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Lipopolysaccharide reduces tactile startle response magnitude but not prepulse inhibition in rats: A dose–response examination

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ABSTRACT

As a result of innate immune system stimulation, lipopolysaccharide (LPS) exposure produces a range of behavioral modifications referred to as "sickness behaviors." This study assessed the effects of multiple doses of LPS on air-puff tactile startle reflex (Startle-Only trials) and acoustic prepulse inhibition (PPI) in adult male rats. Rats were injected intraperitoneally with LPS (300, 200, 100, or 50 μg/kg LPS, $n=9$, 10, 10, and 10 respectively) or saline vehicle ($n=10$) on 2 test days 72 h apart. Magnitude of the startle response was recorded following 15 psi air-puffs (Startle-Only trials) and auditory PPI of the tactile startle response (with prepulses at +3, +6 and +12 dB above background noise). Startle-Only trial analysis suggested a significant dose-dependent effect of LPS on Test Day 1 with the 300 and the 200 μg/kg LPS groups exhibiting significantly reduced startle responses. On the second test day, the control animals displayed significant habituation to the tactile startle stimulus while the LPS animals did not. On the PPI trials, LPS animals exhibited normal prepulse inhibition. The acoustic PPI of the tactile startle response was significantly greater on Test Day 2 than on the first test day, regardless of treatment. These results suggest that "sickness behaviors" induced by high doses of LPS may include decreased non-voluntary motor activity, as measured by the tactile startle response. They also show that sensory processing, as measured by PPI, is not impaired with sickness.

1. Introduction

Lipopolysaccharide (LPS), a component of Gram negative bacterial cell walls, is commonly used to model bacterial infection. Upon first exposure to LPS, the innate immune system is engaged, leading to the development of the "acute phase response" (Berczi et al., 2000; Heumann and Roger, 2002). The acute phase response primarily results from peripheral and central release of the pro-inflammatory cytokines interleukin-1β, interleukin-6 and tumor necrosis factor-α (Kent et al., 1992; Roth et al., 1994; Sagar, 1994; Wilder, 1995; Linthorst et al., 1997; Dantzer et al., 1998a,b; Berczi et al., 2000; Szelenyi, 2001; Rivest, 2003; Harden et al., 2006). The acute phase response encompasses a variety of physiological and neurobiological changes, as well as adaptive behavioral modifications commonly referred to as "sickness behaviors" (Hart, 1988).

Sickness behaviors include but are not limited to, decreased locomotor activity and exploration, hyperalgesia (increased pain sensitivity), anhedonia (lack of pleasure), reduced food and water intake and increased time spent asleep (Bluthe et al., 1992; Kent et al., 1992; Maier et al., 1993; Plata-Salaman and Borkoski, 1993; Yirmiya et al., 1994; Franklin et al., 2003; Cross-Mellor et al., 2004; Ambrosini et al., 2005; Franklin et al., 2007). Other characteristics of the acute phase response include a strong pyrogenic response (fever) and catabolism (resulting in a substantial decrease in body weight) (Hart, 1988; Bluthe et al., 1992; Kozak et al., 1994; Roth et al., 1994; Berczi, 1998; Tollner et al., 2000; Harden et al., 2006). Tolerance to both the behavioral and physiological effects of LPS forms very quickly (Roth et al., 1994; Almeida et al., 1999; Engeland et al., 2001; West and Heagy, 2002; Engeland et al., 2003; Franklin et al., 2003). The development of tolerance to LPS exposure serves to prevent excess inflammation (Ziegler-Heitbrock, 1995), is mediated through decreased macrophage secretions of pro-inflammatory cytokines (e.g., Knopf et al., 1994; Mathison et al., 1990; Zeisberger and Roth, 1998) and, in part, by a decreased responsiveness to the cytokines themselves (He et al., 1992).

Most studies of the effects of LPS have focused on physiological responses and/or behavioral responses such as feeding, voluntary movement, and memory deficits. In particular, a large body of literature reports significant decreases in locomotor activity and locomotor dependent behavior following LPS exposure (Hart, 1988; Kozak et al., 1994; Lacosta et al., 1999; Engeland et al., 2001; Engeland et al., 2003; Franklin et al., 2003; Dunn and Swiergiel, 2005; Harvey et al., 2006; Franklin et al., 2007). This raises the possibility that these LPS-induced decreases in voluntary locomotor activity may result from a general inhibition of motor function or a motor impairment.

