EFFECTS OF STAPHYLOCOCCAL ENTEROTOXIN A ON IMPULSIVITY AND SPATIAL COGNITION IN C57BL/6 MICE

by

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ABSTRACT OF THE THESIS

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Background & Aims: Bacterial antigens have shown to cause cognitive deficits and impulsivity in mice that has been thought to be the result of an activated immune system. However these findings could be due to other factors, such as sickness behavior, which could interfere with the behavioral assessments of cognition and impulsivity.

Staphylococcal enterotoxin A (SEA) is a bacterial superantigen that does not induce sickness behaviour in mice. The aim of this study was to investigate the effects of SEA on spatial cognition and impulsivity. Methods: Saline or SEA (5 µg i.p) was injected in C57BL6 mice (n=8 per group). Changes in impulsivity were assessed on a 24-32 second differential reinforcement of low response rates schedule of reinforcement and changes in cognition were assessed with the Morris water navigation task (hidden platform and
probe trial) after a single or repeated injections of SEA. **Results:** SEA significantly decreased the number of food pellets mice earned (P=.01) but showed no effect on efficiency ratios (reinforced nose pokes / total nose pokes) with respect to controls, when tested on the DRL schedule. Compared with controls, SEA also increased stereotypy like behavior in mice, with increased responding after food reinforcement (P=.001) in the DRL schedule. Morris water navigation task showed decreases in latency and distance traveled to reach platform across days both in single (P< 0.05) and repeatedly (P<0.005) injected mice. However no differences in performance were seen between SEA and controls. **Conclusion:** Injection of SEA does not affect spatial cognition or induce impulsive behavior in mice; however, SEA induces behavioral changes similar to stereotypy.
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Introduction

The innate immune system recognizes and responds to highly conserved bacterial and viral epitopes through antigen presenting cells (APCs) such as macrophages and dendritic cells which possess receptors capable of binding epitopes (Iijima, Mattei et al.). Upon binding, APCs begin phagocytizing any nearby pathogens and present resultant protein fragments on their cell surface within a cleft on the major histocompatibility complex II molecule (MHC II) (Garbi, Hammerling et al. 2010). Only proteins encased within the cleft of the MHC II molecule are capable of stimulating T-cells (Garbi, Hammerling et al. 2010). The combination of peptide fragment and MHC II molecule determines the binding affinity between MCH II molecules and T-cell receptors and subsequently the ability of APCs to stimulate T-Cells (Garbi, Hammerling et al. 2010).

Superantigens are endotoxic proteins approximately 22-29 daltons in size and are produced by viral as well as bacterial microorganisms (Fraser and Proft 2008). Unlike normal antigens that stimulate less than 1%, superantigens stimulate up to 20% of T-cells in the organism (Fraser and Proft 2008; Urbach-Ross and Kusnecov 2009). There are over 20 known superantigens, each of which stimulates the adaptive immune system through different mechanisms. For example Staphylococcal protein A binds to B-cells while Staphylococcal enterotoxins A through K stimulate T-Cells (Anderson 2006). Staphylococcal enterotoxin A (SEA) is secreted by the bacteria Staphylococcus aureus and bypasses the innate immune system by cross linking MHC II molecules to T-cell receptors irrespectively of their affinity (Fraser and Proft 2008). The large number of T-cells activated by SEA results in a massive release of inflammatory cytokines such as IL-1, IL-2, TNFα, and IL-6 (Fraser and Proft 2008; Urbach-Ross and Kusnecov 2009).
Cytokines are proteins secreted by immune cells such as APCs, T-cells, B-cells, endothelial cells and almost all nucleated cells within the body and consequently can be found in the periphery and the central nervous system (CNS) (Miyamoto, Prause et al. 2003; Banks 2009). Cytokines provide intracellular communication between immune and non-immune cells within the organism. While the effect of cytokines ultimately depends on the cells they bind to, in general, they can be classified as inflammatory (IL-1, TNFα, IFN-γ, ect) or anti-inflammatory (IL-4, IL-10, IL-13, ect) (Lee and Lau 2011). Initially the function of cytokines was believed to be limited to the regulation of the immune response by recruiting effectors cells such as macrophages, T-cells and B-cell through direct binding or by facilitating chemotaxis to sites of injury and infection. However, more recent research has shown that cytokines, apart from immune responding, take part in a wide variety of biological processes such as embryogenesis (Saito 2001), synaptic scaling (Steinmetz and Turrigiano; Stellwagen and Malenka 2006), neurogenesis (Chohan, Li et al. 2009; Wolf, Steiner et al. 2009) and behavioral changes (Dantzer, O'Connor et al. 2008).

