Published in final edited form as:

J Neuroendocrinol. 2010 April ; 22(4): 272–281. doi:10.1111/j.1365-2826.2010.01967.x.

Neonatal Programming by Neuroimmune Challenge: Effects on Responses and Tolerance to Septic Doses of Lipopolysaccharide in Adult Male and Female Rats

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Abstract

A mild immune challenge experienced during the neonatal period leads to attenuated febrile responses to a similar challenge experienced later in life. However, the immune response to an endotoxin differs depending upon the severity of the challenge and it is not clear whether a neonatal immune challenge will also affect responses to a severe, potentially life-threatening stimulus, such as sepsis. In the present study, we examined the effects of a neonatal immune challenge with lipopolysaccharide (LPS) on adult sickness responses, as well as the development of endotoxin tolerance, to a septic dose (1 or 3 mg/kg) of the same LPS in male and female rats. We demonstrate significant differences, particularly in males, in the fever profiles of neonatally LPS-treated rats compared to neonatally saline-treated controls. Specifically, male rats treated neonatally with LPS have reduced hypothermic and enhanced hyperthermic responses to both septic doses of LPS in adulthood. A somewhat different profile is seen in females, with neonatally LPS-treated females having reduced hypothermia and enhanced hyperthermia compared to controls with 1 mg/kg but no differences with 3 mg/kg LPS. The results obtained demonstrate that alterations in innate immune responses previously reported for low doses of LPS can, for the most part, also be observed after severe immune challenge in later life.

Keywords

HPA axis; lipopolysaccharide; sickness behaviour; temperature; thermoregulation

An intact febrile response is a crucial part of the host's defence against infection and is integral to survival. Fever has an important role in potentiating immunological responses (1–4), as well as altering the optimal temperature for pathogen growth and therefore slowing proliferation (4). As such, preventing the development of a febrile response after infection can lead to a reduced likelihood of survival (4, 5). Despite this adaptive value, however, an overactive or dysregulated febrile response, such as can occur with cerebral malaria or septic shock, can be lethal.

An early life immune challenge is known to have pronounced long-term effects on an animal's ability to mount a febrile response to an immune challenge (6–11). In particular, we have shown that a single i.p. injection of bacterial endotoxin, lipopolysaccharide (LPS), at postnatal day (PN) 14, programmes an attenuated febrile response to a further, low dose (50 $\mu\text{g/kg}$), immune challenge of the same type in both adult male and female Sprague–Dawley rats (6–8). This programming is associated with concomitant attenuation of the increases in hypothalamic cyclo-oxygenase-2 and circulating pro-inflammatory cytokine levels (12), as well as an enhancement of the corticosterone response, indicating that there are substantial alterations to this immune response.

The immune response to an endotoxin challenge differs, however, depending upon the severity of the challenge. In particular, in response to a high dose of endotoxin, a fever profile is observed that is bi- or poly-phasic, with an initial hypothermic component that is followed either by fever and recovery or continued hypothermia and death (13, 14). The degree and duration of this hypothermic phase is a good indicator of the severity of the challenge and the likelihood of mortality (15, 16). Sepsis is a severe systemic inflammatory state caused by infection or inflammatory agents that typically includes an hypothermic and a febrile component (14, 15). Sepsis can be considered to be life-threatening in humans and rats (17, 18). It is also seriously detrimental to the animal in the sense that it results in pronounced sickness behaviours, potentially reducing predator avoidance as well as food-seeking and mating opportunities (19). As such, we were interested in investigating whether a neonatal LPS challenge would also alter the temperature profile, one reflection of the response to a septic dose of LPS, particularly in the hypothermic phase. We therefore assessed whether neonatally LPS-treated rats (nLPS) would, as adults, have different thermoregulatory profiles to their neonatally saline-treated (nSal) counterparts to a sepsis-producing dose of LPS. The doses of LPS that were used in the present study (1 and 3 mg /kg i.p.) are below those necessary to cause significant mortality, although still sufficiently severe to produce periods of hypothermia as well as fever.