The startle response is a sensorimotor reflex thought to have evolved as a defense mechanism to deflect predatory blows (Yeomans

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et al., 2002). Induced by sudden intense or unexpected stimuli, the mammalian startle response involves the coordinated contraction of skeletal muscles such that the organism will appear to jump (Hoffman and Ison, 1980). The primary startle response interface in the central nervous system is the caudal pontine reticular nucleus (PnC), a brain stem structure containing giant neurons that receive input from multiple senses and project to motor pathways in the spinal cord (Koch, 1999). When stimuli of differing modalities are combined to produce a startle response, the effects of individual modalities appear to summate and produce a startle response of greater magnitude than startle responses with either modality alone (Yeomans et al., 2002).

Prepulse inhibition (PPI), a phenomenon observed in most mammalian species, is one of several ways in which the startle response is modulated (Braff et al., 2001; Swerdlow et al., 2001). PPI is a commonly used measure to operationalize "sensorimotor gating", as it reflects the ability of an animal to allocate attentional resources to more salient stimuli in the environment (Braff et al., 2001). Normal PPI performance requires adequate sensory detection and processing (Koch, 1999). Operationally, PPI measures are the relative decrease in the magnitude of the startle response when the startle event is preceded by a non-startle eliciting stimulus (prepulse) (Braff et al., 1999; Koch, 1999). Thus, the startle response paradigm allows for the evaluation of sensorimotor reflex function. In combination with prepulse inhibition measures, the startle response paradigm can provide valuable insight into several neural processes, including non-voluntary motor function, sensory detection, and sensorimotor gating.

In rodents, acoustic startle response paradigms are most commonly used to elicit startle, employing bursts of white noise, both as the prepulse and the startling stimuli. The response (or reflex) quantified as the variable of interest in rodent research is the force produced by a non-voluntary contraction of the skeletal muscles (Hoffman and Ison, 1980). Non-voluntary defensive reflexes, such as the startle response, would not necessarily be expected to decrease in response to immune insult, as deficits in startle reflexes would result in reduced survival rates (Juszczak et al., 2008). However, a previous study, which examined the acoustic startle response following LPS exposure in rats, found a significant reduction in startle magnitude following administration of high doses of LPS (Lockey et al., 2009). This finding was independent of any evident sensory impairment and it was suggested that the reduced acoustic startle response might have resulted from diminished non-voluntary motor performance.

A major advantage of the startle response paradigm is that it can be studied across species, leading to translational research opportunities (Koch, 2002; Swerdlow et al., 2002). There is however, a general dichotomy in the modalities used to elicit startle. Most rodent research uses auditory stimulation and many human studies use air-puff stimulation. Air-puff tactile startle response is known to elicit dual modality stimulation as there is a significant acoustic component which accompanies the tactile sensation and it is well accepted that the auditory component of air-puff stimuli contributes more to the startle response magnitude than the tactile component (Pilz et al., 2004; Taylor et al., 2005). However, recent animal studies comparing the acoustic startle response to the air-puff tactile startle response have suggested that genetic contributions differentially influence an animal's reactivity to air-puff versus auditory startle stimuli (Torkamanzehi et al., 2008).

PPI can consist of either uni-modal or cross-modal sensory protocols. In the former, both the prepulse and the startle pulse are of the same modality (typically acoustic); in the cross-modal task, the prepulse and the startle pulse activate different sensory modalities, such as acoustic and tactile. Although reduced uni-modal and cross-modal PPI have been reported in schizophrenia (Braff et al., 1992), rodent studies suggest that inhibition in uni-modal PPI tests is greater than inhibition in cross-modal tests (Bullock et al., 1997). Performance differences between uni-modal and cross-modal PPI have been interpreted to reflect differential genetic regulation of PPI (Bullock et al., 1997; Torkamanzehi et al., 2008). There is also evidence that different brain regions are involved in uni-modal and cross-modal PPI (Swerdlow et al., 2001; Yeomans et al., 2006).

The present study determined the acute effects of various doses of LPS on tactile startle response and auditory PPI of the tactile startle response, in young adult male rats. In view of the effects of LPS on uni-modal acoustic startle and PPI (Lockey et al., 2009), it was hypothesized that higher doses of LPS would decrease the size of the tactile startle response but would have minimal effect on PPI. The effects of behavioral tolerance to LPS were also examined by testing the rats over two days, 72 h apart.