Since cytokines are proteins and cannot cross the blood brain barrier it was initially thought that peripherally located immune events were incapable of affecting the CNS (Dantzer, O'Connor et al. 2008; Banks 2009). However latter research in the 1980’s and early 1990’s showed a consistent pattern of behavioral alterations that accompanied peripheral immune activation denoted by malaise, decreased motor activity, anorexia, decreases in water intake, alterations in sleep pattern and anhedonia (Hart 1988; Dantzer, O'Connor et al. 2008; Tizard 2008). This constellation of behavioral changes was
eventually termed sickness behavior and was thought to be inducible by any form of immune stimulation regardless of antigenic source (Exton 1997; Tizard 2008). Cytokines were found to be the primary factors mediating sickness behavior (Bluthe, Crestani et al. 1992; Bluthe, Dantzer et al. 1992; Bluthe, Pawlowski et al. 1994) with receptors on vagal afferents and endothelial cells providing a route for peripherally located cytokines to influence the CNS (Bluthe, Walter et al. 1994). Further investigation throughout the 2000’s showed that immune activation induced not only sickness behavior but also alterations in psychological processes such as cognition and impulsivity. Deficits in cognition have been measured on a variety of behavioral tasks such as the Morris water maze navigation task, the delayed matching to sample task and the passive avoidance task (Hauss-Wegrzyniak, Vraniak et al. 2000; Gahtan and Overmier 2001; Jain, Patil et al. 2002; Sell, Crowe et al. 2003). These changes however may reflect changes in behaviors unrelated to cognition (such as appetite and locomotion) but that are involved in its behavioral assessment (Cunningham and Sanderson 2008). Similarly, while several studies have shown that cytokines such as IL-6 and TNFα increase impulsivity, these studies only examined behaviors that are related to impulsivity (aggression and risk taking) and did not directly measured impulsivity (Zalcman and Siegel 2006; Cortese, Konofal et al. 2008; Patel, Siegel et al. 2010; Sutin, Terracciano et al. 2010). Consequently, the changes reported in impulsivity and cognition following immune stimulation may actually reflect changes in intervening variables such as aggression and motivation but not impulsivity or cognition themselves.
Specific aims

I. Specific Aim I: Determine if SEA has an effect on impulsive behavior.

Increases in cytokines, such as IL-1, TNFα and IL-6 have been associated with changes in behaviors related to impulsivity, such as aggression, anxiety and risk taking, in animal and human studies. However, no direct assessment of the effects of cytokines on impulsivity has ever been made. To determine the effect that a general release of cytokines (like that seen during an infection) has on impulsive behavior, the performance of mice before and after systemic injection of SEA (an antigen that causes an unspecific but massive release of cytokines) will be assessed on a 24-32 second differential reinforcement of low response rates (DRL) schedule, a test traditionally believed to reveal deficits in response inhibition and/or impulsivity by counting the number of reinforced response relative to the total number of responses made. We hypothesize that SEA will increase impulsivity as increases in inflammatory cytokines have been shown to increase behaviors related to impulsivity.