Endotoxin tolerance is the phenomenon whereby prior exposure to endotoxin causes the immune system to become transiently refractory or less responsive to a subsequent endotoxin challenge (20). As such, it acts as a protective mechanism against an overactive inflammatory response and can be protective against the development of sepsis (20). Our previous findings of attenuated immune responses to an LPS challenge in adulthood in nLPS rats (6–8) leads us to predict that the nLPS animals would develop endotoxin tolerance less effectively, leaving them more vulnerable to a subsequent LPS challenge. In this light, we also examined whether the development of endotoxin tolerance, in terms of their temperature responses, was affected by the neonatal LPS.

In the wild, it is important that an animal recovers quickly from an illness and reverts to usual behaviours that are essential for survival, such as food acquisition and consumption and predator avoidance. We also, therefore, measured body weight changes and food intake, as well as behaviours in the open field in a subset of the rats, as further indicators of the degree and duration of sickness after induction of sepsis.

Materials and methods

Animals

Pregnant Sprague–Dawley rats (Charles River, La Salle, QC, Canada) were maintained in a specified pathogen-free facility under a 12 : 12 h light/dark cycle (lights on 07.00 h) at 22 °C with pelleted rat chow and water available *ad lib*. At PN 10, litters were reduced to a maximum of 12 pups. All litters were weaned at PN 21. Male and female rats were kept and housed two animals of the same sex per cage until they reached approximately 9 weeks of age. All procedures were conducted in accordance with the Canadian Council on Animal Care regulations and were approved by the local, University of Calgary, Animal Care Committee.

Neonatal immune challenge

At PN 14, animals were removed from their mothers for approximately 5 min and subjected to i.p. injections of either LPS (*Escherichia coli*, serotype 026:B6; L-3755, Sigma, St Louis, MO, USA; 100 µg/kg) in 1 ml/kg pyrogen-free saline, or an equivalent volume of pyrogen-free saline. Approximately equal numbers of rats from each litter received LPS or saline. Ears were clipped for identification. Pups were then returned to their home cages and left undisturbed, except for weaning and the usual cleaning and feeding procedures, until experimentation. These experiments involved 92 rats from eight litters and, for each set of experiments, animals were selected randomly from several litters.

Adult implantation of temperature monitors

When the rats reached approximately 9 weeks of age, they were briefly anaesthetised with halothane (induced at 4% and maintained at 2%). Sterile, silicone-coated temperature data loggers (SubCue Dataloggers; Calgary, ALB, Canada) were then implanted into the abdominal cavity. Briefly, a small incision was made in the skin and muscle using sterile techniques and a data logger inserted. The muscle layer was then sutured with resorbable sutures, the skin clipped with wound clips and a topical analgesic applied. Each surgery took approximately 5 min. Animals were thereafter housed separately.

Experiment 1: Adult sepsis

Seven days after the implantation of the temperature monitors in the neonatally pre-treated rats, baseline body weight and food consumption were measured (i.e. animals and food weighed) at both 08.00 h and 15.00 h every day for 3 days. An initial pilot study revealed that 5 mg/kg LPS resulted in 50% mortality within 12 h. Because this was an unacceptable outcome, in subsequent experiments, the rats were given either 1 mg/kg or 3 mg/kg LPS i.p. (*E. coli*; same serotype as above) in 1 ml/kg pyrogen-free saline (from which there was no mortality). Injections were given between 08.00 h and 09.00 h. One hour after injection, each rat was subjected to a small nick to the tail vein for extraction of a blood sample into a heparinised capillary tube (approximately 20 µl). Blood was immediately centrifuged and the plasma aliquots snap-frozen in liquid nitrogen and kept at –80 °C until use. Body weight and food consumption were measured between 15.00 h and 16.00 h on the day of injection.

as well as at 08.00 h and 15.00 h for subsequent days. At the end of the experiment, the rats were euthanised and temperature monitors extracted.

Experiment 2: Adult tolerance

A second group of neonatally pre-treated rats was given a 1 mg/kg LPS as described above and left to recover for 7 days. They were then all subjected to a further 3 mg/kg LPS injection and blood samples were taken from the tail vein as described. Weight and food intake were also measured. A subset of these rats was also tested, 1 or 3 h after the final LPS injection, for activity in an open field arena, as an indication of sickness behaviour (21, 22). The circular mini open field was 45 cm in diameter and had green inside walls that were 50 cm in height. The arena was placed in a brightly lit room on an off-white coloured floor with a black grid that divided the area into four sections. Each animal was placed in the centre of the arena and filmed for a period of 10 min. Each test was later assessed by an examiner who was blind to the type of treatment the rats had received. The rats were scored for: locomotion (number of grid-lines crossed), middle arena exploration (number of crosses through the middle), vertical exploration (number of rearings), latency to groom, and instances of and time spent grooming. The open field arena was cleaned with the multi-purpose disinfectant, Virkon (Antc Int. Ltd, Richmond, VA, USA) between tests.

