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Divergent effects of brain interleukin-1ß in mediating fever, lethargy, anorexia and conditioned fear memory

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HIGHLIGHTS

- Brain IL-1ß plays a key role in mediating fever, anorexia and lethargy.
- Fear memory consolidation can occur concurrently with fever, anorexia and lethargy.
- Fear memory consolidation can occur concurrently with elevated brain IL-1ß.

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ABSTRACT

The influence of brain interleukin-1 (IL-1ß) on memory processes includes both detrimental and beneficial effects. To further explore the dynamics of brain IL-1ß in mediating learning and memory during acute sickness, we injected species-homologous rat IL-1ß (100 ng/5 μl) or vehicle (0.1% bovine serum albumin, 5 μl) directly into the cisterna magna (i.c.m.) of male Sprague-Dawley rats. We measured, in parallel, body temperature, food intake, body mass, cage activity, as well as learning and memory using contextual fear conditioning. To investigate the effects of IL-1ß on learning and memory processes we used: (1) a retrograde experiment that involved injecting rats i.c.m. with IL-1ß immediately after training in the novel context, and (2) an anterograde experiment that involved injecting rats i.c.m. with IL-1ß two hours before training in the novel context. In addition, hypothalamic and hippocampal concentrations of IL-1β were measured at several time points following injection. Administration of IL-1ß induced fever, lethargy and anorexia for ~two-to-three days and increased the concentration of IL-1ß in the hippocampus and hypothalamus for at least eight hours. Training in the context immediately before IL-1ß administration (retrograde experiment), did not impair contextual and auditory fear memory. However, when training in the context occurred concurrently with elevated hippocampal IL-1ß levels, two hours after IL-1ß administration (anterograde experiment), contextual, but not auditory, fear memory was impaired. Our results show that there are instances where memory consolidation can occur concurrently with elevated levels of IL-1ß in the hippocampus, fever, anorexia and lethargy during acute short-term sickness.

1. Introduction

A key role for the pro-inflammatory cytokine, interleukin (IL) IL-1ß, in mediating fever, anorexia and lethargy, has emerged from studies showing that blocking the endogenous action of brain IL-1ß following an immune challenge can attenuate each of these individual sickness responses [1–5], while exogenously administering species homologous rat IL-1ß directly into the brains of conscious rats in turn induces fever, anorexia and lethargy [6,7]. Not only has brain IL-1ß been shown to influence physiological processes involved in the regulation of body temperature, feeding and activity, but also those involved in learning and memory [8–10].

The influence of IL-1ß on memory processes, particularly in those regulated in the hippocampus, however appears to be complex with studies showing that the effect of brain IL-1ß

Abbreviations: IL-1ß, interleukin1beta; i.c.m., intra-cisterna magna; i.c.v., intracerebroventricular; c.s.f., cerebrospinal fluid; i.h., intra-hippocampalintra-hippocampal.

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on hippocampal-dependent memory performance, as measured using contextual fear conditioning, appears to follow an inverted U-shaped pattern whereby intracerebroventricular (i.c.v.) administration of 1 ng IL-1ß improves memory, while a ten-fold greater dose (10 ng), or blocking the physiological action of IL-1ß impairs memory [8]. Interestingly we and others have previously shown that i.c.v. administration of the dose of IL-1ß (1 ng) shown to improve memory processes, can however consistently induce fever, anorexia and lethargy [6,11–13]. Together, these findings tend to suggest that, during acute illness there may be a divergence in the effect of brain IL-1ß on memory processes and the processes regulating fever, anorexia and lethargy. Although some studies have been published which lend support to the idea that learning and memory can occur in the presence of other sickness responses, induced by IL-1ß administration [14,15], to date no study has measured brain IL-1ß levels, body temperature, food intake and activity with learning and memory, following central administration of IL-1ß, to concurrently investigate the effect of brain IL-1ß on each individual response.

Thus, we have injected species homologous rat IL-1ß directly into the cisterna magna of rats and measured body temperature, food intake, body mass, cage activity as well as learning and memory, concurrently with brain levels of IL-1ß in the hippocampus and hypothalamus. To investigate the effects of IL-1ß on learning and memory processes we used a retrograde and an anterograde experiment of contextual and auditory fear conditioning. The retrograde experiment involved injecting rats i.c.m. with IL-1ß immediately after training in the context chamber, and the anterograde experiment involved injecting rats i.c.m. with IL-1ß two hours before training in the context chamber. Our results show that, during acute short-term sickness, there are instances where memory consolidation can occur concurrently with elevated levels of IL-1ß in the hippocampus, fever, anorexia and lethargy.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (initial body mass 80–100 g) were obtained from the National Health Laboratory Service (Johannesburg, South Africa) and housed individually in cages at an ambient temperature of 22 ± 1 °C, on a 12-h:12-h light-dark cycle (lights on from 03h00 local time for experiment one and 24h00 local time for experiment two). To allow for interventions to occur during normal daily working hours the light:dark cycle of the rats was reversed over a period of three weeks before the rats were used in experiments one and two (described below). That the rats' circadian rhythm had been shifted to the new light:dark cycle was confirmed before the start of the experimental interventions by evidence of a normal nychthemeral temperature rhythm for each rat. Food (pelleted rat chow, Epol, Johannesburg, South Africa) and water were provided *ad libitum*. All procedures were in accordance with the Animal Ethics and Control Committee of the University of the Witwatersrand animal care regulations and were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (ethics number AESC2013/28/04).