II. Specific Aim II: Determine the effect of SEA treatment on spatial cognition.

Activation of the immune system by bacterial antigens such as LPS, has been shown to create deficits in spatial cognition while injection of cytokines such as IL-1 and IL-6 has been shown to improve spatial cognition. Inconsistencies between studies using bacterial antigens and cytokines may be due to decreased motivation and/or physical impairment following injection of bacterial antigens which mimic decreases in cognitive performance. As SEA elevates cytokines without physical impairment, an improvement
in spatial cognition should take place subsequent to an injection of SEA. To determine the effect that injection of SEA has on spatial cognition, mice will be assessed after systemic injection of SEA (an antigen that causes an unspecific but massive immune activation without motor or motivational impairments in mice) by measuring the time and distance traveled in the Morris water maze navigation task, a test traditionally used to measure spatial cognition. We hypothesize that SEA will improve spatial cognition in a similar manner to IL-1 and IL-6.
Methods

I. Animals:
C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 5-6 weeks of age. All animals were at least 3 months of age at the start of experimentation and were housed four per cage under 12 hr light/dark illumination (lights on at 6:00 A.M.). All experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of Rutgers University.

II. Immune Stimulation:
Mice were injected with either 0.2 milliliters of saline or 5 micrograms of SEA from Toxin Technology (Sarasota, FL) diluted in 0.2 milliliters of saline (25 μg /ml) via intraperitoneal injection 2 hours prior to the third day of DRL reinforcement, the first day of hidden platform training for singly injected mice or prior to the first three out of four days of hidden platform training for repeatedly injected mice.

III. Apparatus:

i. Operant Chambers: Test arenas (model H10-11M-TC) were placed into isolation cubicles and outfitted on the right wall with a single nose poke apparatus and an adjacent food pellet feeder and trough (product numbers H14-23M and H14-01M respectively). Arenas were controlled by Graphic State Software. All equipment and software were acquired from Coulbourn Instruments (Whitehall, PA). Feeders dispensed 20mg Dustless Precession Pellets produced by Bio-Serv (Frenchtown, NJ).
ii. **Morris Water Maze**: Morris water maze testing was conducted in a galvanized steel tub (diameter 110 cm, height 59 cm) that was filled with water to a height of 49 cm and tinted with white Crayola nontoxic paint and maintained at 22º Celsius. The platform was a circular (9 cm diameter) piece of clear Plexiglas with perforations to provide mice with traction affixed to a steel rod and placed 48 cm above the bottom of the tub and 1 cm below the surface of the water. During testing a white-cotton curtain was suspended around the perimeter of the pool to which spatial cues were affixed. Spatial cues were located 5-7 cm above the upper rim of the maze and consisted of a constellation of stars near the ‘east’ quadrant, a large cross near the ‘north’ quadrant, a rectangle of black and white stripes near the ‘south’ quadrant and a Jamaican Flag in the ‘west’ quadrant.

**IV: Procedure:**

i. **Differential Reinforcement of Low Response Rates Schedule (DRL):**

One week prior to the start of DRL testing mice were food deprived to 85% of their free feeding weight. DRL testing moved through four phases and followed the protocol reported by (Logue, Swartz et al. 1998) with individual sessions of all four phases lasting 30 minutes.

1. **Pre-training (Phase 1-2):** Pre-training encompassed phases 1-2 and consisted of progressing mice from a FR1 to a FR3 schedule of reinforcement. The criteria for advancing a mouse from phases 1 and 2 were that a mouse received at least 25 reinforcers during a single session.
2. Training (Phase 3): During phase 3 mice were presented with a 3-second auditory tone during which a single nose poke resulted in the presentation of a single food pellet and tone offset. If a mouse failed to respond within 3 seconds the tone terminated and no reinforcer was delivered. Each tone presentation was separated by a 30-second inter trial interval (ITI) and the criterion for completion of phase 3 was that a mouse made 10 reinforced nose pokes (i.e. one nose poke in 10 separate tone presentations) in a single session.