2. Materials and methods

2.1. Animals

Forty-nine naïve adult male Long Evans rats (Charles River, Quebec) weighing between 270 and 305 g at the start of the experiment were used as subjects. The rats were housed in pairs in a colony room maintained at 21 ± 1 °C under a 12 h/12 h light/dark cycle with lights on at 07:00 h. Rat chow (Prolab) and tap water were available ad libitum, except during test sessions. All of the behavioral experiments and body weight measurements were carried out between 9:00 h and 15:00 h (light phase of the light/dark cycle). All of the experimental procedures were carried out according to the guidelines set out by the Canadian Council on Animal Care (CCAC) and approved by the institutional animal care committee.

2.2. Drugs

All treatments were administered intraperitoneally (i.p.) at a volume of 1.0 ml/kg body weight. Lipopolysaccharide (from *Escherichia coli* 0111:B4, L-2630; Sigma, St. Louis, MO) was dissolved in pyrogen-free 0.9% NaCl to concentrations of 300, 200, 100, or 50 μg/kg LPS. Control treatment was an injection of 0.9% isotonic, pyrogen-free saline vehicle.

2.3. Apparatus

All of the tactile startle response and PPI testing was conducted in 2 separate startle devices (SRLAB, San Diego Instruments, San Diego, CA). Each device consisted of a transparent cylindrical acrylic rat enclosure (10.2 cm outside diameter) mounted on an acrylic platform. The platform sat on a piezoelectric accelerometer which transduced the force of animal movement. The platform was located inside a well ventilated, sound attenuating box containing a mounted fluorescent light and a speaker (on the roof, approximately 11 cm from the top of the cylinder) which emitted the background and prepulse noise stimuli. An 8 mm (internal diameter) copper tube, used to deliver air-puff stimuli, was projected directly downward towards the animal's back. The source of air-puff stimulation was compressed air delivered at a constant pressure of 15 psi, via use of a 2-Stage Regulator (Praxair Canada Inc, Brampton, ON). The air pressure wave then passed through the solenoid valve and into the copper tubing before bifurcating to the 2 startle chambers. The resulting "air-puff" then stimulated the dorsal torso of the animals through a hole in the top of the enclosure. Beginning at startle stimulus onset, data were recorded and stored by a personal computer attached to the accelerometer. The animal's average startle amplitude in response to bursts of air-puff stimulation was recorded and analyzed.

2.4. Procedure

2.4.1. Habituation

Rats were handled and weighed for three consecutive days prior to testing. Two days prior to testing, rats were injected with saline vehicle 60–75 min before being placed in a startle box for an

acclimation period. The acclimation period consisted of the presentation of a 70 dB background noise for 5 min, upon completion of which, rats were returned to their home cages. The test boxes were cleaned with a soapy Alconox solution and rinsed after each session.

2.4.2. Test days

Behavioral measures were assessed for all of the treatment groups on 2 test days, 72 h apart. This repeated testing facilitated a measurement of tolerance and the 72 hour inter-test interval ensured that there was minimal residual LPS in the animal before LPS exposure on Test Day 2. On the test days, all rats were weighed at approximately 09:00 h before administration of 300, 200, 100, 50 μg/kg LPS ($n=10$ for each dose, except 300 μg/kg LPS, $n=9$) or saline ($n=10$). Animals were weighed again 24 h later for determination of percent reduction in body weight.

The rats were injected approximately 60 to 75 min prior to placement in the startle apparatus. The 1 hour injection-to-testing interval was chosen to allow significant immune system activation by LPS (Engeland et al., 2006). After a five minute acclimation period in the startle box with background noise (70 dB), a 13 minute (58 trials) testing session commenced, throughout which the 70 dB background noise was maintained. Four trial types were used in the testing session; Startle-Only trials (consisting of a burst of air-puff stimulation lasting 40 ms in duration), and 3 different prepulse inhibition trial types (each consisting of a 20 ms burst of white noise presented with onset 120 ms prior to the air-puff startle stimulus). The 3 PPI trial types were categorized by intensity of the acoustic prepulse; with the prepulse 3, 6 or 12 dB louder than the 70 dB background noise (73, 76 and 82 dB PPI trial types, respectively).

