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Research report

Lipopolysaccharide causes deficits in spatial learning in the watermaze but not in BDNF expression in the rat dentate gyrus

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Abstract

We investigated the effects of a single injection and a daily injection of lipopolysaccharide (LPS) on spatial learning and brain-derived neurotrophic factor (BDNF) expression in the rat dentate gyrus. LPS is derived from the cell wall of Gram-negative bacteria and is a potent endotoxin that causes the release of cytokines such as interleukin-1 and tumour necrosis factor. LPS is thought to activate both the neuroimmune and neuroendocrine systems; it also blocks long-term potentiation in the hippocampus. Here, we examined the effects of LPS on a form of hippocampal-dependent learning–spatial learning in the water maze. Rats were injected with LPS intraperitoneally (100 μg/kg) and trained in the water maze. The first group of rats were injected on day 1 of training, 4 h prior to learning the water maze task. Groups 2 and 3 were injected daily, again 4 h prior to the water-maze task; group 2 with LPS and group 3 with saline. A number of behavioural variables were recorded by a computerised tracking system for each trial. The behavioural results showed a single injection of LPS (group 1) impaired escape latency in both the acquisition and retention phases of the study, whereas a daily injection of LPS did not significantly impair acquisition or retention. BDNF expression was analysed in the dentate gyrus of all animals. No significant differences in BDNF expression were found between the three groups.

Keywords: Lipopolysaccharide; BDNF; Learning; Memory; Watermaze; Dentate gyrus; LPS tolerance

1. Introduction

Lipopolysaccharide (LPS), a potent bacterial endotoxin, produces neural and endocrine responses that are similar to responses produced by behavioural stress [32]. LPS produces a wide range of non-specific behavioural effects collectively termed 'sickness behaviours' [22]. These behaviours include a reduction in activity, a reduction in exploration, decreased social interaction, fever, a reduction in consumption of food and drink, hypersomnia, activation of the hypothalamic–pituitary–adrenal axis and increased sympathetic activation [17,21,33]. The exact mechanism of action by LPS in the central nervous system (CNS) remains unknown; it is thought to act primarily through the release of cytokines [9,34]. Interleukin-1 (IL-1) and tumour necrosis factor, for example, are thought to play an important role in sustaining the 'sickness behaviours' induced by LPS. It has been proposed that cytokines activate a chain of immune and endocrine responses [10], including an increased release of corticosterone. A direct action by LPS within the CNS is an important possibility [12]: it is possible that LPS can access the brain directly via peripheral nerve transduction [16], the circumventricular organs [1], area postrema [13] or even at the level of the hypothalamus [40,41].

The hippocampal formation plays a critical role in certain types of learning and memory [37]. It is particularly sensitive to stress; cumulative exposure to high levels of glucocorticoids has negative functional and structural effects on the hippocampus [20,26,27]. Recent human studies have shown that people with chronically high cortisol levels show a reduction in

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hippocampal volume and also deficits in hippocampal-dependent memory tasks [24]. This result mirrors previous work in rodents that reports stressed rats show cognitive deficits on hippocampal-dependent tasks such as the water maze [7].

Recent evidence suggests that LPS may disrupt the consolidation of certain memory processes: acute administration of LPS prior to training impairs contextual-cue fear conditioning, a hippocampal-dependent learning paradigm [33]; while a chronic infusion of LPS has been found to impair spatial memory [18,19]. Furthermore, administration of IL-1β impairs performance of rats in the Morris water maze, another hippocampal-dependent learning task [32]. Long-term potentiation (LTP) of synaptic transmission is a popular model of the biological processes that may underlie memory [2,3]. LTP is readily induced in the hippocampus, and studies have shown that blocking LTP causes impairment on the water-maze task [30]. LPS has also been shown to inhibit LTP in the rat dentate gyrus in vitro [6] and we have shown recently that LPS also blocks LTP in the subiculum in vivo [5]. The induction of LTP is also inhibited by behavioural stress [8], and induction of long-term depression in the hippocampus is facilitated by exposure to mild stress [39]. Paired-pulse facilitation in the hippocampal area CA1–subiculum projection is also markedly reduced after exposure to behavioural stress [4].