3. Testing (Phase 4): Within phase 4 tone was again presented for a 3-second interval but ITIs were shortened from 30 to 20-seconds and a variable period ranging in length from 1 to 8-seconds was added after the ITI. During the variable period no stimuli were presented but a nose poke resulted in the randomized selection of another 1 to 8-second variable period thereby delaying onset of the tone. Mice were run in phase 4 for 10 days.

ii. Morris Water Maze Testing:

1. Visible Platform Trials: Visible platform trials were performed for three consecutive days (-3 to -1) to identify any visual or motor deficits that could impair performance during subsequent testing. Daily sessions consisted of placing mice in the maze for four separate trials with an ITI of 2–3 minutes. During individual trials the escape platform was semi-randomly submerged in the center of one of the four quadrants with a circular, three dimensional, multicolored flag indicating its location. Mice were run in squads of four and were towel dried and placed in heated cages between trials.
2. **Hidden Platform Trials**: Hidden platform trials began 2 days after the completion of visible platform trials and consisted of four trials per day with a 2-3 minute ITI. Hidden platform trials were conducted for five consecutive days (1-5) for singly treated subjects and for four consecutive days for repeatedly treated mice (1-4) as well as one moth later (day 36) for singly and repeatedly injected mice. Platform location remained stable during hidden platform testing while a novel start location was given for each trial. Start locations were designated as north, south, east, and west and selected in a semi random manner such that for each mouse all locations were tested on a given day.

3. **Probe Trials**: On the 5th and 36th day of hidden platform testing for singly and repeatedly treated subjects the platform was removed and mice were placed in the maze for 60 seconds.

V. **Behavioral Measures**:

   i. **DRL Testing**:

   DRL measurements were split into ‘overall operant responding’, ‘overall operant response pattern’ and ‘operant responding during individual components of differential reinforcement’.

   1. **Overall Operant Responding**: Overall operant responding included the number of reinforced nose pokes and the total number of nose pokes for each daily session of phase 4. Additionally, an efficiency ratio was calculated by dividing the total number of reinforced responses by the total number of responses for each session. This efficiency
ratio was designed to account for the raw response rate of individuals and correct for any possible differences in motivation.

2. **Overall Operant Response Patterns During Daily Sessions:** Overall operant response patterns during daily sessions were derived by dividing trials into one second bins and summating the number of responses across all trials within a single daily session.

3. **Operant Responding During Individual Components of Differential Reinforcement:** Operant responding during individual components of differential reinforcement (see figure 1) were obtained by subdividing the number of responses for a given session into the different phases of the trial in which they occurred (tone presentation, post food ITI, non food ITI, and pre auditory stimulus interval (PASI)) with measures including the number of responses made in a given phase and the average number of responses per occurrence within a given phase.

   **ii. Morris water maze Testing:**

   All Morris water maze sessions were videotaped and analyzed using SMART software (Spontaneous MotorActivity Recording & Tracking; San Diego Instruments, San Diego) which calculated the latency and distance traveled to locate the escape platform for visible and hidden platform testing, as well as the time and distance traveled in the escape (quadrant formerly containing the escape platform) and other quadrants, for the probe trials.

   **VI. Statistical Analysis:**
For DRL testing trials 3 through 10 (post treatment trials) were analyzed by repeated measures ANCOVA with days 1 and 2 loaded as covariates (SPSS, Ver 18). For MWM visible platform trials, hidden platform trials and initial versus retest probe trials were analized by repeated measures ANOVA (Statview, Ver 5.0.1). An alpha level of 0.05 was set for all statistical analysis.
Results

I. Impulsivity Testing

i. Overall Operant Responding: Across the 10 days of DRL reinforcement mice treated with saline and SEA showed steady decreases in overall number of nose pokes from initial averages of 171±65.27 (mean ± standard error) nose pokes and 143±36.09 (saline, SEA) nose pokes on day one down to 107.5±12.06 and 55.25±12.30 nose pokes by day 10. While decreases were seen in overall nose pokes, number of food pellets increased from averages of 8.25±2.29 and 13.33±3.33 (saline, SEA) on day one to 45.25±6.02 and 22.33±7.22 food pellets by day 10. Corresponding to decreases in nose pokes and increases in number of food pellets efficiency ratios increased from initial averages of 0.113±0.0596 and 0.128±0.471 (saline, SEA) on day one to averages of 0.613±0.125 and 0.468±0.104 by day 10 (figure 2). Repeated measures ANCOVA only showed increases in efficiency ratio across days to be significant \(F(7,12) = 5.016, p < 0.0001\) with neither nose pokes nor amount of food pellets earned significantly changing across days. No main of effect of treatment was seen for any of the measures however a significant interaction between treatment and day was seen with SEA injected mice obtaining less reward \(F(7,12) = 3.431, p < 0.01\) and making fewer nose pokes\(F(7,12) = 2.444, p < 0.05\) in latter trails than saline controls (figure 2).

