



# *Cronobacter sakazakii* infection alters serotonin transporter and improved fear memory retention in the rat

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It is well established that *Cronobacter sakazakii* infection cause septicemia, necrotizing enterocolitis and meningitis. In the present study, we tested whether the *C. sakazakii* infection alter the learning and memory through serotonin transporter (SERT). To investigate the possible effect on SERT, on postnatal day-15 (PND-15), wistar rat pups were administered with single dose of *C. sakazakii* culture (infected group; 10<sup>7</sup> CFU) or 100  $\mu$ L of Luria-Bertani broth (medium control) or without any treatment (naïve control). All the individuals were subjected to passive avoidance test on PND-30 to test their fear memory. We show that single dose of *C. sakazakii* infection improved fear memory retention. Subsequently, we show that *C. sakazakii* infection induced the activation of toll-like receptor-3 and heat-shock proteins-90 (Hsp-90). On the other hand, level of serotonin (5-hydroxytryptamine) and SERT protein was down-regulated. Furthermore, we show that *C. sakazakii* infection up-regulate microRNA-16 (miR-16) expression. The observed results highlight that *C. sakazakii* infections was responsible for improved fear memory retention and may have reduced the level of SERT protein, which is possibly associated with the interaction of up-regulated Hsp-90 with SERT protein or miR-16 with SERT mRNA. Taken together, observed results suggest that *C. sakazakii* infection alter the fear memory possibly through SERT. Hence, this model may be effective to test the *C. sakazakii* infection induced changes in synaptic plasticity through SERT and effect of other pharmacological agents against pathogen induced memory disorder.

**Keywords:** *Cronobacter sakazakii*, animal model, fear memory, Hsp-90, SERT, microRNA-16

## Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated as the modulator of learning and memory with special preference to consolidation of new information into long-term memory (Kandel, 2001). 5-HT play a key role in memory formation by interacting with other neurotransmitters/exerts its effect through their seven (5-HT<sub>1</sub>–5-HT<sub>7</sub>) subclass of receptors (Meneses, 1999, 2007, 2013; Meneses et al., 2011; Perez-Garcia and Meneses, 2009; Hoyer et al., 2002). Although evidence from *Aplysia* to human points at a functional role of serotonergic transmission in learning and memory, the underlying mechanism is depends on the level of 5-HT (Barbas et al., 2003; Meyer et al., 2009), and the depletion of 5-HT could affect the memory formation (Kaang et al., 1993; Barbas et al., 2003; Seyedabadi et al., 2014; Stansley and Yamamoto, 2015).

Serotonin transporters (SERT) play a key role in clearance of the released 5-HT through transport across pre-synaptic membrane and maintain the homeostasis of 5-HT level. In addition, expression status of SERT protein could control the duration and intensity of 5-HT activity at synapse (Gainetdinov and Caron, 2003; Tellez et al., 2012; Yoon et al., 2013; Bravo et al., 2014). Earlier studies reported that expression of SERT protein regulated by the interacting molecules such as ribonucleoprotein (RNP) and sequence specific microRNA (miR; Standart and Jackson, 1994; Wilkie et al., 2003; Bartel, 2009; Croce, 2009; Gyawali et al., 2010; Goldie and Cairns, 2012; Hartley et al., 2012). At this point, heterogeneous nuclear ribonucleoprotein K (hnRNPK) and miR-16 appears to negotiate for the binding site at 3'-untranslated region (UTR) of SERT and regulate the repression/depression of translation (Baudry et al., 2010; Yoon et al., 2013).

Over the past few years, research has been conducted to understand the pathogenicity mechanism, genetic, nature of survival and molecular characterization of virulence in *Cronobacter* spp. (Jaradat et al., 2014). Reports have shown that source of *Cronobacter* infection was the powder infant formula (PIF; Yan et al., 2012), apart from that significant association was found with contaminated home environment (Kandhai et al., 2004), packed foods (Friedemann, 2007) and drinking water (Liu et al., 2013). In parallel, Clinical and laboratory studies reported that they have resistance to heat, desiccation and acid stress growth condition (Breeuwer et al., 2003; Edelson-Mammel et al., 2005; Dancer et al., 2009), and *Cronobacter* infection in neonates and infants cause meningitis, necrotizing enterocolitis (NEC) and sepsis with case fatality rate ranging from 40 to 80% (Muytjens et al., 1983; Block et al., 2002; Hyun-Lee et al., 2011; Yan et al., 2012; Hunter and Bean, 2013). However, *Cronobacter* infection also have been reported in elder patients or immunocompromised persons (Healy et al., 2010), among them 50% had an underlying malignancy (Lai, 2001; See et al., 2007). In addition, *Cronobacter* infection have been linked to conjunctivitis, osteomyelitis, diarrhea, acute cholecystitis, and wound infection (Gosney et al., 2006; Flores et al., 2011; Yan et al., 2012; Tsai et al., 2013). Pathogen induced neuroinflammation can alter the behavior possibly either through hypothalamus pituitary-adrenal (HPA) axis or neurotransmitter system through the interacting molecules (Pérez et al., 2009; Herrero et al., 2015). In fact, several line of studies reporting that responding to the endotoxin (lipopolysaccharide, LPS) produced by the pathogenic bacteria, the host system activate innate immune response, in which different toll-like receptors (TLRs) and heat-shock proteins (Hsp) are part of it (Pandey and Agrawal, 2006; Okun et al., 2009; Chen et al., 2014), TLRs in dendritic cells play critical role (Stanislawski et al., 2004) and 5-HT transmission (Desbonnet et al., 2008; van Heesch et al., 2014; Depino, 2015). Currently, very little information is available on the pathogen infection mediated effect on serotonergic system. Therefore, the present study is designed to examine the effect of *Cronobacter sakazakii* infection on postnatal rats' serotonergic system particularly on SERT and associated changes in learning and memory.

## Materials and Methods

### Bacterial Strain and Media

The bacterial strain *C. sakazakii* was obtained from American Type Cell Culture (ATCC BAA-894). The obtained bacterial strain was cultured on the selective chromogenic *Enterobacter sakazakii* agar medium (Song et al., 2008). The positive blue-green colonies were picked and grown on 1.5% Luria-Bertani (LB) agar. The overnight culture was prepared in the LB broth, which was maintained at 37°C in an incubator shaking at the rate of 145 rpm. Serial dilution and plating method was used to assess the bacterial concentration (Miller, 1972). In detail, 3 h culture of *C. sakazakii* was examined through biophotometer (Eppendorf Inc) at O.D<sub>600</sub>. The bacterial culture was serially diluted and plated on LB agar for colony counting. Based on colony counting assay result, concentration of bacterial cells was calculated. Bacterial concentration of 10<sup>7</sup> CFU was fixed as infectious dose for the present study based on LC<sub>50</sub> analysis.

### Animals

Timed-pregnant wistar rats at gestation day-15 were acquired (Sri Venkateshwara Enterprise, Bangalore, India), acclimated and maintained under controlled ambiance (12 h light/dark cycle; temperature: 22 ± 2°C; humidity: 50 ± 5%). The pregnant rats were housed individually in a standard laboratory cage (43 cm × 27 cm × 15 cm) with saw dust as bedding material, and food and water provided *ad libitum*. This study was carried out in accordance with the recommendation of Institutional Animal Ethics Committee (IAEC), Bharathidasan University (BDU). The animal experimental protocol was approved by IAEC, BDU.

### Experimental Groups

Wistar rat pups at the age of postnatal day-15 (PND-15) were used as host system for the present study. Pups from different litters were randomly divided into three different groups: naïve control (NC), medium control (MC), and infected (IF) group. Rat pups in NC groups were maintained at normal condition without treatment. MC groups were treated with single dose of LB (100 µL) and IF pups with *C. sakazakii* culture (10<sup>7</sup> CFU) on PND-15 by oral gavage. Then the animals were maintained at typical condition with mother.

### Confirmation of Infection

On PND-30, the amygdala region was dissected out as described by Kalin et al. (1994) from NC and IF group rats ( $n = 3$  from each group) and homogenized in phosphate buffer saline (PBS). The homogenate was serially diluted up to 10<sup>-4</sup> with PBS and plated on specific medium to identify *C. sakazakii* (Hirome *E. sakazakii* agar; Himedia cat. No. M1641-100G) and incubated at 37°C for overnight to observe the presence of *C. sakazakii* in brain tissue.