Assessment of plasma corticosterone

A standard corticosterone enzyme linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA), was used to assess plasma corticosterone. The inter-assay variability for this ELISA was 7.8–13.1%, intraassay variability was 6.7–8.4%, with a lower limit of detection of 27 pg/ml. All samples were assayed in duplicate together with their appropriate controls.

Statistical analysis

Mean baseline body weight, open field and corticosterone data were compared using Student's unpaired t-tests. Changes in body weight after injection were compared using one-way ANOVA with repeated measures with neonatal treatment as the between factor and time from injection (day) as the repeated measure. Food consumption was averaged over three days and three nights and the means of nSal and nLPS groups compared using Student's unpaired t-tests. Where no significant effects of neonatal treatment were found, further analysis was not conducted. Temperature data were calculated as a temperature index ($^{\circ}\text{C} \times \text{h}$) for 0–1 h (stress-induced hyperthermia), 1–2 h (hypothermic phase) and 3–9 h (hyperthermic phase) after injection. The temperature at each 15-min interval from 08.00–09.00 h on the day preceding the injection was used to calculate a baseline temperature. The mean change from this baseline was then calculated per hour for each animal and these data summed as appropriate to produce an 'area under the curve' value (temperature index) (6, 8, 12). Temperature indices were then compared using Student's unpaired t-tests. In each case, males and females and 1 and 3 mg/kg were compared separately. $P < 0.05$ was considered statistically significant. Data are presented as the mean $\pm$ SEM.

Results

Effects of neonatal programming with LPS on basal conditions in adulthood

No differences were seen under basal (unstimulated) conditions between the nSal or nLPS adult rats, either male or female, in any of the variables we measured. Both groups of males ($n = 7$ each) weighed approximately 350 g on the first morning of basal measurements (nSal = 345.6 ± 6.5 g, nLPS = 355.3 ± 3.9 g). They lost approximately 3% of this weight during the day and gained approximately 5% of the initial weight back again during the next night, becoming approximately 2% heavier each day (Fig. 1A). Both groups of males ate minimal amounts of rat chow, approximately 2 g, during the day but, at night, consumed approximately 29 g (Fig. 1C). Female nSal ($n = 9$) and nLPS ($n = 7$) rats showed a similar profile of circadian body weight changes to the males despite lower starting weights (nSal = 240.3 ± 4.7 g, nLPS = 236.4 ± 6.3 g; Fig. 1B), and they ate approximately 2 g of rat chow during the day and approximately 18 g during the night (Fig. 1D).

As previously reported (7, 8), neonatal treatment with LPS also did not affect basal circadian temperature rhythms in either the males ($n = 19$ each) or females (nSal, $n = 19$; nLPS, $n = 18$; Figs 1E, F; 2C, D and 3C, D).

Effects of neonatal programming with LPS on 1 mg/kg LPS in adulthood

Body weight and food intake—Exposure to 1 mg/kg LPS resulted in some weight loss in all groups of rats. By the third morning, however, weights had recovered to approach those recorded prior to LPS. No significant differences in weight loss or recovery were seen between nSal and nLPS rats in either males ($n = 7$ each) or females (nSal, $n = 9$; nLPS, $n = 7$; Fig. 2A, B).

At least part of the weight loss after 1 mg/kg LPS can be accounted for by sickness-induced anorexia. Thus, daytime food intake was reduced to approximately 0.5 g in all rats on the day of LPS (males: nSal = 0.3 ± 0.2 g; nLPS = 0.7 ± 0.4 g; females: nSal = 0.4 ± 0.2 g; nLPS = 1.0 ± 0.4 g). Night-time food intake was also reduced from baseline during the first night after LPS (males: nSal = 12.0 ± 3.6 g; nLPS = 12.6 ± 1.6 g; females: nSal = 9.3 ± 1.3 g; nLPS = 10.9 ± 1.4 g). No significant differences were seen between the groups in males or females and all groups had recovered to approximately their usual eating patterns by the third night.