Brain-derived neurotrophic factor (BDNF) plays a critical role in the survival and growth of neurons. High levels of this neurotrophin are found in the hippocampus and have been implicated in hippocampal-dependent learning. Recent studies have found that BDNF also regulates synaptic plasticity; more specifically, the exogenous application of BDNF has been found both to increase synaptic transmission in hippocampal neurons and to enhance LTP [23]. BDNF expression is increased in the hippocampus during spatial learning tasks, lending further support for its role in learning and memory [15,29]. The expression of BDNF in the hippocampus is susceptible to behavioural stress; BDNF is reduced in response to both acute and repeated stress [27,35,36]. Little research has been performed on the effects of LPS on BDNF expression, and the existing research is conflicting. Elkabes et al. [11] found BDNF expression was unaltered in the rat brain in the presence of LPS, whereas Miwa et al. [28] found that LPS stimulated the synthesis of BDNF. The experiments presented in this article examine the effects of LPS on hippocampal-dependent spatial learning and on BNDF expression in the dentate gyrus of the hippocampal formation. We hypothesise that LPS would impair spatial learning in the water maze; specifically, animals repeatedly injected with LPS would perform worse than the single-injected animals. Furthermore, we expect BDNF levels to correlate with learning, such that poor performance in learning the water-maze task would result in diminished BDNF levels.

2. Materials and methods

2.1. Animals

Eighteen male Wistar rats (mean weight, 250 g) were used in this experiment. Rats were pair-housed and maintained on a 12 h:12 h light/dark cycle with free access to food and water.

2.2. Behavioural equipment and procedures

2.2.1. The water maze

The water maze was a black circular pool (2 m diameter, 35 cm deep; water, 20 ± 1°C) filled to 31 cm. Rats could escape the water by climbing on to a hidden platform (29 cm × 9 cm). The hidden platform was placed in the North Western quadrant of the pool and submerged 2 cm below the water surface so it was invisible at water level; the location of the platform was fixed during the experiment. Distal cues were standard room objects (e.g. doors, shelving, and curtains). A computerised digital tracking system recorded escape latencies and swim paths during each trial (EthoVision; Noldus Information Technology, Wageningen, The Netherlands).

2.2.2. Water-maze training

Rats were trained on the water maze for 8 days using standard procedures: 5 days learning (acquisition phase) with 3 days retention testing after a 3-day rest period. The trained rats received five trials per day for 5 days, where they had to search for the hidden platform. The rat was allowed 60 s to find the platform; otherwise, the rat was led to the platform by the researcher. The rat remained on the platform for 15 s. The inter-trial interval was 5 s. All rats entered the maze at a fixed start position. On the last day of the retention task, a probe trial was conducted. During this trial, the platform was removed and the rats swam freely for 60 s in the pool; persistent swimming in the platform quadrant is commonly interpreted as an indication of spatial learning.

2.2.2.1. LPS protocol. LPS (100 μg/kg was obtained from *Escherichia coli* (L-2630, 100 mg: Sigma Chemical Co., St. Louis, MO, USA). The rats were injected intraperitoneally with either 100 μg/kg LPS or saline (0.9% NaCl).

The rats were randomly assigned to one of three groups ($n = 6$ per group).

Single LPS: LPS (intraperitoneally (i.p.), 100 μg/kg) injected once on the first day of the acquisition phase 4 h before training.
Daily LPS: LPS (i.p., 100 μg/kg) injected 4 h before each daily test of the training phase (5 days).
Saline control: Saline (i.p., 0.9%) injected 4 h before each daily test of the training phase (5 days).

2.2.2.2. BDNF enzyme-linked immunosorbent assay protocol (BDNF Emax™ ImmunoAssay System; Promega UK Ltd). All rats were sacrificed on the last day of the retention phase; their brains were removed and tissue was taken from the dentate gyrus. Slices of dentate gyrus were preincubated in 250 μl Krebs solution containing 2 mM $CaCl_2$ for 3 min, and the supernatant was removed and discarded. This step was repeated in a volume of 100 μl, but at the end of the 3 min incubation samples were centrifuged at $1000 \times g$ for 3 min and the supernatant was retained. This step was repeated in the presence of 40 mM KCl to depolarise the slices and supernatant was retained. Both samples of supernatant were stored at −80°C for later analysis of BDNF by two-site immunoassay.