### Behavioral Test

#### Passive avoidance test

Passive avoidance apparatus was constructed following the specification of Zare et al. (2015). The apparatus consisted of equally sized light and dark compartments (20 cm × 40 cm × 20 cm) made up of Plexiglas separated

by a guillotine door (12 cm × 12 cm). The floor of both chambers were made up of stainless steel rods (3 mm diameter) spaced 1 cm apart but the gridded floor of the dark chamber could be electrified using a shock generator. All the experiments were conducted between 09:00 and 18:00 h. All groups (NC,  $n = 14$ ; MC,  $n = 20$ ; IF,  $n = 29$ ) were subjected to step-through passive avoidance test, in which the rats were trained to the criterion and tested for their retention 24 h post-training. Each time after removing the animal, the apparatus was wiped with 70% ethanol to remove odor. During each experiment the experimenter handle the animals for <60 s.

### Exploration and training

On PND-31, each animal was placed in the light compartment of the apparatus facing away from the door and 10 s later the guillotine was raised. The animal was left for 5 min to habituate the apparatus. On PND-32, each animal was trained for the criterion. The rat was placed in the light compartment of the apparatus facing away from the door and 10 s later the guillotine was raised. When the animal had placed at all four paws in the dark compartment, entrance latency to the dark compartment was recorded. Once the animal entered into the dark compartment, the door was closed and an inescapable foot shock (0.5 mA) was applied for 5 s. After 20 s, the animal was retrieved from the dark box and placed back into their home cage. After 2 min, the procedure was repeated. The rat received foot shock each time it placed its four paws into the dark compartment. The training was terminated when the rat remained in light compartment for 120 s consecutively. Number of trials required for training the animal was recorded.

### Retention test

On PND-33, retention test was performed 24 h post training. The rat was placed on the light compartment and 10 s later the door was raised. The step-through latency and time spent in dark compartment was recorded up to 300 s. If the rat did not enter the dark compartment within 300 s, a score of 300 s was assigned.

### Neurotransmitter Analysis

On PND-30, group of rats from NC ( $n = 5$ ), MC ( $n = 5$ ), and IF ( $n = 5$ ) were euthanized, and the amygdala region was dissected as described elsewhere (Kalin et al., 1994) and frozen on dry ice. The tissue samples were weighed and homogenized in a glass homogenizer with 0.1 M perchloric acid containing 4.5 mM Na<sub>2</sub>EDTA and 1.6 mM reduced glutathione. The homogenates were centrifuged at 12,000 rpm for 20 min at 4°C. The supernatants were collected in a fresh tube and stored at -70°C. The level of 5-HT was estimated with a 5-HT ELISA kit (Biosource, Europe S.A., Belgium) by following the manufacturer's instructions. The concentrations of 5-HT in each tissue samples were calculated by comparing the optical density of the sample (mean for duplicates) with that of the standard curve.

### Sample Preparation

On PND-30, group of rats from NC ( $n = 5$ ), MC ( $n = 5$ ), and IF ( $n = 5$ ) groups were euthanized and amygdala region was dissected out from and divided into two part for the

preparation of total RNA and protein. Total RNA was isolated from the tissue samples following the manufacture' instructions (Trizol method; Merck, Bangalore, India) and stored at -70°C with RNase inhibitor (1U/μL; Rnasin, Promega, Madison, WI, USA). Total RNA (1 μg) was converted into cDNA by following manufacture' instructions (QuantiTect® Reverse Transcription Kit; catalog no. 205311, Qiagen, Germany). Tissue samples were homogenized in 300–400 μL of ice cold lysis buffer (150 mM NaCl, 50 mM Tris-HCl; pH 7.5, 5 mM EDTA, 0.1% v/v NP-40, 1 mM DTT, 0.2 mM sodium orthovanadate, 0.023 mM PMSF) with protease inhibitor cocktail (10 mg/mL; Sigma-Aldrich, USA), and incubated on ice for 30 min. The homogenate was centrifuged at 10,000 g for 30 min at 4°C. The supernatant was collected in a fresh tube and again centrifuged at 12,000 g for 30 min at 4°C. The supernatant was extracted and stored at -70°C.

### Quantitative Real-Time PCR

The quantitative real-time PCR (qRT-PCR) was performed in CFX-96 Touch™ Real-time PCR detection system using SSoAdvanced™ SYBR® green mix (Bio-Rad Laboratories, Inc., USA). The level of mRNA of the selected genes were assessed through qPCR using specific primers: *Tlr-3* (for 5'-ACAATGCCCAACTGAACCTC-3' and rev 5'-CGGAGGCTGTTGTAGGAAAG-3') and *miR-16* (for 5'-CCGCTCTAGCAGCAGCGTAAA-3' and rev 5'-CCCTGTCACACT AAAGCAGC-3'). The level of *Tlr-3* and *miR-16* was normalized with internal control GAPDH (for 5'-AACATCATCCCTGCATCCAC-3' and rev 5'-AGGAACACGGAAGGCCAT GC-3') and U6 SnRNA (for 5'-CTCGCTTCGGCAGCACA-3' and rev 5'-AACGCTTCACGAATT TGCCT-3'), respectively. Thermo cycling conditions for qPCR were as follows: initial denaturation at 92°C for 3 min and then denaturation at 92°C for 5 s, annealing (at 59°C for GAPDH, 62°C for *tlr-3*, 60°C for U6 SnRNA, and 64°C for *miR-16*), for 5 s, extension at 72°C for 5 s, and melt curve analysis at 65–95°C. Amplification of the single PCR product was confirmed by monitoring the dissociation curve followed by melting curve analysis. Each reaction was performed in triplicates with threefold serial dilution of cDNA with normalizing internal control GAPDH/U6 SnRNA. The data are presented as mean fold change of the normalized expression (CFX Manager™ version 2 software; Bio-Rad Laboratories, Inc., USA).

### Western Blotting

An equal concentration of protein (40 μg) was mixed with loading buffer (glycerol, 125 mM Tris-HCl pH 6.8, 4% SDS, 0.006% bromophenol blue, 2% mercaptoethanol) and resolved on 10% polyacrylamide gel (PAGE). The separated proteins were transferred electrophoretically on to the PVDF membrane (Millipore India Pvt. Ltd., India). The membranes were then placed in the blocking solution [5% non-fat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween-20: TBS-T] for 3 h at room temperature (RT). The blocking solution was discarded and the membranes incubated at 4°C overnight with one of the following primary antibodies (Santa-Cruz Biotech, Germany/BD Biosciences, USA): SERT (SC-1458, 1:200), anti-β-actin (SC-130656; 1:1000) affinity purified rabbit polyclonal antibody and

Hsp-90 (SC-5977, 1:200) mouse monoclonal antibody.  $\beta$ -actin was used as control for each samples. The membrane was washed and bound antibodies were detected by incubating for 3 h either with the mouse anti-rabbit (Cat # 621100180011730; 1:2000; MERCK, Bangalore, India) or goat anti-mouse (Cat # 621100480011730; 1:2000; MERCK, Bangalore, India) alkaline phosphatase conjugated antibody. The membrane was washed three times with TBS-T, and alkaline phosphatase activity was detected with 5-bromo-4-chloro-3-indolylphosphate disodium salt (BCIP)/nitro-blue tetrazolium chloride (NBT) following the instructions from the manufacturer (Invitrogen, USA). The images were acquired with Molecular Imager ChemiDoc XRS system (Bio-Rad Laboratories, Inc., USA) and the trace quantity for each band was measured using Quantity One image analysis software (Bio-Rad Laboratories, Inc., USA). The obtained Hsp90 and SERT levels were normalized to  $\beta$ -actin for respective samples.