ii. Operant Responding During Daily Session of DRL Schedule of Reinforcement:

Corresponding to changes observed in overall measures of DRL reinforcement, changes were also seen in the response pattern of saline and SEA injected mice across days (figure 3). Mice initially showed a tendency to time the interval between tone
presentations as evidenced by a sharp increase in responding during PASIs. This
tendency gradually faded over days, as mice slowly increased the number of responses
made during tone presentations and decreased the number of responses made during all
other portions of the trial (figure 3).

**iii: Operant Responding during Individual Components of DRL Schedule of Reinforcement:** As with overall measures, operant responding during individual stages of DRL reinforcement was found to decrease during stages unrelated to food reinforcement (ITIs and PASIs) and increased during tone presentations. Average number of nose pokes during non food ITIs decreased from averages 1.31±0.348 and 1.44±0.531 (saline, SEA) on day one down to averages of 0.587±0.220 and 0.220 ±0.560 by day 10. Also the total number of responses made during PASIs decreased from totals of 84.14±21.03 and 57.63±19.375 (saline, SEA) on day one down to totals of 24.166±16.414 and 8.125±5.108 by day 10. As animals responded less during non food ITIs and PASIs, the percentage of tones to which animals responded increased from initial averages of 14.4%±3.1 and 26.4%±6.7 on day one up to averages of 47.67%±13.7 and 37.1±8.57 by day 10. As with overall food reward, a significant interaction was seen for the percentage of tones to which animals responded ($F(7,12) = 3.391$, $p < .005$), with SEA treated animals responding to fewer tones that saline controls in later trials (figure 4). However after receiving a food pellet mice injected with SEA made significantly more nose pokes during the ITI than saline controls($F(7,12) = 8.822$, $p < 0.001$) with the greatest discrepancy on day 5 with saline injected mice averaging
0.25±0.08 nose pokes per post food ITI and SEA injected mice averaging 1.07±0.26 nose pokes per post food ITI.

II. Cognition Testing

   i. Visible Platform Testing:

   Prior to any treatment, mice destined for a single injection of saline or SEA showed similar decrease in average latency from 33.50±3.54 (mean ± standard error) and 35.50±2.231 seconds (saline, SEA), on day -3 of testing, down to latencies of 7.0 ±0.1 and 6.125 ±0.6 seconds on day -1. Decreases in latencies to reach the platform were found to be significant by repeated measures ANOVA across trials (F(2,14)=85.147, P<0.0001) but no difference between future treatment groups were seen.

   Repeatedly treated subjects also showed a decrease in the average latency from 28.3±2.7 and 27.6 ±5.1 seconds (saline, SEA) on day -3 of visible platform trials down to averages of 6.6± 0.21 and 8.5 ± 0.9 seconds by day -1. Repeated measures ANOVA revealed this decrease across days to be significant (F(2,14)=42.253, P<0.0001) but showed no effect associated with future treatment.

   ii. Hidden Platform Testing:

   After a single injection of SEA, or saline, mice showed comparable decreases in latency and distance traveled to find the platform over hidden platform trials. Figure 5a shows that latencies decreased from 23.7±3.9 (mean ± standard error) and 27.04±5.273 seconds
(saline, SEA) on day one down to latencies of 16.0±2.8 and 16.4±4.1 seconds by day five while distance to reach the escape platform decreased from initial averages of 375.8±51.9 and 439.9±84.7 cm (saline, SEA) on day one down to averages of 222.5±47.324 and 220.1±57.4 cm by day five (figure 5c). Repeated measures ANOVA showed decreases in time and distance across days to be significant (F(4,14)=2.660, P<0.05), (F(4,14)=4.824, P<0.005) but showed no effect of treatment, with both groups learning at equivalent rates. Day 36 hidden platform testing showed that both groups were still capable of locating the escape platform with mice taking 24.9±5.9 and 25.6±4.9 seconds (saline, SEA) and traveling 293.7±82.5 and 367.5±43.3 cm to locate the escape platform.

