5-2015

Genetic and Environmental Interactions on Schizophrenia-Like Phenotypes in CHL1 Deficient Mice

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GENETIC AND ENVIRONMENTAL INTERACTIONS ON SCHIZOPHRENIA-LIKE PHENOTYPES IN CHL1 DEFICIENT MICE

by

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Thesis submitted in partial fulfillment
of the requirements for the degree

of

DEPARTMENTAL HONORS

in

Psychology
in the Department of Psychology

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Spring 2015

Acknowledgements

I would like to thank Dr. Catalin Buhusi and Dr. Mona Buhusi for mentoring me as I conducted this research. They have provided me with countless hours of their time for which I am extremely grateful. Also I would like to thank them for funding my research and for allowing me to use their equipment. I would also like to thank them for helping me in the process of learning the skills needed to carry out this research, and for taking a chance on me when they let me come into their lab. I would like to thank Brooke N. Hansen and Alysha Westwood for genotyping the mice used in this experiment. Finally, I would like to thank Chance Christensen for setting up FreezeScan on the computers, and getting it to a working state. I would like to thank Bret Guercio for his willingness to let me discuss academic papers that I had found with him, and for sharing papers he had found with me. I would also like to thank Nicholas Borgogna for helping at the beginning of this experiment.

Abstract

Schizophrenia is a debilitating disorder which is often characterized by dysregulation of the processing of sensory information. Schizophrenia has been shown to have a strong genetic component, as well as a strong environmental component. As such, a number of hypotheses such as the diathesis stress hypothesis have been developed to explain the etiology of schizophrenia. As most of these theories attempt to account for a genetic and an environmental factor, they are often viewed as double-hit models of schizophrenia. Several theories have emerged as potential explanations for the symptoms of schizophrenia. The dopamine hypothesis suggests that the basal level of dopamine transmission within the mesolimbic and mesocortical pathways is increased in schizophrenia. The glutamate hypothesis suggests that increased glutamate transmission in the striatum combined with NMDA receptor hypofunction could result in some of the symptoms of schizophrenia. Both the dopamine and the glutamate hypothesis draw on the idea that individuals with schizophrenia show heightened neural activation as compared with non-schizophrenics. The developmental theory of schizophrenia posits that insults to the brain occurring during development may causes changes in the brain which result in the symptoms of schizophrenia later in life. In this study, CHL1 deficient mice, an animal model of schizophrenia were compared with their wild type littermates on measures of neuronal activation and latent inhibition, a measure of normal attentional and sensory processing. Additionally, some mice from both genotypes were selected to receive stress. It was found that all unstressed mice as well as the wild type stressed mice showed latent inhibition. The stressed CHL1 deficient mice did not show latent inhibition, the absence of which is associated with the positive symptoms of schizophrenia. These results provide support for a double hit (environment x genetic) account of schizophrenia.

Introduction

Schizophrenia is a neurological disorder characterized by delusions, hallucinations, disorganized behavior and speech, and attentional control deficits that can lead to severe impairments in adaptive function. Schizophrenia is believed to affect as many as 4 out of every 1,000 individuals at some point in their life (Saha, Chant, Welham, & McGrath, 2005). Schizophrenia has a large economic impact on the affected individual as well as society as a whole, with an estimated economic cost of over $32 billion in the United States alone (Knapp, Mangalore, & Simon, 2004). There are a number of genetic and environmental factors which have been identified that can affect an individual's susceptibility to schizophrenia. Some environmental factors such as gender and location do not affect the prevalence of schizophrenia (Saha et al., 2005). The instance of schizophrenia however, has been shown to differ on basis of both gender and location. Men show a higher incidence of schizophrenia than women, and individuals living in urban areas show a higher incidence of schizophrenia than individuals in suburban or rural areas (McGrath et al., 2004). Additionally, it has been found that immigrant populations have a higher incidence rate than native populations (McGrath et al., 2004). This suggests that environmental factors may play a role in the onset of schizophrenia. In further support of the idea that environmental factors play a role in the incidence of schizophrenia, joint estimates have suggested that 11% of the liability to schizophrenia may be controlled by environmental factors (Sullivan, Kendler, & Neale, 2003). In addition to risk factors in the environment, the heritability of schizophrenia has been estimated to be as high as 81% (Sullivan et al., 2003). Taken together, these studies suggest strong interplay between genetic factors and the environment in the development of schizophrenia.

On a neuroanatomical level, schizophrenia has been characterized by changes in brain morphology including: enlarged ventricles (Johnstone et al., 1989), localized decreases in the density of cortical gray matter (Suddath, Christison, Torrey, Casanova, & Weinberger, 1990), and decreases in the size of the thalamus (Andreasen et al., 1990) and the caudate nuclei (Mion, Andreasen, Arndt, Swayze, & Cohen, 1991). Additionally, abnormalities are often reported in the medial temporal lobe (McCarley et al., 1999), particularly in the entorhinal cortex (Baiano et al., 2008; Joyal et al., 2002), the hippocampus and the amygdala (Keshavan et al., 2002; Velakoulis et al., 2006). Interestingly, some limbic regions such as the basal ganglia (Gur et al., 1998) and the nucleus accumbens (Lauer, Senitz, & Beckmann, 2001) show increased volume in schizophrenics treated with typical neuroleptics.