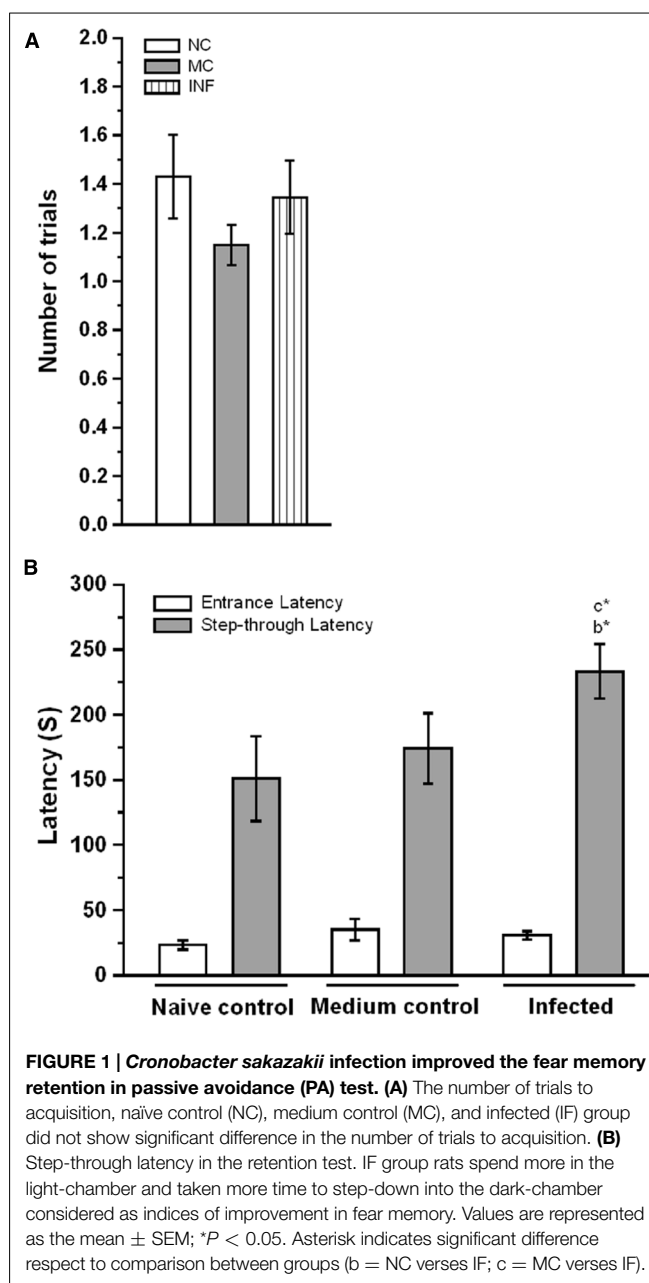
### Statistical Analysis

Data were presented as a mean  $\pm$  standard error of the mean (SEM) and plotted with KyPlot (version 1.0) for graphical representation. The obtained data were evaluated by one-way analysis of variance (ANOVA) to detect differences between groups (SigmaStat; version 3.1) followed by Bonferroni *post hoc* test was performed. Differences were considered significant if  $p < 0.05$ .

## Results

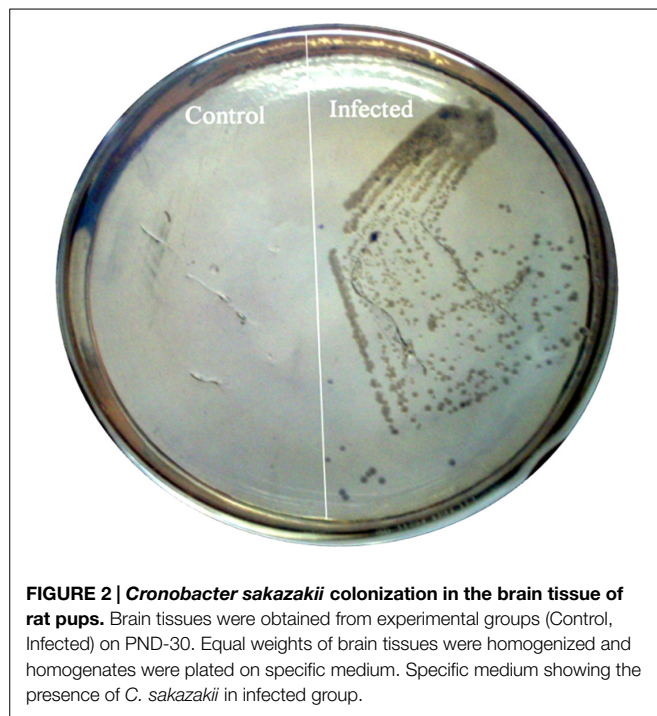
### C. sakazakii Infection Alters the Fear Memory Retention

To determine whether the *C. sakazakii* infection affects cognitive function, we compare the fear memory retention between the experimental groups. We first assessed the performance of experimental groups during the training session of the light-dark passive avoidance task, there was no significant difference in the number of acquisition trials between NC ( $1.42 \pm 0.17$ ) and MC ( $1.15 \pm 0.08$ ) groups [ $F_{(1,33)} = 2.56$ ;  $P > 0.05$ ]. Similarly, *C. sakazakii* infection did not change the number of trials in IF group ( $1.35 \pm 0.15$ ) from NC [ $F_{(1,42)} = 0.113$ ;  $P > 0.05$ ] and MC group [ $F_{(1,47)} = 1.00$ ;  $P > 0.05$ ; **Figure 1A**]. Further, Bonferroni test revealed that the acquisition trials required by IF group was not significantly different from NC ( $P = 0.739$ ) and MC group ( $P = 0.322$ ). In comparison, there was no significant difference between NC and MC groups ( $P = 0.011$ ). Similarly, there was no significant difference in entrance latency between NC ( $23.21 \pm 3.36$  s) and MC ( $35.10 \pm 8.13$  s) group [ $F_{(1,32)} = 1.36$ ;  $P > 0.05$ ]. When the IF group ( $30.62 \pm 3.0$  s) compared to NC [ $F_{(1,42)} = 2.26$ ;  $P > 0.05$ ] and MC [ $F_{(1,47)} = 0.344$ ;  $P > 0.05$ ], no significant difference was found (**Figure 1B**). Bonferroni test confirmed that the entrance latency of IF group was not significantly different from NC ( $P = 0.144$ ) and MC group ( $P = 0.62$ ). In addition, it showed that NC group was not significantly different from MC group ( $P = 0.160$ ). However, there was a significant difference between groups in step-through latency during testing. Our analysis revealed that the IF group ( $237.34 \pm 19.35$  s) rats showed significantly higher latencies



to enter the dark box compared to NC ( $150.92 \pm 30.5$  s) [ $F_{(1,42)} = 4.883$ ;  $P < 0.05$ ] and MC ( $171.8 \pm 26.38$  s) group [ $F_{(1,47)} = 4.26$ ;  $P < 0.05$ ]. When we compare the latency exhibited by the NC and MC groups, there was no significant difference between them [ $F_{(1,32)} = 0.344$ ;  $P > 0.05$ ]. Further, Bonferroni test showed that the IF group took significantly more time to step into the dark box than NC ( $P = 0.021$ ) and MC group ( $P = 0.044$ ), but there was no significant difference between NC and MC group ( $P = 0.596$ ). The observed data showed that *C. sakazakii* infection did not alter their learning during acquisition but IF group exhibited higher step-through latency during retention test, which showed the persistence of fear memory.