Body temperature—By contrast to our previous studies using low febrile doses of LPS (50 μ g/kg) in adulthood (7, 8), 1 mg/kg LPS did not lead to an attenuated febrile response in nLPS rats (Fig. 2C–F). In male rats ($n = 13$ each), we saw no significant differences in the initial hypothermic phase, and nLPS rats displayed an accentuated rather than attenuated response in the febrile phase ($P = 0.0002$, $t = 4.44$; Fig. 2C, E). Female nSal ($n = 15$) and nLPS ($n = 13$) rats, by contrast, were notably different in that nLPS rats did not become hypothermic, unlike nSal ($P < 0.0001$, $t = 8.93$) and female nLPS rats also showed an accentuated febrile response to the 1 mg/kg LPS ($P < 0.0001$, $t = 5.98$; Fig 2D, F). It should also be noted that stress (injection)-induced hyperthermic responses were not different between the groups in either males or females (Figs 1E, F; 2C, D and 3C, D).

Corticosterone—Again, in contrast to our previously reported results with a lower dose of LPS (12), plasma corticosterone levels 1 h after 1 mg/kg LPS were not significantly different between the nSal or nLPS groups for either male (nSal = 67.1 ± 13.3 ng/ml, n = 10; nLPS = 71.9 ± 20.1 ng/ml, n = 8) or female (nSal = 94.9 ± 24.8 ng/ml, n = 6; nLPS = 116.2 ± 38.5 ng/ml, n = 5) rats.

Effects of neonatal programming with LPS on 3 mg/kg LPS in adulthood

Body weight and food intake—As with 1 mg/kg LPS, an adult LPS dose of 3 mg/kg caused a loss of total body weight in nSal and nLPS rats that had stabilised by the third morning (nSal males n = 6, females n = 4, nLPS males n = 6, females n = 5; Fig. 3A, B). No significant differences between groups were seen. Both day-time and night-time food intake were also markedly reduced, but were at no time significantly different between the nSal and nLPS groups in either males or females (data not shown).

Body temperature—In male rats, 3 mg/kg LPS lead to a slightly but significantly reduced hypothermia in the nLPS group than in the nSal ($P = 0.011$, $t = 3.12$, n = 6 each). As with the 1 mg/kg LPS dose, we also saw an accentuated hyperthermic response in the nLPS animals ($P = 0.0002$, $t = 5.55$, Fig. 3C, E). Females, by contrast, displayed similar hypothermic and febrile responses to adult 3 mg/kg LPS, irrespective of their neonatal treatments (nSal, n = 4; nLPS, n = 5; Fig. 3D, F).

Corticosterone—As with the 1 mg/kg LPS dose, no significant differences were seen between the groups in plasma corticosterone 1 h after 3 mg/kg LPS in either male (nSal = 61.7 ± 15.7 ng/ml, n = 6; nLPS = 87.1 ± 10.6 ng/ml, n = 6) or female (nSal = 117.1 ± 10.2 ng/ml, n = 5; nLPS = 150.6 ± 11.3 ng/ml, n = 5) rats.

Effects of neonatal programming with LPS on tolerance to a second dose of LPS in adulthood

Body weight and food intake—Prior adult treatment with 1 mg/kg in rats given 3 mg/kg LPS as adults resulted in effects on body weight and food intake after the second injection that were similar to those seen with 1 mg/kg or 3 mg/kg alone. That is, all the rats lost weight and were anorexic for the first 1–2 days before recovering to pre-injection weights, and no significant differences were seen between nSal and nLPS groups in either males (n = 7 each) or females (nSal, n = 9; nLPS, n = 7; Fig. 4A, B).

Body temperature—Male rats (n = 13 each) demonstrated an apparent tolerance to the stress of being injected; their temperature indices for injection-induced hyperthermia to the second injection being half or less than half those to the first injection (Fig. 1E and 4C, E). A significantly smaller injection-induced hyperthermia was seen in nLPS rats ($P = 0.0005$, $t = 4.0$). Females, on the other hand (nSal, n = 15; nLPS, n = 13), showed no such tolerance and no such differences between nSal and nLPS groups. They displayed essentially identical hyperthermic responses to the stress of injection (Fig. 4D, F).