In addition to morphological changes, functional changes in the physiology of the brain have been postulated based in part on efficacy of neuroleptics and atypical neuroleptics in alleviating the positive and negative symptoms of schizophrenia. One such theory has often been referred to as the dopamine hypothesis. In its earliest variation, the dopamine (DA) hypothesis postulated that dopamine levels in the brain were increased resulting in the symptoms of schizophrenia (Meltzer & Stahl, 1976). This postulate was based on the observation that the clinical efficacy of neuroleptics could be predicted based upon the DA D_2 receptor affinity of the drug (Creese, Burt, & Snyder, 1976, 1996). The assumption that increased dopamine levels in the brain were to blame for the symptoms of schizophrenia was challenged when it was found that DA D_2 receptors were upregulated in subcortical regions (caudate nucleus) in schizophrenic individuals (Mita et al., 1986) as this would suggest decreased DA flow in subcortical regions, as opposed to

increased DA flow. As a result, the DA hypothesis was amended to account for tonic DA release controlled by the glutamate efferents from the prefrontal cortex (PFC) (Grace, 2000; Karreman & Moghaddam, 1996; Sesack & Carr, 2002; Spencer, 1976) as well as phasic DA release controlled by DA neuron activation. The mechanism whereby the PFC controlled tonic DA release was postulated to be through glutamate efferents to the nucleus accumbens (NA) and the striatum, as it had been noted that NMDA antagonists ketamine (Kapur & Seeman, 2002) and phencyclidine (PCP) (Egerton et al., 2008; Itil, Keskiner, Kiremitci, & Holden, 1967; Kapur & Seeman, 2002) can induce symptoms of schizophrenia. PCP induces symptoms of schizophrenia both through increasing phasic DA release (Grace, 1991; Vickroy & Johnson, 1983) as amphetamines do (Carboni, Imperato, Perezzani, & Di Chiara, 1989; Daberkow et al., 2013; Pontieri, Tanda, & Di Chiara, 1995), as well as by decreasing tonic DA release through negative allosteric modulation at NMDA receptors (Wroblewski, Nicoletti, & Fadda, 1987). Other possible mechanisms whereby tonic DA could be regulated would be through glutamate efferents from the amygdala (Grace, 1991) and hippocampus (Belujon, Patton, & Grace, 2013; Grace, 1991, 2012) to the NA and striatum.

More recently the cause of the upregulated DA D_2 receptors has again been called into question as it has been noted that chronic treatment with neuroleptics can cause upregulation of subcortical DA D_2 receptors (Silvestri et al., 2000). This would suggest that the original DA hypothesis of increased DA transmission in individuals with schizophrenia may have been correct as many of the early studies were conducted in populations that had been treated chronically with neuroleptics. More evidence for this is found in studies that have confirmed elevated levels of tyrosine hydroxylase in the substantia nigra of schizophrenic patients (Howes et al., 2013), suggesting that schizophrenics have increased capacity to synthesize DA. Additionally, recent studies using single-photon emission computed tomography (SPECT) have shown increased dopamine release in the striatum of schizophrenic patients (Abi-Dargham, van de Giessen, Slifstein, Kegeles, & Laruelle, 2009), further calling into question previous assessments suggesting that dopamine release was reduced in subcortical areas in individuals with schizophrenia. Finally, it has been shown that individuals with schizophrenia spectrum disorders but not schizophrenia also demonstrate increased capacity for dopamine synthesis in the striatum (Reith et al., 1994). Combined, these studies provide increased support for the idea that schizophrenics do indeed experience increased dopamine synthesis and transmission within the striatum and potentially within other subcortical regions as well.

An alternative hypothesis to the dopamine hypothesis of schizophrenia is the glutamate hypothesis of schizophrenia. The glutamate hypothesis of schizophrenia is based in the observation that drugs which act as antagonists to NMDA receptors can trigger the onset of both the positive and negative symptoms of schizophrenia. In rats in particular it has been noted that administration of the NMDA antagonist PCP causes behavioral deficits which mimic some of those seen in schizophrenia (Egerton et al., 2008). Further, glutamate levels in the caudate nucleus have been found to be abnormally elevated in individuals with a genetic risk of developing schizophrenia (Tandon et al., 2013), as well as in unmedicated schizophrenics (de la Fuente-Sandoval et al., 2011). The results of these studies suggest that glutamate dysregulation may be involved in schizophrenia, however due to the current lack of effective antipsychotic medications aimed at the glutamatergic system it remains unclear if elevated glutamate levels serve only as a biomarker or as a precursor to the disease. Additionally it remains unclear if

NMDA receptors would be effective targets for future pharmacological interventions to treat schizophrenia.