BDNF concentration was determined in the supernatant. To prepare the samples, dentate gyri were homogenised in ice-cold Krebs solution 25 times. Samples were centrifuged and the supernatant retained. Protein was assessed and samples were diluted to give equal protein concentrations and stored at −80°C. Ninety-six-well plates were coated with 100 μl anti-BDNF monoclonal antibody diluted (1:1000) in 0.025 M carbonate–bicarbonate buffer. Plates were covered, incubated overnight at 4°C and plates were subjected to interceding washes to remove excess antibody. Plates were blocked for non-specific binding for 1 h at room temperature and washed (composition of wash buffer (mM): Tris–HCl, 20; NaCl, 150 containing 0.05% Tween (v/v); pH 7.6). Samples of dentate gyrus (50 μl), supernatant (50 μl) or BDNF standards (50 μl; ranging from 0.0078 to 1 ng/ml) were added to the wells, which were covered, incubated for 2 h at room temperature with shaking and incubation overnight at 4°C, and washed. Aliquots (100 μl) of anti-human BDNF pAb (diluted 1:500) were added to the wells, plates were incubated for 2 h at 37°C and washed. Aliquots (100 μl) of anti-immunoglobulin Y horseradish peroxidase (1:2000 dilution) were added to wells and incubated for 1 h at 37°C. During this incubation, the enzyme substrate was prepared. Plates were washed and 100 μl of this substrate was added to the wells, and incubated for approximately 15 min until a blue colour formed in the wells. The reaction was stopped by the addition of 100 μl of 1 M phosphoric acid to the wells. Plates were read a 450 nm in a 96-well plate reader, and BDNF concentrations were estimated for the standard curve (expressed as ng/mg protein).

2.2.2.3. Statistics. The behavioural data and BDNF enzyme-linked immunosorbent assay values were analysed using a one-way analysis of variance (ANOVA); a value of $P < 0.05$ was considered to be significant. Post-hoc comparisons were made using Tukey's method.

3. Results

3.1. Water-maze training and retention analysis

3.1.1. Direct swim analysis

A direct swim to the hidden platform was defined as a swim path that remained inside a corridor 30 cm wide from the start position to the platform. Fig. 1a demonstrates that there was a significant difference between groups on days 5 and 6 (day 5, ANOVA $F(2, 82) = 6.325$, $P < 0.01$; day 6, ANOVA $F(2, 28) = 3.421$, $P < 0.05$). Post-hoc tests showed the single LPS group performed worse than the other two groups on day 5, and on day 6 the single LPS group performed worse than just the saline controls ($P < 0.05$). Fig. 1b shows representative swim paths taken from each group on days 5 and 8.

3.1.2. Overall escape latency analysis

Fig. 2a demonstrates that escape latencies for all groups decreased during the training period, with the saline control group finding the platform faster than the LPS groups. Overall significant differences were found on days 4 and 8 (day 4, ANOVA $F(2, 82) = 7.53$, $P < 0.001$; day 8, ANOVA $F(2, 82) = 5.648$, $P < 0.01$). Post-hoc tests revealed the single LPS group was slower than both the daily LPS group and the saline controls on day 4 of the training task and day 8 of the retention period ($P < 0.05$).

3.1.3. Cumulative distance analysis

The cumulative mean distance per day for each group was calculated. Fig. 2b shows that the daily LPS group swam the shortest distance to reach the platform while the rats in the single LPS group swam the furthest. Overall significant differences were found on days 1 and 5 (day 1, ANOVA $F(2, 82) = 8.507$, $P < 0.001$; day 5, ANOVA $F(2, 420) = 3.811$, $P < 0.05$). Post-hoc tests revealed the daily LPS group swam a significantly shorter distance than the saline control group on day 1 and the single LPS group on day 5 of training ($P < 0.05$). Fig. 2b also shows that the rats in the single LPS group swam further to reach the platform during the retention period. There was an overall significant difference between the groups on days 6, 7 and 8 (day 6, ANOVA $F(2, 505) = 4.649$, $P < 0.01$; day 7, ANOVA $F(2, 590) = 5.244$, $P < 0.001$; day 8, ANOVA $F(2, 671) = 6.895$, $P < 0.001$). Post-hoc tests revealed

that, on days 6, 7, and 8, the rats in the single LPS group swam further than the daily LPS-injected group.