In the testing session, the first 10 stimuli were Startle-Only trials and these trials were not used in later analyses. The next 48 trials (presented in pseudo-random order) consisted of 12 Startle-Only (air-puff-alone) and 36 PPI trials (12 each of the 3 different PPI trial types). All of the trials were separated by an inter-trial interval (ITI) of 8–23 s in length (mean ITI = 15 s). The startle parameters used were consistent with previously described procedures (Swerdlow and Geyer, 1998; Bakshi and Geyer, 1999; Slawecki et al., 2006; Torkamanzehi et al., 2008). For each trial, responses were recorded by the computer for 100 ms immediately following the onset of the air-puff stimulus.

2.5. Data analysis

Tactile startle response magnitude was calculated as an average force produced in the 100 ms following the air-puff startle stimulus. Startle-Only responses were analyzed using Mixed-Design Analysis of Variance (ANOVA), using Test Day (2 levels) as the within-subjects variable and Drug (5 levels — 300, 200, 100, 50, and 0 μg/kg LPS) as the between-subjects factor. Univariate ANOVAs of Startle-Only trials were also conducted for each test day.

Prepulse inhibition was calculated as the "percent difference from control" for each prepulse level, where control refers to the average response on Startle-Only trials (Blumenthal et al., 2004). % PPI = 100 * (Startle-Only startle magnitude − PPI startle magnitude) / Startle-Only startle magnitude. In some groups the 73 dB prepulse produced non-significant facilitation of the startle response magnitude (increased tactile startle response) instead of the expected inhibition. Because of this, all of the PPI analyses were conducted using only the 76 and 82 dB prepulse trials. Due to greater than 10% facilitation rather than inhibition in some animals, group sizes were reduced by one animal ($n=9$ for all treatments except the 300 μg/kg group, $n=8$). PPI was analyzed using a Mixed-Design ANOVA with Test Day (2 levels) and/or Prepulse Intensity (2 levels — 76 and 82) as within-subjects factors and Drug (5 levels) as the between-subjects factor. An average of the %PPI scores from the 76 and 82 dB prepulse trials (Mean % PPI) was used to examine the effect of test day on PPI.

Body weight data were converted into a 24 hour percent reduction in body weight: % Body Weight Reduction = 100 * (24 h post Test Day Body Weight − Test Day Body Weight) / Test Day Body Weight. % Body Weight Reduction data were then analyzed using Univariate ANOVAs for each test day with Drug as the between-subjects factor.

Dose of LPS (with saline as 0 μg/kg LPS) was used to test for a dose–response effect in each data set subjected to ANOVA (for Startle-Only, PPI and body weight data). Linear contrasts were examined for a significant relationship between LPS dose and the relevant dependent variable. In addition, Startle-Only data on Test Day 1 were analyzed with *a priori* *t*-tests for pair-wise comparisons of LPS groups to the saline control group, based on previous findings of reduced day 1 auditory startle magnitude with higher doses of LPS (Lockey et al., 2009). LSD post hoc tests were performed to assess differences among groups following ANOVA procedures. Significance criterion used was $\alpha=0.05$.

3. Results

3.1. Tactile startle response

3.1.1. Startle-Only

Scores for Startle-Only trials are shown in Fig. 1. A significant Day × Drug interaction $F(4, 44)=6.00$, $p<0.01$ in the Mixed-Design ANOVA showed that the animals displayed different modulations of tactile startle responses across test days depending on treatment. *A priori* *t*-tests for Drug effects on Test Day 1 revealed that the animals in the both the 300 and 200 μg/kg LPS groups exhibited significantly lower startle response magnitudes relative to the saline animals, $t(17)=2.11$, $p<0.05$ and $t(18)=2.76$, $p<0.05$, respectively (see Fig. 1). There was also an overall decrease in startle reactivity on Test Day 2 compared to Test Day 1 which was reflected by a significant main effect of Day $F(4, 44)=4.23$, $p<0.05$. Post hoc tests revealed that animals in the saline condition displayed significantly reduced startle responses on Test Day 2 relative to Test Day 1 ($p<0.01$). Further univariate analyses were conducted for each test day. On Test Day 1, there was no significant main effect of Drug $F(4, 44)=2.31$, $p=0.073$, but there was a significant linear contrast ($p=0.012$) suggesting a dose-dependent modulation of tactile startle response with LPS exposure. Analysis of Test Day 2 startle responses revealed no significant effects.