Neither group of male rats demonstrated a hypothermic response to 3 mg/kg LPS 1 week after 1 mg/kg LPS. However, a hyperthermic response was developed and, again, this was significantly greater in nLPS rats ($P < 0.0001$, $t = 4.92$; Fig. 4G).

Female rats also did not develop hypothermia in response to 3 mg/kg LPS 1 week after 1 mg/kg LPS. The initial 3 mg/kg LPS induced response was, instead, slightly hyperthermic, significantly more so in nLPS-treated rats ($P = 0.0016$, $t = 3.90$). As with the single 3 mg/kg dose of LPS, the female febrile response that developed in the period thereafter was similar in nSal and nLPS rats (Fig. 4H).

Corticosterone—Pre-treatment with 1 mg/kg LPS lead to severely blunted corticosterone responses to 3 mg/kg LPS in nSal male rats compared to the nLPS group. nLPS male rats, however, had corticosterone responses very similar to those displayed in response to the initial 1 mg/kg or 3 mg/kg LPS dose (nSal, $n = 6$; nLPS, $n = 7$; $P = 0.0003$, $t = 5.19$; Fig. 5A). Female rats showed no such differences, with both groups displaying low plasma corticosterone after the second adult LPS treatment (nSal, $n = 9$; nLPS, $n = 7$; Fig. 5B).

Open field—Behavioural tests in the open field revealed no significant differences between nSal and nLPS males ($n = 4$ each) in any of the parameters measured either at 1 or 3 h after the second LPS injection (Fig. 5C, E, G). The female nSal rats ($n = 5$) showed a trend towards more vertical exploration (rearing; $P = 0.071$, $t = 2.08$; Fig. 5F) and did show significantly more locomotor activity (number of lines crossed; $P = 0.015$, $t = 3.09$; Fig. 5D) and more middle arena exploration ($P = 0.013$, $t = 3.17$; Fig. 5H) than the nLPS ($n = 5$) at 1 h. These female nSal rats also spent significantly less time grooming in the 1 h trial (nSal = 9.5 ± 3.9 s, nLPS = 25.5 ± 2.8 s, $P = 0.01$, $t = 3.33$). None of these differences were seen in the open field at 3 h post-injection.

Discussion

In the present study, we show that neonatal immune challenge with LPS attenuates the hypothermic and accentuates the febrile responses to a septic dose of the same LPS in adulthood, particularly in male rats, although this effect is not associated with alterations in other indices of health, such as weight loss, food intake or activity.

As expected from our previous findings (7, 8, 23), the nSal and nLPS groups were similar in all of the basal parameters measured. However, both male and female nLPS rats treated as adults with 1 mg/kg LPS, displayed attenuated or absent hypothermia. Because the hypothermia response to LPS is often seen in rats given high doses of LPS at sub-thermoneutral temperatures, (14) and is usually correlated with tumour necrosis factor (TNF) α levels (24–26), this finding would be compatible with our previous observations (12) that nLPS results in attenuated cytokine responses in adults subsequently re-exposed to LPS. It is uncertain why there remained a subsequent slightly enhanced febrile response in these animals. Perhaps the more thermogenic cytokines such as interleukin (IL)-6 also were reduced in nLPS animals but were still elevated enough after this high dose of LPS that their levels had little influence on temperature, and it is the balance between TNF α and IL-6 that affects the subsequent hyperthermic phase.

It could be argued that the attenuated hypothermic and accentuated febrile responses to septic doses of LPS in nLPS rats reflect a positive adaptation in these animals. The hypothermic component of the response to a septic dose of endotoxin is often regarded to be a dysregulated, maladaptive response and those individuals who do not display such reductions in temperature demonstrate better survival rates and recover more quickly (27, 28). As suggested in the Introduction, the febrile response is considered to be an important adaptive phenomenon, aiding the organism in combating invading pathogens (3–5, 29). Thus, the absence of hypothermia and / or enhanced fever in these nLPS-treated rats after 1 mg/kg LPS in adulthood may reflect an enhanced ability to combat a severe immune challenge. On the other hand, we also observed a lack of tolerance in our male rats that may represent a cost of such an adaptation, indicating that the effects on nLPS on septic doses of LPS in later life are not wholly beneficial.