3.1.4. Velocity analysis

The mean speed per day for each group was calculated. Fig. 2c shows that the daily LPS group swam the slowest during the training period while the single LPS group swam the fastest. On days 1, 2, 3 and 5, there was a significant difference between all groups (day 1, ANOVA $F(2, 82) = 13.25$, $P < 0.01$; day 2, ANOVA $F(2, 82) = 5.883$, $P < 0.01$; day 3, ANOVA $F(2, 82) = 5.284$, $P < 0.01$; day 5, ANOVA $F(2, 82) = 6.832$, $P < 0.01$). Post-hoc tests showed that there were significant differences between all groups on day 1. The daily LPS group swam the fastest and the single LPS group swam the slowest ($P < 0.05$). On days 2, 3, and 5, the single LPS group swam significantly faster than the daily LPS group. There was an overall significant difference between groups on days 6 and 7 during the retention period (day 6, ANOVA $F(2, 82) = 3.186$, $P < 0.05$; day 7, ANOVA $F(2, 82) = 4.13$, $P < 0.05$). Post-hoc tests confirmed that the single LPS group swam faster than the other two groups ($P < 0.05$).

3.2. Water-maze probe trial analysis

3.2.1. Velocity analysis

Fig. 3a shows that the groups swam at significantly different speeds during the probe trial (ANOVA $F(2, 14) = 4.059$, $P < 0.05$). The post-hoc test revealed that the daily LPS group swam slower than the single LPS group ($P < 0.05$). This finding is consistent with the training and retention phases (see earlier).

3.2.2. Distance analysis

An overall difference in total distance swam during the probe trial was found (ANOVA $F(2, 14) = 3.929$, $P < 0.05$). The post-hoc test revealed that the daily LPS group swam the shortest distance than the single LPS group (see Fig. 3b). This finding is also consistent with the training and retention phases (see earlier).

3.2.3. BDNF expression

BDNF concentration was determined in the dentate gyrus from all animals in each group. No significant differences were found between groups (ANOVA $F(2, 15) = 0.479$, $P > 0.05$; see Fig. 4).

4. Discussion

We investigated whether the administration of LPS affected spatial learning in the water maze and also whether LPS affected the expression of BDNF in the rat dentate gyrus. A learning deficit was apparent after a single injection of LPS compared with a daily LPS injection or saline-injected controls. The single LPS group both swam faster and further, and spent the least amount of time on direct route to the platform compared with the daily LPS group and saline control group. We also found that there were no differences in BDNF concentration between the different treated groups and controls. Here, we reveal that animals that received a daily injection of LPS swam the slowest and also swam the shortest distance during both the acquisition and retention phases. This may suggest a manifestation of some of these behavioural sickness symptoms,