During hidden platform trials repeatedly injected mice showed a decrease in latency to find the escape platform from initial averages of 23.0±4.1 and 18.125±2.5 seconds (saline, SEA) on day one down to averages of 4.8±0.6 and 4.7±1.1 seconds by day four. Average distances also dropped from 408.9±60.6 and 413.3±58.7 cm (saline, SEA) on day one down to averages of 103.4±12.6 and 127±15.3 cm by day 4. Decreases in both time and distance traveled across days were significant (F(3,14)=23.740, P<0.001), (F(3,14)=22.717, P<0.005) but no effect of treatment was seen with both saline and SEA treated mice showing equivalent learning rates. Day 36 hidden platform testing showed that both groups took more time (15.7±3.6 and 22.8±2.1 (seconds, saline, SEA) and distance (311.6±63.7 and 246.9±39.0 cm) to locate the platform than they did on day 4.
iii. **Probe Trials:**

During probe trials mice that received a single injection spent significantly less time and traveled significantly less distance in the escape quadrant on day 36 than on day 5.

Figure 5e and 5h show that mice initially spent 25.6±2.9 (mean ± standard error) and 28.4±3.6 seconds (saline, SEA) in the escape quadrant on day 5 and 14.5±3.1 and 21.9±2.8 seconds on day 36 and traveled 395.0±32.0 and 422.4±52.2 cm (saline, SEA) in the escape quadrant on day 5 and 222.4±47.4 and 313.9±30.5 cm in the escape quadrant on day 36. Repeated measures ANOVA showed both, decreases in time (F(1,14)=6.738, P<0.05) and distance (F(1,14)=9.318, P<0.01), to be significant.

In terms of probe trials mice that received repeated injections spent equivalent amounts of time and traveled similar distances in the escape quadrant on day 5 and day 36. Figure 5f and 5i show that mice initially spent 24.8±2.6 and 29.5±1.9 seconds (saline, SEA) in the escape quadrant on day 5 and 24.2±1.9 and 30.3±2.5 on day 36 and traveled 448.6±42.18 and 548.0±30.2 cm (saline, SEA) in the escape quadrant on day 5 and 486.3±42.08 and 561.3±52.0 cm on day 36. Repeated measures ANOVA failed to show effect of day or treatment, for either time or distance spent in the escape quadrant, in repeatedly injected subjects.
Discussion