The results obtained in the present study also demonstrate that there may be a sex difference in how nLPS affects adult ability to combat severe immune challenge in terms of their temperature responses to both single and sequential high (3 mg/kg) LPS challenges. Whereas responses in male rats to 3 mg/kg LPS were identical to those seen after 1 mg/kg, nLPS and nSal female rats had identical hypothermic and febrile responses to this initial 3 mg/kg dose. The underlying mechanism for the male–female difference is unknown, although it is interesting that both hypothermic and hyperthermic phases were unaltered in female, whereas both were affected in males. This lends credence to the idea advanced above that it may be the cytokine profile elaborated during the hypothermic phase that is partly responsible for the magnitude of the hyperthermic phase. Furthermore, it is interesting that the corticosterone response in male rats (at least when we measured it after the second LPS injection) was elevated in nLPS males and not in nLPS females compared to nSal treated rats. High corticosterone could be responsible for a reduction in TNF α in males (12) that accounts for their reduced initial hypothermic responses.

The possibility of differences between the sexes is also evident in the rats' response to the second of two high LPS doses in adulthood. Male rats of both groups appeared to have developed some tolerance, as indicated by the lack of hypothermic response to the second challenge and the reduced hyperthermic response relative to the first exposure. However, the febrile response of the nLPS rats remained high, as did the corticosterone. The high corticosterone is consistent with that previously observed at much lower LPS doses (12) and, as indicated above, may have reduced the initial TNF α levels and, consequently, abolished the initial hypothermia. However, in females of both groups, the initial hypothermia was reversed to a small hyperthermia in the tolerant animals, although differences in corticosterone levels were not seen, and so appear not to be responsible for differences in either phase of the response. Conversely, the behavioural response of the nLPS female rats to this second dose of LPS was more severely affected than that of the nSal. Although we saw no differences in open field behaviour between the groups in the males, nLPS females showed reduced exploration of the total and middle of the open field arena compared to nSal females at 1 h post-injection. They also spent significantly more time grooming. These findings, demonstrating less activity in the open field in nLPS female rats, are suggestive of more sickness behaviour in these animals (30–33). We have previously seen that nLPS alters some basal indices of anxiety (21) and at least one other group has found increased anxiety

after adult psychological stress in adult rats treated neonatally with LPS (34). It is therefore also possible that our female rats are displaying increased anxiety levels in response to the adult LPS challenge.

Despite the differences in temperature responses of the male and female nLPS rats, we did not see differences in other indices of health. For example, weight loss and suppression of food intake remained consistent between treatment groups. It may be argued that differences in health and sickness behaviour may manifest themselves in the face of more ethologically relevant tests, such as if the rats were forced to forage for food.

A possible mechanism to explain our findings is changes to the arginine vasopressin (AVP) system. Septic doses of LPS cause a rapid drop in blood pressure that provides a strong stimulus for the release of AVP; thus, AVP is strongly elevated in the plasma in the early stages of sepsis. This elevation is thought to be due to the actions of inducible nitric oxide synthase (iNOS), catalysing nitric oxide production, which then acts directly on the hypothalamus to stimulate AVP release (35). Arginine vasopressin is a key player in antipyresis and is therefore considered to play a principal role in the hypothermic phase of the temperature response in sepsis (35, 36). It has previously been demonstrated that at least one neonatal manipulation, neonatal handling, can lead to permanent down-regulation of central NO production (37). It is conceivable that neonatal LPS could also permanently down-regulate the iNOS response to LPS, which would result in reduced AVP release after septic doses, leading, in turn, to a reduced hypothermic and accentuated hyperthermic response. This latter mechanism could also account for the potential sex differences in these responses. For example, male, but not female rats subjected to maternal separation as neonates display reduced numbers of AVP immunoreactive cells in the paraventricular nucleus of the hypothalamus as adults (38), demonstrating that alterations in this system have the potential to be sexually dimorphic. Other factors such as sex hormones or inflammatory cytokines could also account for the apparent differences that were found between males and females in the present study. For example, ovarian hormones and IL-1ra are both expected to suppress febrile responses to LPS more effectively in females than in males (39–41). It is possible that these prevent the nLPS-facilitated enhancement of the febrile response to septic doses of LPS.