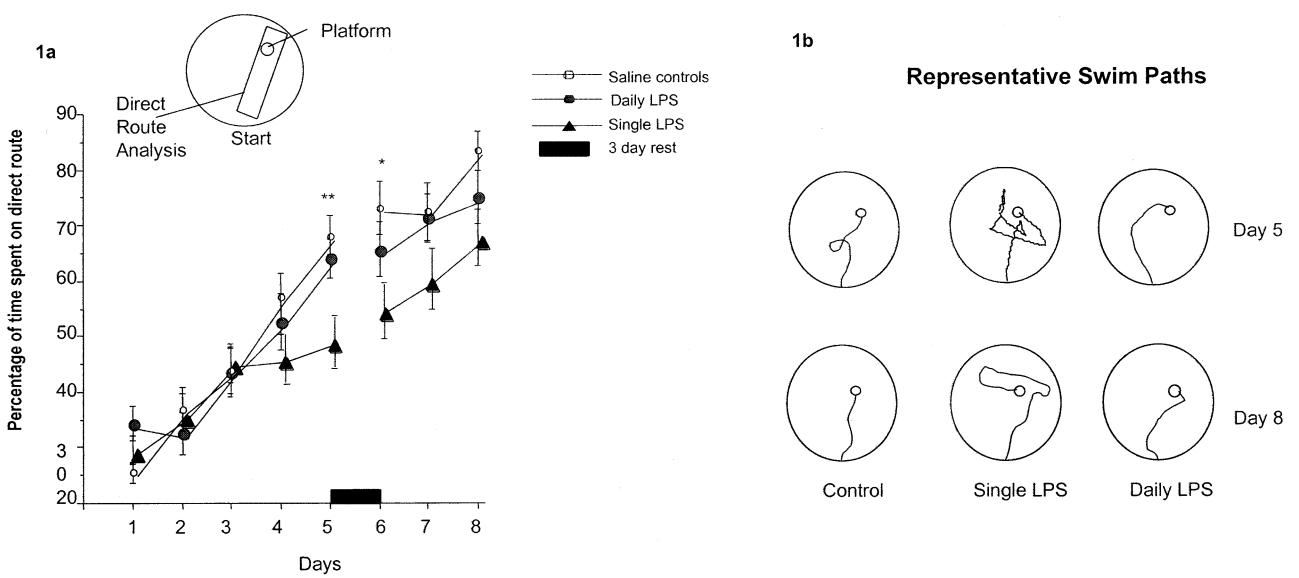

Fig. 1. (a) Percentage time spent in direct route to platform (see inset) for all groups across the acquisition and retention periods. (b) Representative swim paths taken from the final day of acquisition (day 5) and the final day of retention (day 8) for each group ($n = 6$ per group; * $P < 0.05$, * $P < 0.01$).