### *C. sakazakii* Entered into the Brain

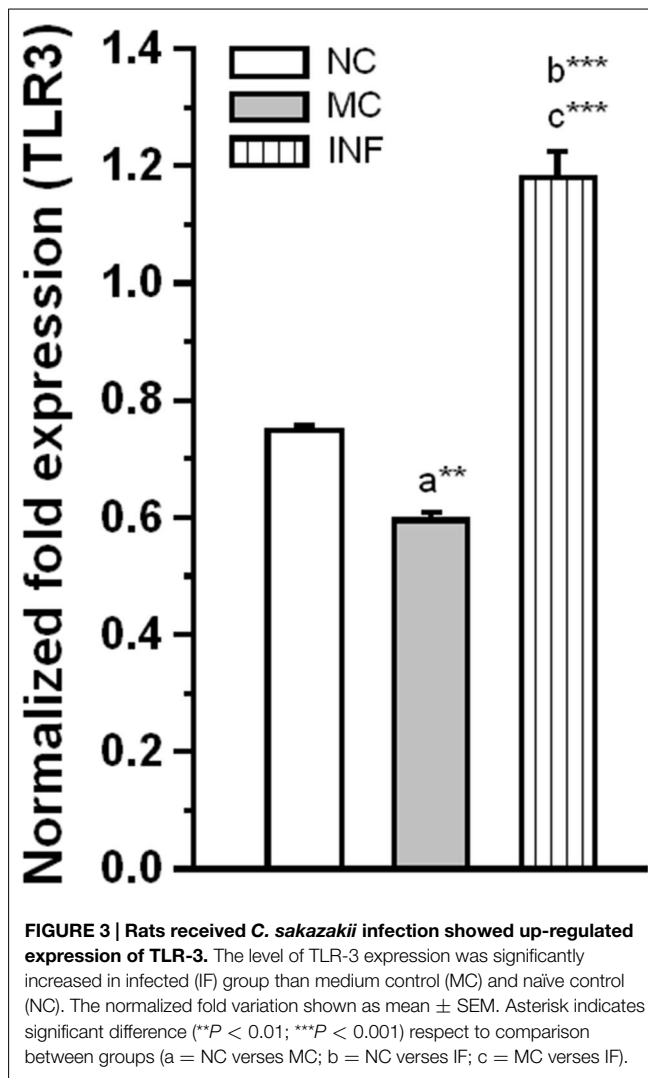
To confirm the observed behavioral phenotype was due to the single dose of *C. sakazakii* infection on PND-15, we tested the presence of *C. sakazakii* in brain. When we plated the brain tissue homogenates in *Enterobacter* medium plate, we found the growth of blue-green color colonies from the IF group samples but not in the naïve control (Figure 2). This result suggested that single dose of oral treatment of *C. sakazakii* during post-natal day is enough to induce the infection at brain.

### *C. sakazakii* Infection Activates TLR-3

To further evaluate the effect of *C. sakazakii* infection on activation of TLR-3. Our analysis revealed that *C. sakazakii* infection significantly increased the expression level of TLR-3 (Figure 3), the Ct values of TLR-3 for each group followed by GAPDH (NC:  $23.24 \pm 0.038$ ;  $12.11 \pm 0.041$ ; MC:  $23.24 \pm 0.026$ ;  $11.77 \pm 0.064$ ; IF:  $22.36 \pm 0.122$ ;  $12.12 \pm 0.054$ ). The estimated level of TLR-3 was significantly higher in IF group than MC group [ $F_{(1,9)} = 167.19$ ;  $P < 0.001$ ] and NC group [ $F_{(1,9)} = 103.78$ ;  $P < 0.001$ ]. Similarly, there was a significant difference between MC and NC groups, but the difference obtained by the reduction of TLR-3 expression was significant in MC group than NC group [ $F_{(1,9)} = 23.68$ ;  $P < 0.01$ ]. These results suggesting that *C. sakazakii* infection activated TLR-3 expression.

### *C. sakazakii* Infection Up-Regulate Hsp-90

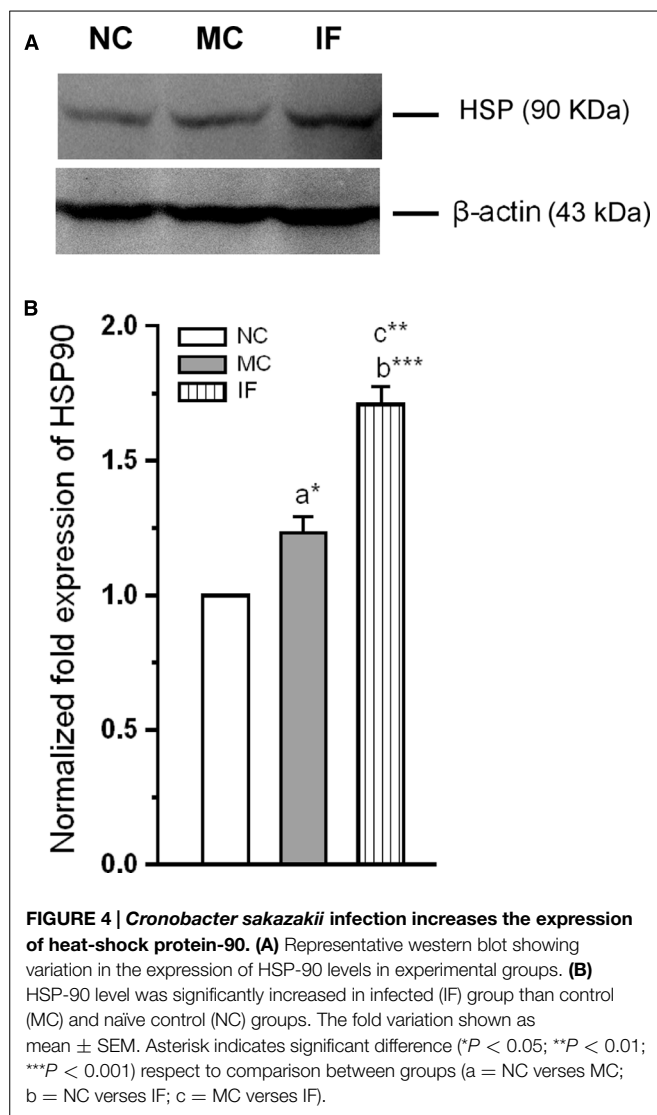
We next examined along with activation TLR-3, whether the Hsp-90 also activated following *C. sakazakii* infection. When examined the level of Hsp-90 in the experimental groups (Figure 4), we found that the *C. sakazakii* infection significantly alter the Hsp-90. The estimated level was significantly high in IF group than MC [ $F_{(1,9)} = 188.64$ ;  $P < 0.001$ ] and NC group [ $F_{(1,9)} = 1424.96$ ;



$P < 0.001$ ). However, there was no significant difference between MC and NC groups [ $F_{(1,9)} = 6.28$ ;  $P = 0.052$ ]. Our analysis revealed that *C. sakazakii* increased Hsp-90 expression.

### *C. sakazakii* Infection Modulates Serotonin and SERT Protein Level

In addition to the activation of TLR-3 and Hsp-90, we estimated the level of 5-HT, and expression level of SERT in experimental group rats. As shown in Figure 5, the basal levels of 5-HT was significantly affected by *C. sakazakii* infection [ $F_{(1,9)} = 9735.27$ ;  $P < 0.001$ ] compared to NC and MC [ $F_{(1,9)} = 236.78$ ;  $P < 0.001$ ]. In addition, levels of 5-HT was significantly lower in MC group than NC group [ $F_{(1,9)} = 9.12$ ;  $P < 0.05$ ]. Further, our analysis revealed that the expression of SERT was significantly reduced in IF group [ $F_{(1,9)} = 51.85$ ;  $P < 0.001$ ] than NC group, but not significantly different from MC group [ $F_{(1,9)} = 4.8$ ;  $P = 0.07$ ]. When we compare the expression level of MC and NC groups, they were not significantly different [ $F_{(1,9)} = 4.1$ ;  $P = 0.074$ ]. Our analysis suggests that *C. sakazakii* infection reduced the levels of 5-HT and expression of SERT protein.