Impulsivity can refer to a wide variety of behaviors but is generally used to refer to either the premature performance of a behavior, the execution of a contextually inappropriate behavior or the execution of a behavior with long term negative consequences. While often considered solely in terms of its role in the etiology of pathologic behavior, impulsivity plays an important role in an animal’s behavioral repertoire, as it allows for the initiation of novel reactions to environmental situations (Evenden 1999). As there are a variety of behavioral manifestations of impulsivity there are also a wide variety of effects of impulsive responding. For example impulsivity can result in the diminishment, delay or withholding of a reward, or the elicitation of a negative outcome. Negative outcomes are commonly considered when impulsivity results in addictive or aggressive behaviors, while diminishment of reward is more often associated with premature responding (Evenden 1999). Pathologic levels of impulsivity are often classified by their underlying mechanisms. A common classification of impulsivity divides impulsivity into: motor impulsivity (initiation of an action before any thought towards a behavior can occur), attentional impulsivity (initiation of a premature or inappropriate behavior due to a decreases in attention towards the behavioral task), and cognitive impulsivity (lack of evaluation of the impact of a behavior, like that of quick or “hasty” decisions) (Buss and Plomin 1975; Dickman 1993; Patton, Stanford et al. 1995; Barratt 1994; Patton, Stanford et al. 1995). A common method used to evaluate these types of impulsivity is the DRL schedule. DRL testing assesses the ability of mice to select and time the appropriate moment and manner in which to respond to a stimulus and consequently can be used to assess all three aspects of impulsivity (motor, attentional and cognitive).
In this study, mice injected with saline and SEA showed evidence of learning over the course of DRL reinforcement, as mice made fewer overall responses but earned more food pellets across days, resulting in an improvement of the efficiency scores of saline and SEA treated mice. Corresponding to increases in efficiency scores, saline and SEA treated mice gradually shifted from responding before tone onset to waiting until tone to respond. The responding during individual stages of DRL reinforcement, for saline and SEA treated mice, also indicated learning, as responding decreased during stages when tone was absent (ITIs and PASIs) and increased during tone presentation. Effect of treatment was seen with mice that were injected with SEA. SEA treated mice decreased the number of tones to which they responded, and subsequently earned fewer food pellets, relative to saline controls in the later trails. While increased responding during post food ITIs by mice injected with SEA relative to saline controls was aberrant, it does not indicate an increase in impulsivity, as this response pattern was neither premature nor it resulted in a negative outcome. Rather, increases in responding were focused during a portion of the schedule which did not result punishment. This focused response pattern is inconsistent with the attentional, motor or cognitive deficits which form the basis of impulsivity (Buss and Plomin 1975; Dickman 1993; Evenden 1999; Sanabria and Killeen 2008) as mice were still mindful to decrease their responding prior to tone onset and showed no change in their efficiency ratios relative to saline controls. The increase in responding during post food ITIs and the decrease in responding to tone presentations are more consistent with SEA inducing stereotypy like behavior as mice responded in a “coordinated, patterned, repetitive… and purposeless but seemingly purposeful” manner, which is in line with the definition of stereotypy. The interpretation that SEA induces an
increase in stereotypy is consistent with prior studies in which injection of cytokines such as IL-2, IL-6 and IL-1 induce behaviors similar to stereotypy (such as climbing) (Zalcman 2002) and potentiate stereotypy induced by dopamine agonists (Zalcman, Savina et al. 1999; Zalcman 2001).

Similarly to impulsivity, cognition is an abstract term that refers to different mental processes not very well defined in the literature but is often used synonymously with intelligence (Cromwell and Panksepp 2011). Frequently, it is divided into specific subdomains according to type of mental process and their associated brain regions (Henson and Gagnepain 2010). Common subdomains are speed of processing, attention, working memory, problem solving, and verbal and spatial cognition (Nuechterlein, Barch et al. 2004; Persson, Wallin et al. 2009). Recently, some researchers have also included emotional and social cognition as additional subdomains where previously they have been treated as non cognitive processes (Nuechterlein, Barch et al. 2004).

Spatial cognition is dependant on the encoding of spatial features of the environment into a cognitive map (Olton 1977; Suzuki, Augerinos et al. 1980). Successful formation of a cognitive map requires encoding of both individual spatial cues within the environment and spatial relations of between cues (Suzuki, Augerinos et al. 1980). Extensive research has shown that the neurons in the hippocampus known as place cells provide the neural basis for cognitive maps as they fire in response to an animal’s specific orientation within a given environment (O'Keefe and Dostrovsky 1971; Brown, Frank et al. 1998). One of the most frequent means for assessing spatial cognition (particularly the relation of
immune stimulation to spatial cognition) has been the Morris water navigation task (Cunningham and Sanderson 2008).

In this study, data from visible platform testing of the Morris water navigation task, showed no group differences prior to treatment and that mice did not possess any visual or motor deficits which could hamper performance during hidden platform testing. Days 1-5 of hidden platform testing showed that neither single nor repeated injections of SEA impaired spatial cognition of the mice, as SEA and saline treated mice showed nearly identical decreases in latency and distance to reach the escape platform across days. Results obtained from the probe trials on day 5 demonstrate that mice used a spatial path finding strategy to locate the hidden platform, as mice that received single or repeated injections of saline or SEA spent and traveled similar amounts of time and distance in the escape quadrant. Probe trials on day 36 also failed to show differences between mice singly and/or repeatedly injected with SEA relative to saline injection. However mice that received repeated injections of SEA or saline spent more time and traveled more distance in the escape quadrant on day 36 probe trials than mice singly injected with saline or SEA.