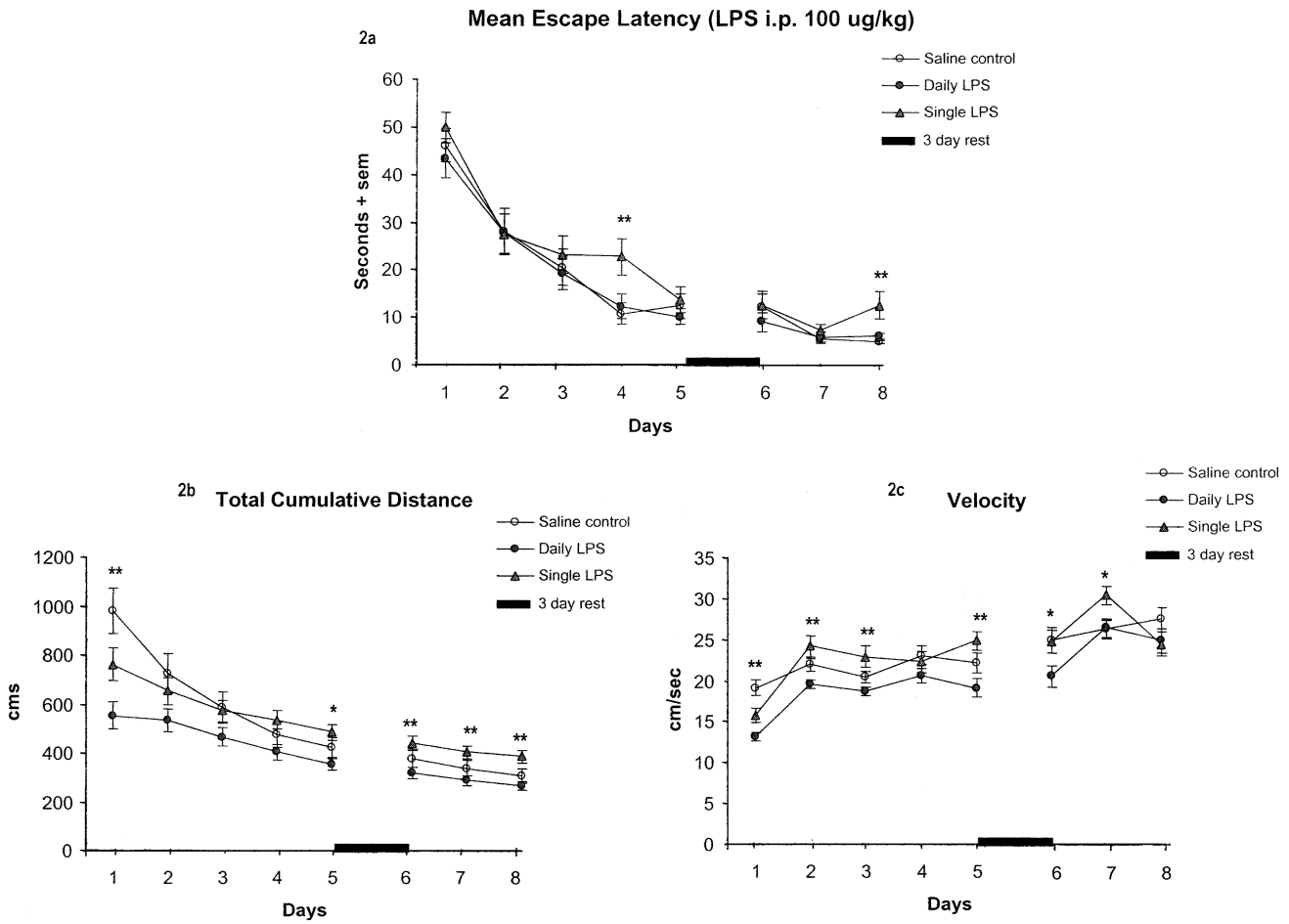

Fig. 2. (a) Mean escape latencies (seconds ± S.E.M.) for the three groups across the acquisition and retention periods. (b) Total distance swam within 60 s allowed for each group across the acquisition and retention periods. (c) Total velocity of each group within 60 s allowed across the acquisition and retention period ($n = 6$ per group; * $P < 0.05$, ** $P < 0.01$).

i.e. a reduction in activity and a reduction in exploratory behaviour. These symptoms were supported by the probe test. The daily LPS group swam significantly shorter distances and was slower than the single LPS group within the 60 s allowed per trial. Although animals with a single injection of LPS swam the fastest, they also swam the furthest indicating an inability to locate the platform and thus revealing a subtle spatial learning deficit. This group also showed impairments in the overall escape latency measure and in their direct swim paths, which revealed that the single LPS group spent significantly less time in the direct route area than the controls and the daily LPS group. We have confirmed a further possible relationship between synaptic plasticity and learning, consistent with the hypothesis outlined by Martin et al. [25], which states that synaptic plasticity underlies the biological consolidation of memories. We have also demonstrated the important regulatory impact of the neuroimmune system on cognitive processing.

Recent research has indicated that cognitive processing may be disrupted following either endotoxin or cytokine administration. *Legionella pneumophila* (an endotoxin) and IL-1β impair water-maze performance in rodents [14,32]. Furthermore, the disruption of learning by endotoxins appears to be dose dependent; Pugh et al. [33] demonstrated that 0.125 and 0.25 mg/kg LPS interfered with contextual fear conditioning, while 0.5 mg/kg had no effect. Similarly, LPS differentially affects the hypothalamic–pituitary axis in that a higher dose of LPS stimulates the sustained release of corticosterone [31,33]. This appears to be only true for acute exposure to LPS; repeated injections of LPS have no such effect on corticosterone levels [38]. Repeated LPS exposure also resulted in lower Il-1β activation; this may be due to an alteration in peripheral sympathetic responsiveness [41]. It is very possible that repeated exposure to LPS pro-