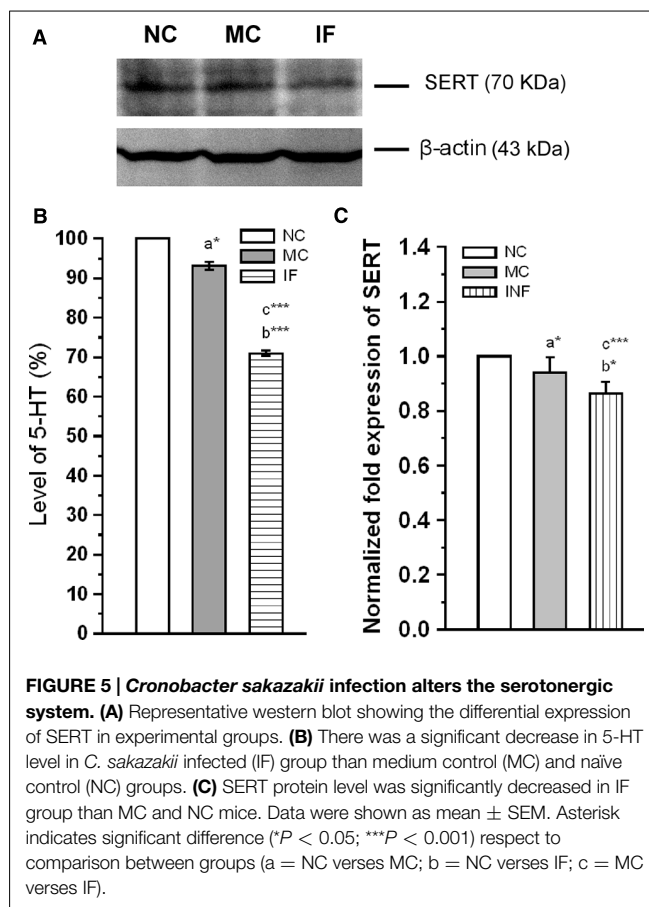


### C. sakazakii Infection Modulates miR-16 Expression

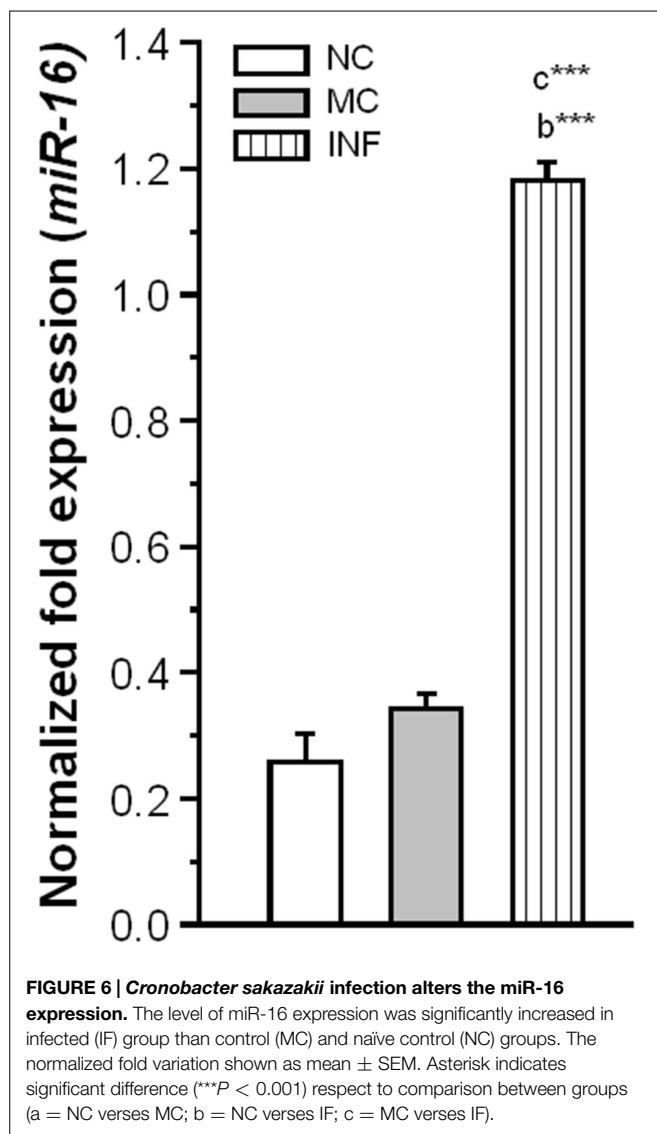
Subsequently, we explored the possible role of miR-16 in *C. sakazakii* mediated regulation of SERT protein expression. We observed that the level of miR-16 expression was significantly elevated in IF (Ct:  $23.21 \pm 0.054$ ) group than NC [ $F_{(1,9)} = 8348.15$ ;  $P < 0.001$ ] and MC group [ $F_{(1,9)} = 8117.98$ ;  $P < 0.001$ ]. However, there was no significant difference between the MC (Ct:  $24.73 \pm 0.103$ ) and NC (Ct:  $25.34 \pm 0.260$ ) groups [ $F_{(1,9)} = 1.68$ ;  $P = 0.084$ ; Figure 6]. Our results suggested that up-regulation of miR-16 by *C. sakazakii* infection possibly suppressed the translation of SERT.

## Discussion

*Cronobacter sakazakii* has been associated with human infection especially in newborn and infants (Joseph and Forsythe, 2011; Cruz-Córdova et al., 2012). Earlier, Hyun-Lee et al. (2011) demonstrated that *C. sakazakii* can cross the blood-brain barrier (BBB) in postnatal mice (PND-3.5), possibly through exploiting immature dendritic cells (Townsend et al., 2007; Mittal et al., 2009;



Emami et al., 2011). In the present study, we IF the postnatal rats on PND-15, during the onset of “brain growth spurt” period (Dobbing and Sands, 1979). During this period, changes like axonal outgrowth and dendritic maturation, establishment of neuronal connections and proliferation of glial cells occurred accompanying with myelination (Kolb and Wishaw, 1989). At first, we showed the presence of *C. sakazakii* in PND-30 rats’ brain. Further, we demonstrated that single dose of *C. sakazakii* infection in postnatal rats did not alter their learning efficiency but improved the fear memory retention. Our results adding support to the earlier study and suggest that postnatal Wistar rats may be used as animal model for human neonatal *C. sakazakii* infections. Earlier studies discussed how the infection and inflammation lead to changes in brain (Goehler et al., 2007; Jaradat et al., 2014), in which TLRs are a part. TLRs are conserved from sponges to human and very much present in neuronal cells (Barton, 2007; Tang et al., 2007; Wiens et al., 2007). It has been stated that as a pro-inflammatory or a comprehensive neuroprotective response, TLR-3 is activated (Bsibsi et al., 2006; Kim et al., 2008), whereas its activation has not yet been established under normal condition (Okun et al., 2011). In brain, TLR-3 has broad effect on the cognitive function based on injury and/or disease. Studies in animal models reported that TLR-3 deficient mice showed improved contextual and extinction of fear memory (Okun et al., 2010). Interestingly, we found that rats with *C. sakazakii* infection showed elevated level of TLR-3 expression compared to other groups and they displayed improved fear memory. Supporting to



this, earlier study demonstrated that TLR-3 activation possibly negatively regulate ERK-CREB signaling, thus, activation of TLR-3 contribute to cognitive impairment and other behavioral disorders (Okun et al., 2010).

Earlier studies reported that as a innate immune response exposure to pathogen/LPS activate TLR and Hsp90 (Stanislawski et al., 2004; Xie et al., 2015), in many observations expression of Hsp90 facilitates the pathogenesis (Qin et al., 2010; Smith et al., 2010; Shapiro et al., 2012). TLR-3 can also respond to the endogenous ligands such as Hsp-90, especially in dendritic cells during pathogenesis (Stanislawski et al., 2004). Similarly, we found that *C. sakazakii* infection induced the expression of Hsp90, the estimated level was higher than the other experimental group. Hsp90 is one of the molecules that interact with serotonergic system, especially with SERT. In fact, N- or C-terminus of SERT protein known to interact with many regulatory proteins, they play critical role in folding of SERT protein (El-Kasaby et al., 2010, 2014; Zhong et al., 2012). When we tested the expression pattern of SERT, the level of SERT protein was significantly

low in IF group than other experimental groups. Although, it is interesting that the infection appears to have changed 5-HT level and SERT protein, the SERT effect appeared to be rather modest and it is unclear whether the alternation of SERT or any other interacting molecules in altering the individual's behavior in IF group. However, earlier *in vitro* report demonstrated that over expression of Hsp90 interact with SERT protein and alter the folding trajectory of SERT protein (El-Kasaby et al., 2014). On the other hand, expression of SERT could be exerted by microRNAs, particularly miR-16 (Baudry et al., 2010; Yoon et al., 2013). Specific miRNAs activation/inactivation patterns are critically regulated by the presence of bacterial effector proteins and localization of the pathogen (Zhu et al., 2010; Al-Quraishy et al., 2012; Izar et al., 2012). Although, there is a differential expression of miR-16 following pathogen infection, we found that miR-16 expression was increased after *C. sakazakii* infection. Further, our analysis suggests that *C. sakazakii* infection up-regulate the expression of miR-16, which also interact with the 3'UTR of SERT and down-regulate the translation process. Supporting to our behavioral observations, SERT knock-out animals showed impaired fear extinction (Wellman et al., 2007; Narayanan et al., 2011; Hartley et al., 2012). The down-regulated SERT expression could affect the reuptake of released 5-HT, and then the level of 5-HT. Our analysis revealed that the level of 5-HT significantly decreased following *C. sakazakii* infection. Supporting to this, *in vivo* and *in vitro* studies demonstrating that exposure to pathogens/pathogen produced endotoxin alter the level of 5-HT and behavior (Esmaili et al., 2009; Martin et al., 2009; Shin and Liberzon, 2010; van Heesch et al., 2014). In addition, we observed difference between the naïve control and MC in molecules we tested in this study but not in the behavior. The observed difference in this study is possibly by the micronutrients in the bacterial medium, which may alter the gut microbiota of the individuals. They have the capacity to can influence precursor pool for 5-HT (Desbonnet et al., 2008).