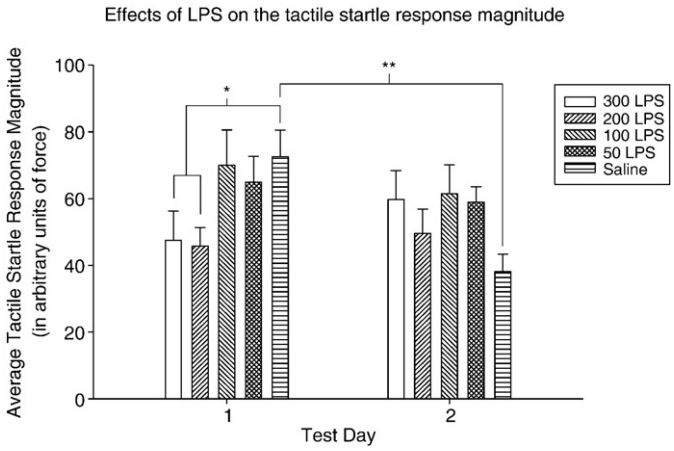

Fig. 1. Mean tactile startle response magnitude on Startle-Only trials demonstrates Day by Drug, and Drug effects, 60–75 min following i.p. injections of LPS (LPS; 300, 200, 100 and 50 μg/kg, $n=9$, 10, 10 and 10 respectively) or saline vehicle (0.9% NaCl, $n=10$). *$p<0.05$ (compared within test day), **$p<0.01$ (compared across test days). Significant dose–response (significant liner contrast, $p<0.05$) evident on Test Day 1. Values represent means ± S.E.M. (standard error of the mean).

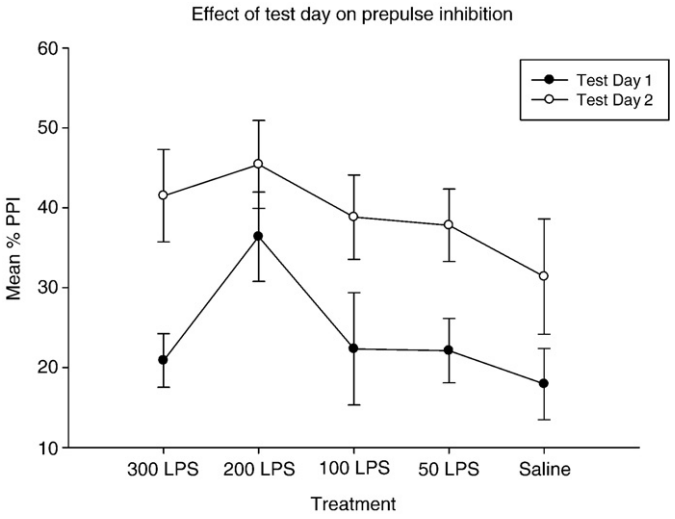

Fig. 2. Mean percent prepulse inhibition (mean % PPI) of the TSR as a function of Drug (% PPI averaged over the 76 and 82 dB trial types), 60–75 min following i.p. injections of LPS (LPS; 300, 200, 100 and 50 μg/kg, $n=8$, 9, 9 and 9 respectively) or saline vehicle (0.9% NaCl, $n=9$). % PPI is plotted to depict the significant main effect of Test Day on PPI ($p<0.001$). Values represent means ± S.E.M.