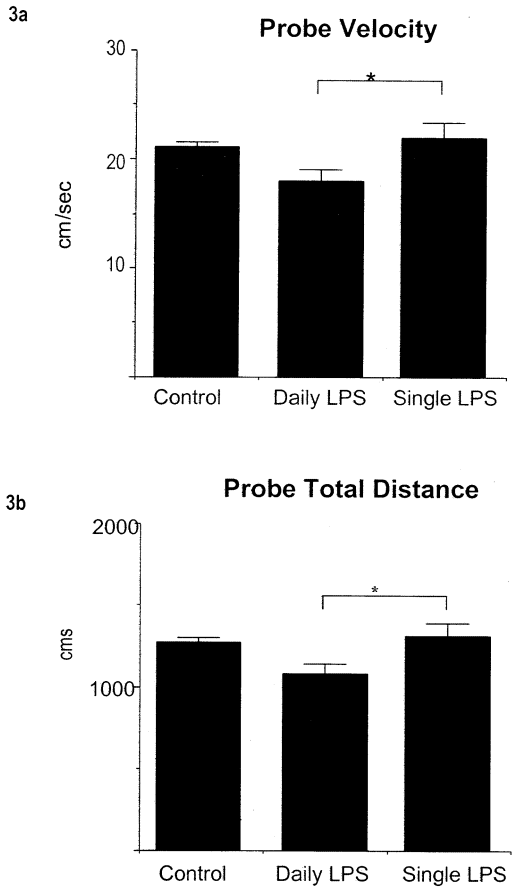

Fig. 3. (a) Total velocity of each group during the probe test. (b) Total distance swam during the probe test for each group ($n = 6$ per group; * $P < 0.05$, ** $P < 0.01$).

duces a tolerance effect, as suggested by the lower Il-1β activation, which might account for the behavioural changes we have observed here. The notion

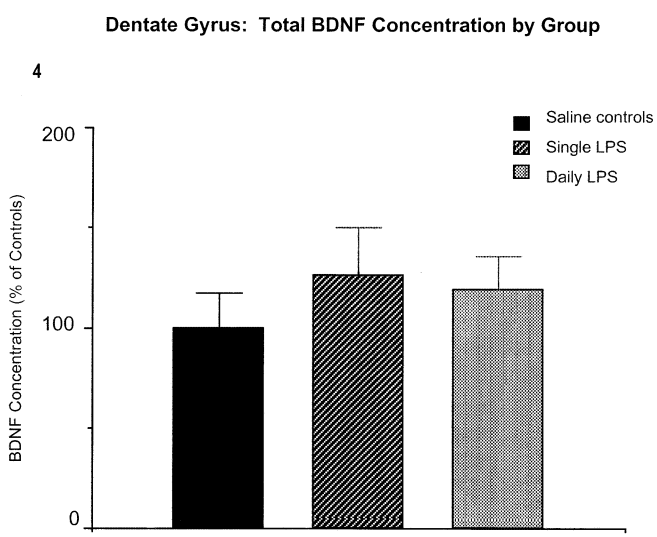

Fig. 4. Mean concentration of BDNF in dentate gyrus for each group ($n = 6$ per group; * $P < 0.05$; ** $P < 0.01$).

of a differential effect from a single and repeated challenge to the neuroimmune system needs further extensive investigation.

No significant differences of BDNF concentration in the dentate gyrus were found between groups. Previous research on the effects of LPS on BDNF expression is controversial. Elkabes et al. [11] found BDNF expression to be unaltered in the rat brain in the presence of LPS, whereas Miwa et al. [28] found that LPS stimulates the synthesis of BDNF. Miwa et al, [28] looked at the expression of BDNF in microglia cultured from embryonic rat brains that were exposed to LPS. They concluded that the LPS-activated microglia participate in neuronal regeneration via production of neurotrophins; thus, this increase in BDNF seems to be neuroprotective in the embryonic brain. We administered a single and daily dose of LPS, and then examined its effect on BDNF concentration in the dentate gyrus on the last day of the water-maze task (day 10). We expected to find a difference between groups because BDNF is implicated in learning and memory, but no difference in BDNF concentration was apparent. A possible explanation is that our dose was too small to cause any neuronal damage that would result in the activation of BDNF synthesis. An alternative reason for this may be that by the time the tissue samples were taken, the LPS was completely metabolised. If BDNF is neuroprotective and if there were no circulating levels of LPS in the animals, then an elevation of BDNF would not be expected. Similarly, if repeated LPS exposure produces a tolerance effect, then normal levels of BDNF would be expected. It would be of interest in future experiments to determine the half-life of LPS after acute administration and to investigate the interaction between different doses of LPS and learning, and on BDNF concentration.

In summary, we have shown that a single injection of LPS impairs learning while a daily injection does not. We have also shown that LPS (either a single or daily dose) does not affect on BDNF concentration in the dentate gyrus. Our findings implicate an important regulatory impact of the neuroimmune system on cognitive processing, synaptic plasticity and BDNF concentration in the hippocampal formation.

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