In conclusion, our results demonstrates that *C. sakazakii* infection enhanced the fear memory retention. Although, further study needed to establish the mechanism of this effect, based on our data, we hypothesize that observed changes in SERT expression may have caused this effect, possibly through the interaction of Hsp-90 and miR-16. Further, the present study suggest that *C. sakazakii* infection in postnatal rats may be used an animal model to examine the effect of bacterial infection mediated changes in synaptic plasticity through SERT and effect of other pharmacological agents against pathogen induced memory disorder.

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## References

- Al-Quraishy, S., Dkhil, M. A., Delic, D., Abdel-Baki, A. A., and Wunderlich, F. (2012). Organ specific testosterone-insensitive response of miRNA expression of C57BL/6 mice to *Plasmodium chabaudi* malaria. *Parasitol. Res.* 111, 1093–101. doi: 10.1007/s00436-012-2937-3
- Barbas, D., DesGroseillers, L., Castellucci, V. F., Carew, T. J., and Marinesco, S. (2003). Multiple serotonergic mechanisms contributing to sensitization in *Aplysia*: evidence of diverse serotonin receptor subtypes. *Learn. Mem.* 10, 373–386. doi: 10.1101/lm.66103
- Bartel, D. P. (2009). MicroRNA target recognition and regulatory functions. *Cell* 136, 215–233. doi: 10.1016/j.cell.2009.01.002
- Barton, G. M. (2007). Viral recognition of Toll-like receptors. *Semin. Immunol.* 19, 33–40. doi: 10.1016/j.smim.2007.01.003
- Baudry, A., Mouillet-Richard, S., Schneider, B., Launay, J. M., and Kellermann, O. (2010). miR-16 targets the serotonin transporter: a new fact for adaptive responses to antidepressants. *Science* 329, 1537–1541. doi: 10.1126/science.1193692
- Block, C., Peleg, O., Minster, N., Bar-Oz, B., Simhon, A., Arad, I., et al. (2002). Cluster of neonatal infections in Jerusalem due to unusual biochemical variant of *Enterobacter sakazakii*. *Eur. J. Clin. Microbiol. Infect. Dis.* 21, 613–616. doi: 10.1007/s10096-002-0774-5
- Bravo, J. A., Dinan, T. G., and Cryan, J. F. (2014). Early-life stress induces persistent alterations in 5-HT1A receptor and serotonin transporter mRNA expression in adult rat brain. *Front. Mol. Neurosci.* 7:24. doi: 10.3389/fnfmol.2014.00024
- Breeuwer, P., Lardeau, A., Peterz, M., and Joosten, H. M. (2003). Desiccation and heat tolerance of *Enterobacter sakazakii*. *J. Appl. Microbiol.* 95, 967–973. doi: 10.1046/j.1365-2672.2003.02067.x
- Bsibsi, M., Persoon-Deen, C., Verwer, R. W., Meeuwse, S., Ravid, R., and Noort, J. M. V. (2006). Toll like receptor-3 on adult human astrocytes triggers production of neuroprotective mediators. *Glia* 53, 688–695. doi: 10.1002/glia.20328
- Chen, Y., Wang, B., Liu, D., Li, J. J., Xue, Y., Sakata, K., et al. (2014). Hsp90 chaperone inhibitor 17-AAG attenuates A $\beta$ -induced synaptic toxicity and memory impairment. *J. Neurosci.* 34, 2464–2470. doi: 10.1523/JNEUROSCI.0151-13.2014
- Croce, C. M. (2009). Causes and consequences of microRNA deregulation in cancer. *Nat. Rev. Genet.* 10, 704–714. doi: 10.1038/nrg2634
- Cruz-Córdova, A., Rocha-Ramirez, L. M., Ochoa, S. A., González-Pedrajo, B., Espinosa, N., Eslava, C., et al. (2012). Flagella from five *Cronobacter* species induces pro-inflammatory cytokines in macrophage derivatives from human monocytes. *PLoS ONE* 7:e25091. doi: 10.1371/journal.pone.0052091
- Dancer, G. I., Mah, J. H., Rhee, M. S., Hwang, I. G., and Kang, D. H. (2009). Resistance of *Enterobacter sakazakii* (*Cronobacter* spp.) to environmental stresses. *J. Appl. Microbiol.* 107, 1606–1614. doi: 10.1111/j.1365-2672.2009.04347.x
- Depino, A. M. (2015). Early prenatal exposure to LPS results in anxiety- and depression-related behaviors in adulthood. *Neuroscience* 299, 56–65. doi: 10.1016/j.neuroscience.2015.04.065
- Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., and Dinan, T. G. (2008). The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. *J. Psychiatr. Res.* 43, 164–174. doi: 10.1016/j.jpsyres.2008.03.009
- Dobbing, J., and Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early. Hum. Dev.* 3, 79–83.
- Edelson-Mammel, S. G., Porteous, M. K., and Buchanan, R. L. (2005). Survival of *Enterobacter sakazakii* in a dehydrated powdered infant formula. *J. Food. Prot.* 68, 1900–1902.
- El-Kasaby, A., Just, H., Malle, E., Stolt-Bergner, P. C., Sitte, H. H., Freissmuth, M., et al. (2010). Mutations in the carboxy-terminal SEC24 binding motif of the serotonin transporter impair folding of the transporter. *J. Biol. Chem.* 285, 39201–39210. doi: 10.1074/jbc.M110.118000
- El-Kasaby, A., Koban, F., Sitte, H. H., Freissmuth, M., and Susic, S. (2014). A cytosolic relay of heat shock proteins HSP70-1A and HSP90 $\beta$  monitors the folding trajectory of the serotonin transporter. *J. Biol. Chem.* 289, 28987–29000. doi: 10.1074/jbc.M114.595090
- Emami, C. N., Mittal, R., Wang, L., Ford, H. R., and Prasadarao, N. V. (2011). Recruitment of dendritic cells is responsible for intestinal epithelial damage in the pathogenesis of necrotizing enterocolitis caused by *Cronobacter sakazakii*. *J. Biol. Chem.* 286, 7067–7079. doi: 10.1074/jimm.11010108
- Esmaili, A., Nazir, S. F., Borthakur, A., Yu, D., Turner, J. R., Saksena, S., et al. (2009). Enteropathogenic *Escherichia coli* infection inhibits intestinal serotonin transporter function and expression. *Gastroenterology* 137, 2074–2083. doi: 10.1053/j.gastro.2009.09.002
- Flores, J. P., Medrano, S. A., Sánchez, J. S., and Fernández-Escartín, E. (2011). Two cases of hemorrhagic diarrhea caused by *Cronobacter sakazakii* in hospitalized nursing infants associated with the consumption of powdered infant formula. *J. Food. Prot.* 74, 2177–2181. doi: 10.4315/0362-028X.JFP-11-257
- Friedemann, M. (2007). *Enterobacter sakazakii* in food and beverages (other than infant formula and milk powder). *Int. J. Food. Microbiol.* 116, 1–10. doi: 10.1016/j.ijfoodmicro.2006.12.018
- Gainetdinov, R. R., and Caron, M. G. (2003). Monoamine transporters: from genes to behavior. *Annu. Rev. Pharmacol. Toxicol.* 43, 261–284. doi: 10.1146/annurev.pharmtox.43.050802.112309
- Goehler, L. E., Lyte, M., and Gaykema, R. P. A. (2007). Infection-induced viscerosensory signals from the gut enhance anxiety: implications for psychoneuroimmunology. *Brain Behav. Immun.* 21, 721–726. doi: 10.1016/j.bbi.2007.02.005
- Goldie, B. J., and Cairns, M. J. (2012). Post-transcriptional trafficking and regulation of neuronal gene expression. *Mol. Neurobiol.* 45, 99–108. doi: 10.1007/s12035-011-8222-0
- Gosney, M. A., Martin, M. V., Wright, A. E., and Gallagher, M. (2006). *Enterobacter sakazakii* in the mouths of stroke patients and its association with aspiration pneumonia. *Eur. J. Intern. Med.* 17, 185–188. doi: 10.1016/j.ejim.2005.11.010
- Gyawali, S., Subaran, R., Weissman, M. M., Hershkowitz, D., McKenna, M. C., Talati, A., et al. (2010). Association of polyadenylation polymorphism in the serotonin transporter and panic disorder. *Biol. Psychiatry* 67, 331–338. doi: 10.1016/j.biopsych.2009.10.015
- Hartley, C. A., McKenna, M. C., Salman, R., Holmes, A., Casey, B. J., Phelps, E. A., et al. (2012). Serotonin transporter polyadenylation polymorphism modulates the retention of fear extension memory. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5493–5498. doi: 10.1073/pnas.1202044109
- Healy, B., Cooney, S., O'Brien, S., Iversen, C., Whyte, P., Nally, J., et al. (2010). *Cronobacter* (*Enterobacter sakazakii*): an opportunistic foodborne pathogen. *Foodborne Pathog. Dis.* 7, 339–350. doi: 10.1089/fpd.2009.0379
- Herrero, M. T., Estrada, C., Maatouk, L., and Vyas, S. (2015). Inflammation in Parkinson's disease: role of glucocorticoids. *Front. Neuroanat.* 9:32. doi: 10.3389/fnana.2015.00032
- Hoyer, D., Hannon, J. P., and Martin, G. R. (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* 71, 533–554. doi: 10.1016/S0091-3057(01)00746-8
- Hunter, C. J., and Bean, J. F. (2013). *Cronobacter*: an emerging opportunistic pathogen associated with neonatal meningitis, sepsis and necrotizing enterocolitis. *J. Perinatol.* 33, 581–585. doi: 10.1038/jp.2013.26
- Hyun-Lee, H. A., Hong, S., Park, H., Kim, H., and Kim, O. (2011). *Cronobacter sakazakii* infection induced fatal clinical sequels including meningitis in neonatal ICR mice. *Lab. Anim. Res.* 27, 59–62. doi: 10.5625/lar.2011.27.1.59
- Izar, B., Mannala, G. K., Mraheil, M. A., Chakraborty, T., and Hain, T. (2012). MicroRNA response to *Listeria monocytogenes* infection in epithelial cells. *Int. J. Mol. Sci.* 13, 1173–1185. doi: 10.3390/ijms13011173
- Jaradat, Z. W., Mousa, W. A., Elbetiha, A., Nabulsi, A. A., and Tall, B. D. (2014). *Cronobacter* spp.—opportunistic food-borne pathogens. A review of their virulence and environmental—adaptive traits. *J. Med. Microbiol.* 63, 1023–1037. doi: 10.1099/jmm.0.073742-0
- Joseph, S., and Forsythe, S. J. (2011). Predominance of *Cronobacter Sakazakii* sequence type 4 in neonatal infections. *Emerg. Infect. Dis.* 17, 1713–1715. doi: 10.3201/eid1709.110260
- Kaang, B. K., Kandel, E. R., and Grant, S. G. (1993). Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in *Aplysia* sensory neurons. *Neuron* 10, 427–435.
- Kalin, N. H., Takahashi, L. K., and Chen, F. L. (1994). Restraint stress increases corticotropin—releasing hormone mRNA content in the amygdala and paraventricular nucleus. *Brain Res.* 656, 182–186.
- Kandel, E. R. (2001). The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 294, 1030–1038. doi: 10.1126/science.1067020
- Kandhai, M. C., Reij, M. W., Gorris, L. G. M., Guillaume-Gentil, O., and van Schothorst, M. (2004). Occurrence of *Enterobacter sakazakii* in food production environments and households. *Lancet* 363, 39–40. doi: 10.1016/S0140-6736(03)15169-0