Despite indications from the present study suggesting that temperature and selected physiological and behavioural responses to a septic dose of LPS are not exacerbated by neonatal immune challenge, some previous studies indicate that neonatal immune challenge may be detrimental to the adult health of an animal. For example, we have shown that neonatal LPS can lead to exacerbated colon damage and body weight loss in a model of ulcerative colitis (42), greater brain damage after global ischaemia (43) and hyperalgesia (44). Bilbo *et al* (45, 46) showed that rats perform less well in a contextual fear conditioning test for learning and memory after adult LPS if they have been previously exposed to an *E. coli* infection as young neonates. Neonatal immune challenge can also predispose an animal to periodontal disease in adulthood (47) and lead to increased tumour colonisation and impaired activity of natural killer cells after induction of tumour metastases (48). Conversely, neonatal immune challenge is also possibly protective, and there is evidence that it may confer protection in some models of inflammatory disease, such as with adjuvant-

induced arthritis (11). It therefore appears that any favourable or detrimental effects of a neonatal immune challenge appear to be very specific to the physiological parameter being studied.

In the present study, we found evidence that neonatal treatment with LPS has a long-term effect on the animals' response to a septic dose of LPS. However, despite many subtle changes in numerous aspects of physiology, we find that neonatal immune challenge is unlikely to confer a substantial disadvantage in the face of a severe immune challenge later in life.

Acknowledgments

We thank Dr Mio Tsutsui for technical assistance. This work was supported by the Canadian Institutes of Health Research (CIHR). S.J.S. held an Alberta Heritage Foundation for Medical Research (AHFMR) postdoctoral fellowship and was also supported by a personnel award from the Heart and Stroke Foundation of Canada, the Canadian Stroke Network, CIHR and AstraZeneca Canada Inc. E.F. was an AHFMR and CIHR postdoctoral fellow. Q.J.P. is an AHFMR Medical Scientist and University Professor.

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Fig. 1.

Basal measurements: (A, B) Body weight; percentage of baseline (percentage of first day of measurement). (C, D) Food consumption during day- and night-time. Values are averaged over 3 days. (E, F) Temperature indices for stress (injection)-induced hyperthermia, 0–1 h after injection with 1 or 3 mg/kg lipopolysaccharide (LPS). nSal, neonatally saline-treated, nLPS, neonatally LPS-treated.

Fig. 2.

One milligram per kilogram lipopolysaccharide (LPS) measurements: (A, B) Body weight; percentage of the morning of injection with 1 mg/kg LPS. (C, D) Temperature changes in the hours after injection. Injection = 08.00 h, arrow. (E, F) Temperature indices for the hypothermic (1–2 h) and hyperthermic (3–9 h) phases of the response to 1 mg/kg LPS. nSal, neonatally saline-treated; nLPS, neonatally LPS-treated. ***P < 0.0005.

Fig. 3.

Three milligram per kilogram lipopolysaccharide (LPS) measurements: (A, B) Body weight; percentage of the morning of injection with 3 mg/kg LPS. (C, D) Temperature changes in the hours after injection. Injection = 08.00 h, arrow. (E, F) Temperature indices for the hypothermic (1–2 h) and hyperthermic (3–9 h) phases of the response to 3 mg/kg LPS. nSal, neonatally saline-treated; nLPS, neonatally LPS-treated. * $P < 0.05$, *** $P < 0.0005$.

Fig. 4.

Tolerance measurements; 3 mg/kg lipopolysaccharide (LPS) 1 week after 1 mg/kg LPS: (A, B): Body weight; percentage of the morning of injection with 3 mg/kg LPS. (C, D) Temperature changes in the hours after injection. Injection = 08.00 h, arrow. (E, F) Temperature indices for stress (injection)-induced hyperthermia, 0–1 h after injection. (G, H) Temperature indices for the hypothermic (1–2 h) and hyperthermic (3–9 h) phases of the response to 3 mg/kg LPS. nSal, neonatally saline-treated; nLPS, neonatally LPS-treated. ** $P < 0.005$, *** $P < 0.0005$.

Fig. 5.

Tolerance measurements; 3 mg/kg lipopolysaccharide (LPS) 1 week after 1 mg/kg LPS: (A, B) Plasma corticosterone 1 h after injection. (C, D) Locomotor activity in the open field 1 and 3 h after injection i.e. number of lines crossed in the 10-min period. (E, F) Vertical exploration (i.e. rearing in the open field). (G, H) Middle exploration in the open field (i.e. number of crosses through the middle). nSal, neonatally saline-treated; nLPS, neonatally LPS-treated. *P < 0.05, ***P < 0.0005.