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Lipopolysaccharide produces dose-dependent reductions of the acoustic startle response without impairing prepulse inhibition in male rats

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ABSTRACT

This study examined the dose-dependent effects of Lipopolysaccharide (LPS) on the acoustic startle response and prepulse inhibition (PPI) in male Long-Evans rats. LPS is known to stimulate the innate immune system and result in behavior modifications referred to as "sickness behaviors". The purpose of this study was to assess the ability of LPS to modulate sensorimotor reflexes (Startle-Only trials) and/or sensory processing (PPI trials). Rats were injected intraperitoneally with LPS (50, 100 or 200 μg/kg LPS, $n = 9$/group) or saline vehicle ($n = 14$) on 2 test days 72 h apart. Subjects were placed in a familiar startle box apparatus where startle response magnitudes were recorded following 115 dB Startle-Only trials and PPI trials (with prepulses at +3, +6 and +12 dB above background noise). Analysis of Startle-Only trials indicated a significant dose-dependent effect of LPS on Test Day 1. The 200 μg/kg LPS group exhibited significantly reduced startle response magnitude relative to all other treatments. On the PPI trials no LPS groups displayed significantly different performance from vehicle controls. Also, Day × Drug interactions for both Startle-Only and PPI trial types indicated behavioral tolerance to LPS. LPS reduced the acoustic startle response in a dose-dependent manner on Test Day 1. From the PPI data, it is evident that all treatment groups elicited near-normal inhibition levels indicating adequate sensory function. In combination, the results suggest that the range of sickness behaviors following LPS-administration to adult rats includes decreased non-voluntary motor activity as reflected by reduced startle magnitude.

1. Introduction

Lipopolysaccharide (LPS), the smallest immune system activating component of gram negative cell bacterial wall, is commonly used to model bacterial infection in both animals and humans. LPS administration, upon first exposure, engages the innate immune system leading to the development of the acute phase response (APR; Berczi et al., 2000; Heumann and Roger, 2002). The APR occurs as a result of immune activation, consisting primarily of the peripheral release of pro-inflammatory cytokines by circulating macrophages and monocytes. In this fashion, peripheral LPS exposure results in cytokine release that is distributed both throughout the periphery and in the central nervous system (Sagar, 1994; Linthorst et al., 1997; Dantzer et al., 1998a; Szelenyi, 2001; Rivest, 2003). Specifically, release of the cytokines interleukin–1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF–α) result in modified physiological and neural function (Kent et al., 1992; Roth et al., 1994; Wilder, 1995; Dantzer et al., 1998a,b; Berczi et al., 2000; Harden et al., 2006). The hypothalamic-pituitary-adrenal axis is also activated upon peripheral exposure to LPS and contributes to the development of the APR (Fricchione and Stefano, 1994; Torpy and Chrousos, 1996; Takemura et al., 1997; Berczi, 1998b; Beishuizen and Thijs, 2003; Johnson et al., 2003). The APR has also been observed to exert region specific modulations of several neurotransmitter systems including serotonergic, noradrenergic, and to a lesser degree dopaminergic systems (Dunn, 1992; Dunn et al., 1999; Lacosta et al., 1999).

Following LPS exposure a specific range of behavioral changes generally referred to as "sickness behaviors" manifests. These sickness behaviors primarily include, but are not limited to: hyperalgesia (increased sensitivity to pain), anhedonia (lack of pleasure), decreased locomotor activity and exploration, reduced food and water intake and increased time spent asleep (e.g. 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; Gaykema et al., 2008). Other characteristic responses to LPS include a strong pyrogenic response (fever) and a substantial decrease in body weight (e.g. Hart, 1988; Bluthe et al., 1992; Kozak et al., 1994; Roth et al., 1994; Berczi, 1998a; Tollner et al., 2000; Harden et al., 2006). After the first exposure to LPS animals develop an adaptive immune response and the activity of the innate immune system is diminished. As a result of this, upon secondary LPS exposure, tolerance develops and a decrease in both physiological and behavioral measures of sickness

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are often observed (Roth et al., 1994; Almeida et al., 1999; Engeland et al., 2001; West and Heagy, 2002; Franklin et al., 2003).

A consistent finding following LPS exposure is the substantial reduction of locomotor activity (Hart, 1988; Kozak et al., 1994; Lacosta et al., 1999; Engeland et al., 2001; Franklin et al., 2003; Dunn and Swiergiel, 2005; Harvey et al., 2006). This raises the possibility of a more generalized motor inhibition or motor deficit following LPS administration. It may then be expected that decreased performance on a variety of non-voluntary, motor responses or reflexes such as the acoustic startle response may also be evident. The acoustic startle response, a sensorimotor reflex, engages a defensive motor response to an intense burst of auditory stimulation (usually white noise at about 100–120 dB). The resulting reflex is a non-voluntary contraction of the skeletal muscles such that the subject will appear to jump (Hoffman and Ison, 1980). It has been thoroughly documented that the primary sensorimotor interface involved in the generation of the acoustic startle response is the caudal pontine reticular nucleus (PnC), a structure containing giant neurons which receive input from multiple senses and project to motor areas of the spinal cord (Koch, 1999).