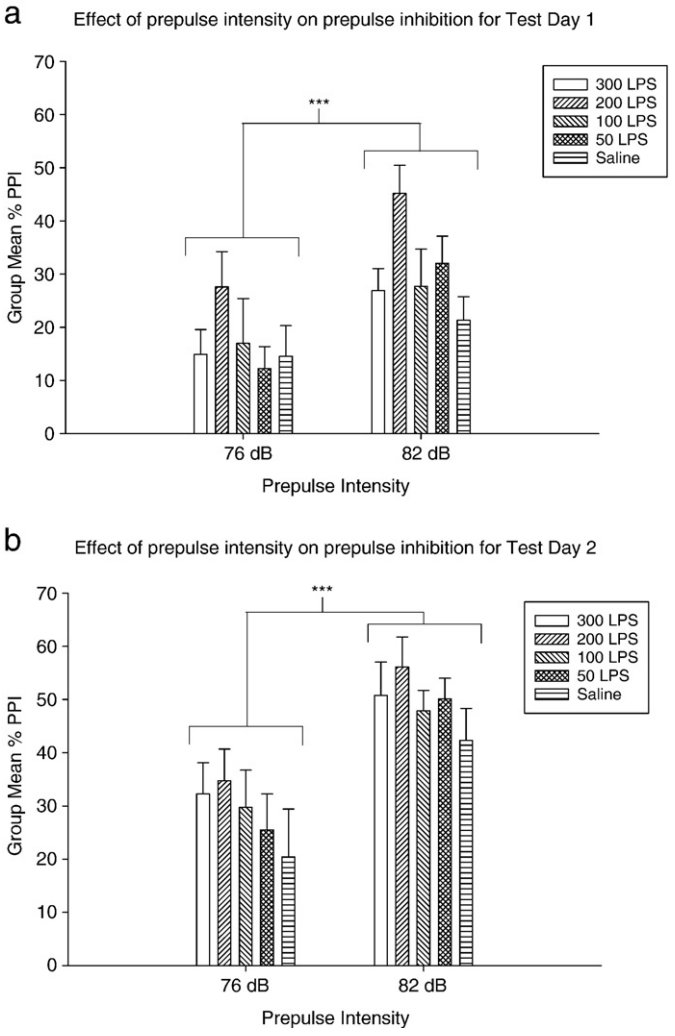

Fig. 3. Group means of percent prepulse inhibition (% PPI) of the TSR at prepulse intensities of 76 and 82 dB, 60–75 min following i.p. injections of LPS (LPS; 300, 200, 100 and 50 μg/kg, $n=8$, 9, 9 and 9 respectively) or saline vehicle (0.9% NaCl, $n=9$). (a) Test Day 1 and (b) Test Day 2 ***$p<0.001$ (comparison between prepulse intensities within a test day). Values represent means ± S.E.M.

3.1.2. Prepulse inhibition of the tactile startle response

Prepulse inhibition measures were calculated using only the 76 and 82 dB PPI trials. When analyzed across test days using a Mixed-Design ANOVA, an effect of Day $F(1, 39)=34.774$, $p<0.001$ was found. The ability of acoustic prepulses to inhibit air-puff startle increased significantly on Test Day 2 (see Fig. 2). A significant Prepulse Intensity effect was also revealed $F(1, 39)=117.09$, $p<0.001$, reflecting the fact that louder prepulses consistently elicit greater levels of inhibition than less-intense prepulses. Univariate ANOVAs for each test day independently, yielded effects of Prepulse Intensity on Test Day 1 $F(1, 39)=31.86$, $p<0.001$ and Test Day 2 $F(1, 39)=101.91$, $p<0.001$ confirming that the expected 'prepulse intensity to inhibition' relationship (Koch, 1999) is not altered with LPS treatment (see Fig. 3a and b for Test Days 1 and 2, respectively). There was no main effect of Drug on either Test Day. Although the %PPI of the 200 μg/kg LPS group was noticeably greater in comparison to the control animals, the groups were not examined with a post hoc test as there was no main effect of Drug and no dose-related trends to justify such comparisons.

3.2. Reduction in body weight

Fig. 4 displays the 24 hour percent reduction in body weight recorded following each test day. A significant Day × Drug interaction $F(4, 44)=7.77$, $p<0.001$ was found with generally less weight lost after Test Day 2 than Test Day 1, suggesting that tolerance to LPS occurred. There were also significant main effects of Day $F(1, 44)=56.03$, $p<0.001$ and Drug $F(4, 44)=28.81$, $p<0.001$. Further post hoc analyses revealed a significant effect of Drug for each Test Day independently, Test Day 1 $F(4, 44)=20.06$, $p<0.001$, and Test Day 2 $F(4, 44)=5.47$, $p<0.01$. A significant dose–response effect was found on each test day when analyzed by linear contrasts ($p<0.001$ for each). All 4 LPS treatments produced significantly more body weight change than saline on Test Day 1 ($p<0.001$ for each). On Test Day 2, each of the 300, 200 and 100 μg/kg LPS groups elicited significantly greater change in body weight than the saline ($p<0.01$) and 50 μg/kg LPS conditions ($p<0.05$).

