned stimulus. Animals were randomly divided into 3 groups and followed a water deprivation regimen (day –7 to day 5) consisting of a daily drinking session (8:30, 15 min). On the afternoon of day –5, an osmotic mini pump was subcutaneously (s.c.) implanted and connected to the intraventricular cannula in order to chronically deliver IL-1ra or vehicle (veh) into the brain.

**Fig. 1.** Behavioral conditioning protocol. **a** Experiment 1: animals were submitted to a water deprivation regimen starting on day –7, allowing fluid access for 15 min at 8:30. A single association trial took place on day 0 and a single evocation forced-choice test occurred on day 5. CS = Conditioned; CSo = conditioned not evoked; NC = not conditioned; LPS = 0.1 mg/kg S. abortus equi lipopolysaccharide i.p. in 0.5 ml sterile saline; Sal = 0.5 ml sterile saline i.p. **b** Experiment 2: on day –16, a cannula was implanted to reach the lateral ventricle of the brain and a temperature data logger was implanted i.p. Animals were submitted to a water deprivation regimen starting on day –9, allowing fluid access for 15 min at 8:30. On the afternoon of day –5, an osmotic mini pump was subcutaneously (s.c.) implanted and connected to the intraventricular cannula in order to chronically deliver IL-1ra or vehicle (veh) into the brain.
Fig. 2. Behavioral and thermoregulatory responses during taste-LPS conditioning. a Consummatory fluid intake during taste-LPS conditioning protocols. Data are presented from experiments 1 and 2 to compare behavioral responses. Since conditioned (CS) and conditioned not evoked (CSo) groups received the same treatment until day 4, fluid intake of these 2 groups is plotted together except on day 5 (evocation). In experiment 2, IL-1ra or vehicle (veh) was chronically infused into the lateral ventricle of the brain from experimental day –5 to day 2, covering the association phase. NC = Not contingent conditioned. b Body temperature during experimental day 0 (association) on which all animals were exposed to a single learning trial contingently pairing Sac (dashed arrow) with a 0.1-mg/kg LPS i.p. injection (continued arrow). All animals were chronically infused into the lateral ventricle of the brain with either IL-1ra (■ = CS + IL-1ra) or vehicle (□ = CS + veh) from experimental day –5 to day 2, completely covering the association phase. Data are expressed as means ± SEM. * p ≤ 0.05.
Ten animals were submitted to stereotaxic surgery (day –16) under deep anesthesia (70 mg/kg ketamine + 6 mg/kg xylazine i.p.) where an L-shape cannula (model 328OP/DW/S; Plastic One, Roanoke, Va., USA) was implanted to reach the left lateral ventricle of the brain. A nylon wire was placed on the cannula in order to keep it patent. Two stainless screws were used as anchors to help cannula fixation with dental cement (3M ESPE, Seefeld, Germany). A sterilized and biocompatible temperature data logger (5 g total weight; SubCue, Calgary, Canada) was implanted i.p. through a small abdominal incision. Muscular layer was closed first with reabsorbable suture and then stainless clips were employed to suture the skin. The head incision was closed using similar suture clips. Postoperative care included analgesics and antibiotics the following 3 days. Seven days after surgery animals started a water deprivation regimen similar to that described in experiment 1 (fig. 1b). On day –5, all animals were quickly but started a water deprivation regimen similar to that described in experiment 1 (fig. 1b). On day –5, all animals were quickly but deeply anesthetized (isoflurane; Abbott, Chicago, Ill., USA) and the head wound was reopened. The dummy wire from the cannula was removed and an osmotic mini pump (model 2001; Alzet, Cupertino, Calif., USA) was implanted to reach the left lateral ventricle, however, did not affect the taste-LPS engram formation, since the receptor antagonist-treated animals (CS + IL-1ra) also displayed a significant avoidance to the taste, comparable to the CS or CS + veh groups. In parallel to these behavioral effects we tested the biological activity of LPS employed by assessing cytokine plasma levels. A single 0.1-mg/kg i.p. LPS challenge did induce a strong IL-1 and TNF-α cytokine response peaking at 90 min after challenge (data not shown), confirming our previous report [24]. In addition, the biological activity of IL-1ra, as well as the correct function of the pump-catheter-cannula system was confirmed by the lack of a fever response in the IL-1ra-treated rats starting 5 h after i.p. LPS administration (unconditioned stimulus) compared to animals infused i.c.v. with vehicle (fig. 2b). Importantly, a modest but significant (p < 0.05) increase in temperature was elicited after the single evocation trial.
Central cytokines have also been involved in taste-immune association. It has been reported that the central infusion of IL-1 or its central induction by LPS or by high mobility group box 1 (HMGBI) can be associated with a given postprandial taste memory, resulting in a strong aversive behavior after reexposing the animals to the gustatory stimuli [33, 34]. In this regard, it has been well documented that i.c.v. and regional brain IL-1 microinjections can mimic most of the aspects of an acute-phase response elicited by peripheral LPS challenge [35–38]. In addition, blocking brain IL-1 signaling with i.c.v. administration of its antiserum or its receptor antagonist (IL-1ra) reduces or eliminates many facets of the acute-phase response to peripheral inflammatory stimuli, such as LPS [39, 40]. However, the present data clearly indicate that although central IL-1 is relevant and might contribute, it seems not to be the only important signal inducing a stable taste-immune engrm resulting in an aversive behavior when a peripheral LPS challenge is employed as unconditioned stimulus. In this regard, such endotoxin challenge induces several other cytokines apart from IL-1, including TNF-α and IL-6 [41, 42]. In addition, it should be indicated that peripheral cytokines may act synergistically, affecting neuroendocrine responses [43]. For instance, systemic IL-1β and TNF-α dose-dependently and synergistically disrupted consumption of a highly palatable food and increased plasma corticosterone levels [44].