2.2. Body temperature and cage activity

As previously described [16] core body temperature and cage activity of rats were measured using sterile, wax-coated temperature-sensitive radiotransmitters (TA10TA, Data Sciences, St. Paul, MN, USA) which had been implanted intra-abdominally under general anesthesia induced by an intramuscular injection of a combination of ketamine hydrochloride (100 mg/kg, Anaket-V, Bayer, South Africa) and xylazine (5 mg/kg, Chanazine, Bayer, South Africa). All rats weighed between 150 and 200 g at the time of surgery. Before surgery rats received an intramuscular injection of an analgesic (Meloxicam 10 mg/ml; Ingelheim Pharmaceuticals, South Africa). After surgery each rat received a single injection of atipamezole hydrochloride (100 mg/kg, Pfizer, South Africa) to promote recovery from anaesthesia and a second intramuscular injection of an analgesic (Meloxicam 10 mg/ml; Ingelheim Pharmaceuticals, South Africa). The radiotransmitters were calibrated in a water bath at 35 °C and 39 °C by the manufacturer (Data Sciences, St. Paul, MN, USA) to an accuracy of 0.01 °C. After surgery for implantation of the radiotransmitters, all animals were returned to their cages and allowed a week to recover before receiving any intervention.

2.3. Food intake and body mass

Food intake and body mass were measured daily approximately three hours before the beginning of the dark cycle using an electronic scale (Diamond, US) accurate to one gram. Food intake was quantified by subtracting the food remaining in the food container and on the cage floor from the amount of food measured at the preceding time point.

2.4. Rat recombinant IL-1ß administration

Rat recombinant IL-1β (501-RL-010, R&D Systems, Minneapolis, MN, USA) was reconstituted in a vehicle of pyrogen-free saline containing 0.1% bovine serum albumin (BSA, fatty acid free, low endotoxin, Roche, Germany) and injected via the cisterna magna at a dose of 100 ng in a volume of 5 μl. The IL-1β dose was chosen based on a previous study in which i.c.v. administration of 100 ng IL-1ß induced fever, lethargy and anorexia in rats [6]. The endotoxin content of the recombinant IL-1ß was <0.01 EU per 1 μg of the protein as measured in a Limulus Amoebocyte Lysate test. Before i.c.m. injections rats were placed into a gas chamber and anaesthetized with gaseous isoflurane administered in conjunction with oxygen (initial concentration 5%, maintenance concentration 2%; Safe Line Pharmaceuticals, South Africa). Once anesthetized the dorsal aspect of the rats' skull was shaved and cleaned with 70% ethanol. A 26-gauge needle (Kendon Medical Supplies, South Africa), which was connected to a 50 μl Hamilton gastight microliter syringe (Hamilton, Switzerland) by polyethylene tubing (0.38 mm inner diameter, 1.09 mm outer diameter), was inserted into the cistern magna. To confirm the correct positioning of the needle in the cistern magna, 3 μl of cerebrospinal fluid was drawn up to verify fluid clarity (i.e., no blood traces). After verification of entry into the cistern magna 5 μl of the IL-1β or vehicle was administered and the needle was left in the cisterna magna for 30 s to ensure total dispersal of the substance.

2.5. Contextual and auditory fear conditioning

As previously described [17] we used a modified version of contextual and auditory fear conditioning to assess learning and memory in rats. When a rat is placed into a chamber and receives an electrical shock to its feet that is preceded by an auditory cue (tone), it will later display a natural defensive response termed freezing (immobility, shallow breathing, increased heart rate and pilo-erection) when it is re-exposed to either the original conditioning chamber in which the shock occurred (contextual fear conditioning) or to the tone that preceded it (auditory cue fear conditioning) [18,19]. The auditory cue fear conditioning is commonly tested in a different context so that the freezing can be attributed to conditioning to the cue (tone) and not to the context in which the shock occurred. Contextual fear conditioning appears to depend