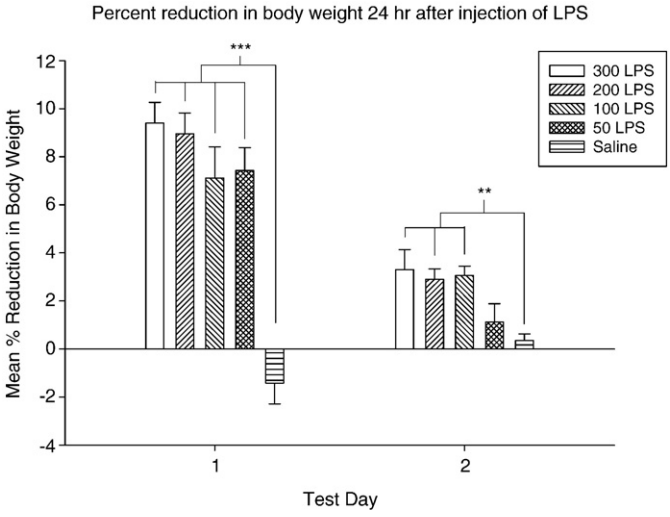

Fig. 4. Twenty-four hour percent reduction in body weight, 60–75 min following i.p. injections of lipopolysaccharide (LPS; 300, 200, 100 and 50 μg/kg, $n=9$, 10, 10 and 10 respectively) or saline vehicle (0.9% NaCl, $n=10$) on each of the 2 Test Days. **$p<0.01$, ***$p<0.001$ (compared with saline injected animals on the same day). Significant dose–response relationship (linear contrasts, $ps<0.001$) evident on Test Days 1 and 2. Values represent means ± S.E.M.

4. Discussion

The present experiment examined the effects of multiple doses of LPS on the air-puff tactile startle response and auditory PPI of the tactile startle response. It was found that, although the startle response magnitude was decreased in a dose-dependent manner following exposure to LPS, only the 300 and 200 μg/kg doses of LPS elicited significantly reduced tactile startle responses relative to controls. Habituation to the testing paradigm occurred in the vehicle control condition, but not with any of the LPS treatments. The normal PPI performance in the LPS treated animals also showed that "sickness" does not impair sensorimotor gating. On the basis of within group comparisons across test days, there was little evidence of behavioral tolerance to LPS on either the Startle-Only or PPI trials. However, when comparing day 2 startle magnitude between the LPS and saline control group, a relative level of tolerance could be observed. A reduction in the level of body weight loss was observed on the second test day, indicating physiological tolerance with the second exposure to LPS. Interestingly, in all of the treatment groups PPI measures were observed to increase from Test Day 1 to Test Day 2, suggesting increased sensitivity to the cross-modal prepulses on Test Day 2.

The present results revealed a significant dose-dependent relationship of decreasing tactile startle response magnitude with increasing dose of LPS on Test Day 1. The *a priori t*-tests revealed that both the 300 and the 200 μg/kg LPS doses exhibited decreased startle responses relative to vehicle controls on Test Day 1. The lower LPS doses exhibited non-significant decreases in tactile startle response magnitude, suggesting that high doses of LPS are necessary to produce this effect. These results confirm and extend previous findings that LPS administration is capable of reducing startle response magnitude in a dose-dependent fashion and that a relatively large dose of LPS (≥200 μg/kg in both studies) is required to produce impairment in the startle response (Lockey et al., 2009).

Within group behavioral tolerance to LPS was not observed, as LPS treated animals performed similarly on Test Day 1 and Test Day 2. However, the vehicle control group exhibited significantly decreased startle response magnitudes (habituation) on Test Day 2 relative to Test Day 1. The observation that the animals in the high dose LPS groups (300 and 200 μg/kg) showed non-significant inhibition in startle magnitude across test days indicates that there was less inhibition of startle on Test Day 2. This increase is suggestive of a tolerance effect, while the opposite pattern in the low dose LPS groups could represent a lack of habituation in these animals experiencing a reduced degree of sickness.