Several experimental data have documented the ability of Pavlovian conditioning paradigms to modify thermoregulatory responses [10, 45, 46]. The conditioned hyperthermic response here reported was smaller, yet faster, compared to the LPS-induced fever response, which is also in agreement with previous reports [9, 10]. The present data further indicate that animals which did not show an LPS-induced fever response during the association phase did not elicit hyperthermia as part of the conditioned response. Our data suggest that the postprandial taste memory may be associated with particular peripheral immune input after LPS administration, forming multiple but specific engrams. In this regard, our data confirm the relevant role of central IL-1 signaling within fever response [40], but in addition indicate that central IL-1 is not necessary to form stable taste-immune engrams storing hedonic values. The blockade of central IL-1 signaling seems not to have mnemonic effects as reported earlier when hippocampal-dependent learning paradigms were employed [22, 23, 47]. However, further experiments should be performed in order to test the role of central IL-1 in regard

**Discussion**

The results of the present experiments confirm that postprandial taste memory can be associated with the effects of the peripheral injection of the bacterial endotoxin in LPS, even after a single pairing. The evocation of this association resulted in a strong conditioned avoidance behavior as well as in a mild conditioned increase in body temperature. Furthermore, the central infusion of IL-1ra did not block the taste-LPS association, indicating that central IL-1 is not the only relevant signal to induce such engrams. More importantly, our data show that animals that did not experience an LPS-induced fever response during association phase were unable to elicit a conditioned hyperthermia.

Food intake induces several postprandial responses, including immune and endocrine effects [3]. In this regard, the naturalistic relation of food ingestion with its possible immunotoxicological consequences results in food categorization as safe or dangerous, affecting long-term individual food selection [26]. In addition to behavioral responses, other neural, endocrine and immune modifications might be elicited after taste-immune associations are retrieved [7, 8]. The present data confirm previous reports indicating a significant reduction of the consummatory fluid intake behavior (in a single choice test), after a relevant gustatory clue has been contingently associated with the effects of peripheral LPS administration in rats [11, 13, 15]. Analogous behavioral conditioned responses have been elicited after pairing gustatory stimulation with peripherally administered specific cytokines [27–29]. However, not all cytokines seem to be able to induce a similar degree of association; TNF-α requires a much higher dose and more association trials than IL-1β [30], and pairing taste with IFN-α may not induce a stable association [31]. Moreover, the relevant role of the vagus nerve within the afferent cytokine-brain pathway has been well documented [32]. Specifically, subdiaphragmatic vagal transection both attenuates acquisition and facilitates extinction of conditioned taste aversions induced by i.p. administration of either IL-1β or TNF-α [30], indicating the importance of this afferent pathway within the taste-immune engram formation.

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of the strength and durability of taste-immune en-
grams.

In summary, we corroborate the associability of gusta-
tory stimuli with the effects of peripheral endotoxin ad-
ministration. More importantly, we demonstrate that central IL-1 seems not to be the only relevant input for the taste-LPS engram formation. In addition, it was docu-
mented that animals, although displaying conditioned avoidance behavior to saccharin, were not able to mount a conditioned hyperthermia when they did not experi-
ence a fever response at association time. These data in-
dicate that taste-LPS pairing may induce multiple spe-
cific engrams (that is, taste-avoidance/taste-hyperther-
mia), which may be subsequently recalled independently under appropriate settings.

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