Mammalian startle responses exhibit significant plasticity, in terms of prepulse inhibition (PPI), latency facilitation, habituation and fear potentiation (Braff et al., 2001; Swerdlow et al., 2001). PPI is a neural phenomenon producing a relative decrease in the magnitude of the startle response when the startle event is preceded 50–200 ms by a non-startle eliciting prepulse stimulus (Braff et al., 1999; Koch, 1999). PPI is a commonly used measure to operationalize "sensory gating", a process that allows the animal to allocate attentional resources to more salient stimuli in the environment (Braff et al., 2001). PPI deficits are common in patients suffering from psychosis and cognitive fragmentation (e.g. schizophrenia and Alzheimer's disease). Normal PPI performance requires adequate sensory detection, sensory processing and attentional capacity (Koch, 1999). Because of this clinical relevance, PPI measures have received considerable study in the recent literature.

Most studies on LPS have focused on physiological responses, behavioral responses such as feeding and voluntary movement, or memory deficits. Aside from a few studies (Fortier et al., 2007; Juszczak et al., 2008), the acute effects of LPS on non-voluntary motor tasks such as the startle response have received limited attention. Some experimental paradigms have used prenatal LPS exposure to model schizophrenia and have shown subsequent sensorimotor gating deficits (Fortier et al., 2004, 2007; Romero et al., 2006), but this reflects neurodevelopmental abnormalities rather than acute LPS effects. LPS administration has been observed to decrease locomotor activity, but it is unclear whether the impaired motor performance generalizes to specific reflexes of survival value. Thus, it is desirable to examine the effects of acute immune activation on non-voluntary reflexes (such as the acoustic startle response) in order to fully characterize the range of behaviors that LPS can modify. This will help in our understanding of motor based measures of sickness behaviors and the contribution of motor/physical deficits versus sensory/attentional influences.

Two studies recently examined the acute of effects of LPS on the acoustic startle response and PPI in rodents and concluded that neither the startle response nor PPI was influenced by LPS administration (Fortier et al., 2007; Juszczak et al., 2008). The pattern of results obtained by Fortier et al. (2007) revealed a non-significant trend towards decreasing startle response following LPS administration in adult rats. Juszczak et al. (2008) used a relatively low dose of LPS to elicit sickness in mice (1 μg/mouse or approximately 28 μg/kg) and this treatment failed to produce any significant effect on the acoustic startle response despite evident reductions on open-field, voluntary, locomotor behavior.

The present study is the first to examine the dose–response relationship for the acute effects of LPS administration on acoustic startle response and PPI in young adult rats, as well as the influences of behavioral tolerance to LPS. Body weight change was quantified in order to confirm dose effectiveness in eliciting sickness. The results suggest adult administration of LPS affects the startle response in a dose-dependent manner, but that PPI is largely unaffected.

2. Methods

2.1. Animals

Forty-one naïve young adult male Long-Evans rats (Charles River, Quebec) weighing between 272 and 301 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 rat chow) and water were available ad libitum, except during test sessions. All behavioral experiments and body weight measurements were carried out between 09:00 and 15:00 h (light phase of the light/dark cycle). All procedures and experimentation were carried out according to guidelines set out by the Canadian Council on Animal Care (CCAC).

2.2. Drugs

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

2.3. Apparatus

All acoustic startle response and PPI testing was conducted in 2 separate startle devices (SRLAB, San Diego Instruments, San Diego, CA). Each device consisted of a cylindrical, clear 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. This was placed 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 animal enclosure) which emitted the background, prepulse and startle noise stimuli. Beginning at startle stimulus onset, data were recorded and stored by a computer attached to the accelerometer. The animal's average startle amplitude in response to bursts of white noise 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 a 5 min acclimation period (continuous background noise at 70 dB). Upon completion of the acclimation period, the rats were returned to their cages. Enclosures were then cleaned with soapy water and rinsed thoroughly.

2.4.2. Test days

Acoustic startle response and PPI were assessed on 2 test days 72 h apart (Test Day 1 and Test Day 2). This allowed for the determination of tolerance and ensured there was minimal residual LPS in the system on Test Day 2. On test days, all rats were weighed at around 09:00 h before administration of 200, 100, 50 μg/kg LPS ($n = 9$ for each dose) or saline ($n = 14$). Animals were weighed again 24 h later for calculation of percent change in body weight.