The present data showed a pattern of strong habituation for the Startle-Only trials in the vehicle control group, but a blocking of such habituation in the LPS animals. It has been suggested that in some mouse strains tactile stimuli induce a much stronger short-term habituation than acoustic stimuli (Simons-Weidenmaier et al., 2006). As well, long-term habituation was observed to develop faster with tactile than with acoustic startle paradigms (Plappert and Pilz, 2005). Additionally, LPS has been shown to impair memory/learning in several studies (Chan et al., 2009; Cross-Mellor et al., 2009; Pugh et al., 1998; Patil et al., 2003; Sparkman et al., 2005; Tanaka et al., 2006), and there is one study reporting impairment of low-level spinal learning with LPS treatment (Young et al., 2007). The vehicle control rats used in the present study displayed profound across session habituation to air-puff startling stimuli and LPS seemed to effectively block this habituation in the present experiment. Clearly, further investigation into the effects of LPS administration on basic learning paradigms such as habituation and sensitization are warranted.

The present PPI results revealed that the widely accepted relationship of increased inhibition levels with increasing prepulse intensity (Koch, 1999) was observed in each treatment group. This suggests that all of the LPS and saline treated animals were capable of detecting and processing prepulse sensory information in sufficient fashion to produce inhibition of the startle response. Therefore, the observed decrease in startle response magnitude in response to LPS likely reflects inhibition at the motor end of the sensorimotor reflex pathway responsible for startle generation.

Also, the present findings revealed a significant increase in prepulse effectiveness on Test Day 2 relative to Test Day 1, an effect independent of treatment. It has been previously reported that PPI measures in rodents are subject to modulation across acoustic startle response test sessions (Gewirtz and Davis, 1995; Faraday and Grunberg, 2000). However, in those previous examinations inhibition levels were shown to decrease across test sessions. In our previous study which used acoustic stimuli only (Lockey et al., 2009) there was no significant increase in PPI across test sessions suggesting that the sensitization/habituation of PPI may be dependent on the modality of the startle-inducing stimulus. The strongly increased PPI observed in this study on Test Day 2 may not be due to a greater PPI performance on Test Day 2, but rather due to a lower than normal PPI on Test Day 1. The use of a cross-modal acoustic prepulse to inhibit a tactile startling stimulus may require substantial exposure before normal PPI levels are observed. In this sense, the present observations suggest that cross-modal PPI measures may display differential habituation/exposure relationships relative to uni-modal PPI measures. Indeed it has been shown that cross-modal PPI performance varies according to rat strain and the modality employed (Bullock et al., 1997; Aubert et al., 2006; Torkamanzehi et al., 2008).

Findings from our previous study (Lockey et al., 2009), together with the present results, indicate that LPS decreases startle responses in a dose-dependent manner while sensory processing is largely unaffected. In both cases a minimum dose of 200 μg/kg LPS was required to significantly reduce startle response magnitude. The present study examined the inhibitory effects of LPS on air-puff induced startle, a multimodal startle stimulus which potentiates activation of the motor neurons of the startle associated nucleus (PnC) by convergence of the acoustic and tactile modalities (Yeomans et al., 2002). The consistency in findings across modalities strengthens the suggestion that, in response to LPS treatment, the reduction in startle magnitude is likely downstream of sensory inputs and possibly due to inhibition of PnC neurons themselves.

The finding, that startle response magnitude was reduced with sickness while sensory processing was conserved, suggests that inhibition of the motor reflexes is a more adaptive response than reduced sensory function. In times of severe immune compromise, there is a motivational reorganization of behavioral output (Hart, 1988) to reduce energy expenditure. Conservation of energy is thought to promote recovery from illness as it ensures that the immune system will have sufficient resources to mount a strong fever response (see Hart, 1988). By inhibiting the magnitude of the startle response in a dose-dependent fashion, but not abolishing it altogether, the motivational reorganization process may effectively be rationing energy allowance in relation to the extent of the insult (dose of LPS) without leaving the animal defenseless. Function of the sensory systems is likely spared to allow for detection of salient stimuli (e.g., presence of a predator) that can be processed and acted upon.

In conclusion, LPS induces a dose-dependent reduction in tactile startle response magnitude in male rats without affecting PPI of the tactile startle response. It is clear that reductions in the amplitude of the startle response are reliably exhibited in response to high doses of LPS (e.g. 300 and 200 μg/kg LPS), an effect that is not caused by sensory impairment. However, animals experiencing a lesser degree of sickness (e.g. the 100 and 50 μg/kg LPS groups) were observed to display relatively normal startle response profiles. Thus, deficits in non-voluntary motor function should be included in the accepted range of behaviors modified with severe sickness. Further studies examining the effects of LPS on other motor reflexes and low-level learning paradigms, such as habituation or sensitization, are clearly warranted.

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