Difference between singly injected and repeatedly injected mice probably do not reflect differences in treatment schedules, as repeatedly injected mice preformed better than singly injected mice on day 1 of hidden platform testing, at which point both groups had been treated identically. These differences more likely reflect an effect of age, as mice which received repeated injections were approximately 3 months younger at the start of
testing than mice that received single injections. While this difference in age is smaller than what is often reported, other studies have seen similar differences between young and middle aged mice (Francia, Cirulli et al. 2006; von Bohlen und Halbach, Zacher et al. 2006; Pan, Sciascia et al. 2008; Pawlowski, Bellush et al. 2009).

The lack of cognitive deficits following SEA treatment is consistent with data from earlier studies in which injection of bacterial antigens increase the latency to reach escape platform, but not the distance traveled to reach escape platform (Sparkman, Martin et al. 2005; Thomson and Sutherland 2006; Cunningham and Sanderson 2008; Cunningham, Campion et al. 2009). This decrease in speed to locate the escape platform, seen in this and previous studies, indicates that what were previously perceived as cognitive deficits actually reflect decreases in swim speed, that are due to the state of malaise which accompanying any injection of bacterial antigens. Results from this study suggest the need to revise the current model of immune-CNS interactions and for the current findings to be validated across a variety of behavioral measures of cognition.
Conclusion

This study is the first to directly assess the effect of an immune stimulus on impulsivity and to examine the effect of SEA on spatial cognition. It was found that injection of SEA does not lead to changes in either impulsivity or spatial cognition, and suggests that prior findings of cognitive deficits or increases in impulsivity following injection of bacterial antigens or cytokines may reflect changes in other intervening behaviors such as sickness behavior, aggression or stereotypy. In conclusion, injection of SEA does not affect spatial cognition or induce impulsive behavior but does induce a behavior similar to stereotypy.
Figure 1: Graphic representation of DRL Training and Reinforcement.

A) Schematic representation of DRL training. **Tone Presentation (3seconds)**. Nose poke is reinforced with food pellet. Lack of a nose poke results in termination of tone without reinforcement. **Inter-trial Interval (30 seconds)**. No stimuli or reinforced response.

B) Schematic representation of DRL reinforcement. **Tone Presentation (3seconds)**. Nose poke is reinforced with food pellet. Lack of a nose poke results in termination of tone without reinforcement. **Inter-trial Interval (20 seconds)**. No stimuli or reinforcement. **Pre-auditory Stimulus Interval (1-8 seconds)**. Nose poke results in selection of another 1-8 second pre auditory stimulus interval and the delay of tone onset.
Figure 2: Overall operant responding during DRL schedule.
Operant responding of mice (n=8 per group) injected with either saline or 5ug SEA. Graphs show average number of nose pokes (a), total number of reinforced responses (b), efficiency ratio (food pellets/total nose pokes) for each 30 minute daily test session (c). Black arrows represent injection.
Figure 3: Overall Operant Response Pattern during daily sessions.
Distribution of responding (number of nose pokes), across ITIs on day 1 (a), day 4 (b), and day 10 (c).
ITI= Intertrial Interval (sec 1-20); PASI= pre-auditory interval (sec 1-8).
x axis=time in seconds.
Figure 4

Figure 4: Operant responding during individual components of DRL schedule. Operant responding of mice after injection with either saline (n=8) or 5ug of SEA (n=8) on day 3 of testing. Graphs show average number of nose pokes per each post food ITI (a), non food ITI (b), total number of nose pokes per pre-auditory stimulus interval (c), and tone presentation (d).
**Figure 5: Morris Water Maze navigation task.** Hidden platform and probe trials of mice injected either with saline or SEA (single or repeated injection), n=8 per group. Graphs show latency (a, b) distance traveled to platform (c, d), time spent per quadrant in probe trial (e, h), and distance traveled per quadrant in probe trial (f, i). Arrows represent injection of saline or SEA (solid) or only saline (dashed). Q= quadrant.
References