- Kim, H., Yang, E., Lee, J., Kim, S. H., Shin, J. S., Park, J. Y., et al. (2008). Double-stranded RNA mediates interferon regulatory factor 3 activation and interleukin-6 production by engaging Toll-like receptors 3 in the human brain astrocytes. *Immunology* 124, 480–488. doi: 10.1111/j.1365-2567.2007.02799.x
- Kolb, B., and Whishaw, I. Q. (1989). Plasticity in the neocortex: mechanisms underlying recovery from early brain damage. *Prog. Neurobiol.* 32, 235–276.
- Lai, K. K. (2001). *Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature. *Medicine (Baltimore)*. 80, 113–122. doi: 10.1097/00005792-200103000-00004
- Liu, L., Yang, Y., Cui, J., Liu, L., Liu, H., Hu, G., et al. (2013). Evaluation and implementation of a membrane filter method for *Cronobacter* detection in drinking water. *FEMS Microbiol. Lett.* 344, 60–68. doi: 10.1111/1574-6968.12155
- Martin, E. I., Ressler, K. J., Binder, E., and Nemeroff, C. B. (2009). The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology. *Clin. Lab. Med.* 30, 865–891. doi: 10.1016/j.psc.2009.05.004
- Meneses, A. (1999). 5-HT system and cognition. *Neurosci. Biobehav. Rev.* 23, 1111–1125.
- Meneses, A. (2007). Stimulation of 5-HT1A, 5-HT1B, 5-HT2A/2C, 5-HT3 and 5-HT4 receptors, or 5-HT uptake inhibition: short and long memory. *Behav. Brain Res.* 184, 81–90. doi: 10.1016/j.bbr.2007.06.026
- Meneses, A. (2013). 5-HT systems: emergent targets for memory formation and memory alterations. *Rev. Neurosci.* 24, 629–664. doi: 10.1515/revneuro-2013-0026
- Meneses, A., Perez-Garcia, G., Ponce-Lopez, T., Tellez, R., and Castillo, C. (2011). Serotonin and memory. *Neuropharmacology* 61, 355–363. doi: 10.1016/j.neuropharm.2011.01.018
- Meyer, J. H., Wilson, A. A., Sagrati, S., Miler, L., Rusjan, P., Bloomfield, P. M., et al. (2009). Brain monoamine oxidase A binding in major depressive disorder: relationship to selective serotonin reuptake inhibitor treatment, recovery, and recurrence. *Arch. Gen. Psychiatry* 66, 1304–1312. doi: 10.1001/archgenpsychiatry.2009.156
- Miller, J. H. (1972). *Experiments in Molecular Genetics*. New York: Cold Spring Harbor Laboratory.
- Mittal, R., Bulgheresi, S., Emami, C., and Prasadarao, N. V. (2009). *Enterobacter sakazakii* targets DC-SIGN to induce immunosuppressive responses in dendritic cells by modulating MAPKs. *J. Immunol.* 183, 6588–6599. doi: 10.4049/jimmunol.0902029
- Muytjens, H. L., Zanen, H. C., Sonderkamp, H. J., Kollée, L. A., Wachsmuth, I. K., and Farmer, J. (1983). Analysis of eight cases of neonatal meningitis and sepsis due to *Enterobacter sakazakii*. *J. Clin. Microbiol.* 18, 115–120.
- Narayanan, V., Heiming, R. S., Janses, F., Lesting, J., Sachser, N., Pape, H.-C., et al. (2011). Social defeat: impact on fear extinction and amygdala-prefrontal cortical theta synchrony in 5-HTT deficient mice. *PLoS ONE* 6:e22600. doi: 10.1371/journal.pone.0022600
- Okun, E., Griffioen, K. J., and Mattson, M. P. (2011). Toll-like receptor in neural plasticity and disease. *Trends Neurosci.* 34, 269–281. doi: 10.1016/j.tins.2011.02.005
- Okun, E., Griffioen, K. J., Lathia, J. D., Tang, S. C., Mattson, M. P., and Arumugam, T. V. (2009). Toll-like receptors in neurodegeneration. *Brain Res. Rev.* 59, 278–292. doi: 10.1016/j.brainresrev.2008.09.001
- Okun, E., Griffioen, K., Barak, B., Roberts, N. J., Castro, K., Pita, M. A., et al. (2010). Toll-like receptor 3 inhibits memory retention and constrains hippocampal neurogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15625–15630. doi: 10.1073/pnas.1005807107
- Pandey, S., and Agrawal, D. K. (2006). Immunobiology of toll like receptors: emerging trends. *Immunol. Cell. Biol.* 84, 333–341. doi: 10.1111/j.1440-1711.2006.01444.x
- Pérez, A. R., Bottasso, O., and Savino, W. (2009). The impact of infectious diseases upon neuroendocrine circuits. *Neuroimmunomodulation* 16, 96–105. doi: 10.1159/000180264
- Perez-Garcia, G., and Meneses, A. (2009). Memory time-course: mRNA 5-HT1A and 5-HT7 receptors. *Behav. Brain Res.* 202, 102–113. doi: 10.1016/j.bbr.2009.03.027
- Qin, Z., DeFée, M., Isaacs, J. S., and Parsons, C. (2010). Extracellular Hsp90 serves as co-factor for MAPK activation and latent viral gene expression during *de novo* infection by KSHV. *Virology* 403, 92–102. doi: 10.1016/j.virol.2010.03.052
- See, K. C., Than, H. A., and Tang, T. (2007). *Enterobacter sakazakii* bacteraemia with multiple splenic abscesses in a 75-year-old woman: a case report. *Age Ageing* 36, 595–596. doi: 10.1093/ageing/afm092
- Seyedabadi, M., Fakhfouri, G., Ramezani, V., Mehr, S. E., and Rahimian, R. (2014). The role of serotonin in memory: interactions with neurotransmitters and downstream signaling. *Exp. Brain Res.* 232, 723–738. doi: 10.1007/s00221-013-3818-4
- Shapiro, R. S., Sellam, A., Tebbji, F., Whiteway, M., Nantel, A., and Cowen, L. E. (2012). Pho85, Pcl 1, and Hms1 Signaling governs *Candida albicans* morphogenesis induced high temperature or Hsp90 compromise. *Curr. Biol.* 22, 461–470. doi: 10.1016/j.cub.2012.01.062
- Shin, L. M., and Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology* 35, 169–191. doi: 10.1038/npp.2009.83
- Smith, D. R., McCarthy, S., Chrovian, A., Olinger, G., Stossel, A., Geisbert, T. W., et al. (2010). Inhibition of heat shock protein 90 reduces Ebola virus replication. *Antiviral Res.* 87, 187–194. doi: 10.1016/j.antiviral.2010.04.015
- Song, K.-Y., Hyeon, J. Y., Shin, H. C., Park, C. K., Choi, I. S., and Seo, K. H. (2008). Evaluation of a chromogenic medium supplemented with glucose for detecting *Enterobacter sakazakii*. *J. Microbiol. Biotechnol.* 18, 579–584.
- Standart, N., and Jackson, R. J. (1994). Regulation of translation by specific protein/m-RNA interactions. *Biochimie* 76, 867–879. doi: 10.1016/0300-9084(94)90189-9
- Stanislawski, J., Interewicz, B., and Olszewski, W. L. (2004). Influence of bacterial antigens on activation of human splenic dendritic cells. *Ann. Transplant.* 9, 54–57.
- Stansley, B. J., and Yamamoto, B. K. (2015). Behavioral impairments and serotonin reductions in rats after chronic L-dopa. *Psychopharmacology (Berl)*. 232, 3203–3213. doi: 10.1007/s00213-015-3980-4
- Tang, S. C., Arumugam, T. V., Xu, X., Cheng, A., Mughal, M. R., Jo, D. G., et al. (2007). Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. *Proc. Natl. Acad. Sci. U.S.A.* 104, 13798–13803. doi: 10.1073/pnas.0702553104
- Tellez, R., Gómez-Viquez, L., and Meneses, A. (2012). GABA, Glutamate, dopamine and serotonin transporter expression on memory formation and amnesia. *Neurobiol. Learn. Mem.* 97, 189–201. doi: 10.1016/j.nlm.2011.12.002
- Townsend, S. M., Hurrell, E., Gonzalez-Gomez, I., Lowe, J., Frye, J. G., Forsythe, S., et al. (2007). *Enterobacter sakazakii* invades brain capillary endothelial cells, persists in human macrophages influencing cytokine secretion and induces severe brain pathology in the neonatal rat. *Microbiology* 153, 3538–3547. doi: 10.1099/mic.0.2007/009316-0
- Tsai, H.-Y., Liao, C. H., Huang, Y.-T., Lee, P.-I., and Hsueh, P.-R. (2013). *Cronobacter* infections not from infant formula, Taiwan. *Emerg. Infect. Dis.* 19, 167–169. doi: 10.3201/eid1901.120774
- van Heesch, F., Prins, J., Konsman, J. P., Korte-Bouws, G. A., Westphal, K. G., Rybka, J., et al. (2014). Lipopolysaccharide increases degradation of central monoamines: an *in vivo* microdialysis study in the nucleus accumbens and medial prefrontal cortex of mice. *Eur. J. Pharmacol.* 725, 55–63. doi: 10.1016/j.ejphar.2014.01.014
- Wellman, C. L., Izquierdo, A., Garrett, J. E., Martin, K. P., Carroll, J., Millstein, R., et al. (2007). Impaired stress-coping and fear extinction and abnormal corticolimbic morphology in serotonin transporter knock-out mice. *J. Neurosci.* 27, 684–691. doi: 10.1523/JNEUROSCI.4595-06.2007
- Wiens, M., Korzhnev, M., Perovic-Ottstadt, S., Luthringer, B., Brandt, D., Klein, S., et al. (2007). Toll like receptors are part of innate defense system of Sponges (Demospongiae: Porifera). *Mol. Bio. Evol.* 24, 792–804. doi: 10.1093/molbev/msl208
- Wilkie, G. S., Dickson, K. S., and Gray, N. K. (2003). Regulation of mRNA translation by 5'- and 3'-UTR binding factors. *Trends Biochem. Sci.* 28, 182–188.
- Xie, Y., Song, L., Weng, Z., Liu, S., and Liu, Z. (2015). Hsp90, Hsp 60, sHsp families of heat shock protein genes in channel catfish and their expression after bacterial infection. *Fish Shellfish. Immunol.* 44, 642–651. doi: 10.1016/j.fsi.2015.03.027
- Yan, Q. Q., Condell, O., Power, K., Butler, F., Tall, B. D., and Fanning, S. (2012). *Cronobacter* species (formerly known as *Enterobacter Sakazakii*) in powdered infant formula: a review of our current understanding of the biology of

- this bacterium. *J. Appl. Microbiol.* 113, 1–15. doi: 10.1111/j.1365-2672.2012.05281.x
- Yoon, Y., McKenna, M. C., Rollins, D. A., Song, M., Nuriel, T., Gross, S. S., et al. (2013). Anxiety—associated alternative polyadenylation of the serotonin transporter mRNA confers translational regulation by hnRNPK. *Proc. Natl. Acad. Sci. U.S.A.* 110, 11624–11629. doi: 10.1073/pnas.1301485110
- Zare, N., Motamedi, F., Digaleh, H., Khodaghali, F., and Maghsoudi, N. (2015). Collaboration of geldanamycin-activated P70S6K and Hsp70 against beta-amyloid-induced hippocampal apoptosis: an approach to long-term memory and learning. *Cell Stress Chaperones* 20, 309–319. doi: 10.1007/s12192-014-0550-3
- Zhong, H., Sánchez, C., and Caron, M. G. (2012). Consideration of allosterism and interacting proteins in the physiological functions of the serotonin transporter. *Biochem. Pharmacol.* 83, 435–442. doi: 10.1016/j.bcp.2011.09.020
- Zhu, J. Y., Strehle, M., Frohn, A., Kremmer, E., Höfig, K. P., Meister, G., et al. (2010). Identification and analysis of expression of novel microRNAs of murine gamma herpesvirus 68. *J. Virol.* 84, 10266–10275. doi: 10.1128/JVI.01119-10
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