The developmental hypothesis of schizophrenia offers a third explanation for the symptoms of schizophrenia. The developmental hypothesis postulates that an insult to the brain early in development may cause changes in the brain that will lead to the expression of symptoms of schizophrenia later in life (Murray & Lewis, 1987). Additionally, under the developmental hypothesis of schizophrenia abnormalities in the brain could also be accounted for by genetic sources. This hypothesis was born out of evidence for schizophrenia as a heritable disorder. Evidence for a developmental hypothesis of schizophrenia includes reduced neuronal size in the hippocampus, aberrant presynaptic terminal markers in the hippocampus (Harrison & Eastwood, 2001), altered adrenoreceptor binding in the hippocampus (Klimek et al., 1999), decreased spine density in the subicular cortex (Rosoklija et al., 2000) and in deep layer 3 pyramidal neurons of the prefrontal cortex (Glantz & Lewis, 2000). While these studies provide a link between schizophrenia and morphological changes in the brain, they fail to provide direct evidence that a single genetic or environmental factor causes the changes. Stronger evidence for this hypothesis comes from the finding that some retroviral sequences are more commonly found in the cerebral spinal fluid of individuals with recent onset schizophrenia than those with chronic or no schizophrenia (Karlsson et al., 2001). This finding suggests that an environmental factor (retroviral infection) could possibly play a role in the onset of schizophrenia.

A more recent variation on the developmental hypothesis of schizophrenia proposes that in addition to the first insult early in development, a second insult may occur later in development causing the onset of schizophrenia. Stated in a slightly different manner, this theory proposes that a genetic insult occurring early in development coupled with an environment insult occurring at a later time, though still within a critical window for neurodevelopment, may result in the onset of schizophrenia (Keshavan, 1999; Keshavan & Hogarty, 1999).

Another theory related to the neuropathology of schizophrenia involves the reduction of parvalbumin interneurons due to oxidative stress. In accordance with the developmental theory of schizophrenia (Murray & Lewis, 1987), parvalbumin interneurons have been shown to be susceptible to impairment as a result of oxidative stress caused by an early life insult (Cabungcal, Steullet, Kraftsik, Cuenod, & Do, 2013). In schizophrenia, parvalbumin interneurons have been found to be reduced in the prefrontal cortex (Beasley & Reynolds, 1997). A loss of parvalbumin neurons in the prefrontal cortex in turn has been shown to result in a reduced evoked gamma-band oscillation response (Lodge, Behrens, & Grace, 2009). Reduced evoked gamma-band oscillation responses have been noted in unmedicated schizophrenics (Gallinat, Winterer, Herrmann, & Senkowski, 2004). This suggests that reduced gamma-band oscillation responses may be related to the dysfunction observed in schizophrenia. Additionally, it suggests that this reduction can be triggered by oxidative stress in parvalbumin interneurons. Finally, this is consistent with a developmental theory of schizophrenia as insults such as social stress (Schiavone et al., 2009), PCP administration (Radonjic et al., 2010), and impaired glutathione synthesis (Cabungcal et al., 2013) can result in oxidative stress in parvalbumin neurons.

Of particular interest in the characterization of schizophrenia as a disorder of the dopaminergic system is the observed increase in dopamine levels throughout the brain in individuals with

schizophrenia. One reason that this is interesting is that stress has been shown to increase dopamine release both in individuals with schizophrenia (Mizrahi et al., 2012) and those without schizophrenia (Suridjan et al., 2012). The ameliorative effects of stress on individuals with schizophrenia (Myin-Germeys, van Os, Schwartz, Stone, & Delespaul, 2001), and its potential role in triggering the onset of schizophrenia have been well documented (Nicholson & Neufeld, 1992; Walker & Diforio, 1997; Zimmerman, Pfohl, Stangl, & Coryell, 1985). These findings suggest that one way in which stress might trigger schizophrenia-like systems is through a compounding effect on the level of dopamine transmission within the brain of individuals who have a genetic predisposition toward schizophrenia.

Another interesting aspect of the dopamine hypothesis is that given the high heritability of schizophrenia (Sullivan et al., 2003) and the findings that individuals with schizophrenia spectrum disorders also show elevated levels of dopamine transmission (Mizrahi et al., 2012), it is possible that the genetic component of schizophrenia causes a partial dysregulation of the dopamine system. Under this assumption, stressful life events could compound this dysregulation leading to the onset of the symptoms of schizophrenia.

An increased risk of developing schizophrenia in human populations has been associated with a polymorphism in the gene coding for the close homolog of L1 (CHL1) neuronal cell adhesion molecule protein (Chu & Liu, 2010; Sakurai, Migita, Toru, & Arinami, 2002; Tam et al., 2010).

Additionally, in C57BL/6J mice with CHL1 deficiency (Montag-Sallaz, Schachner, & Montag, 2002) a number of morphological and behavioral changes have been noted suggesting that mice with CHL1 deficiency may be a relevant animal model of schizophrenia. CHL1 deficient mice show enlarged ventricles and abnormal hippocampal mossy fiber projections (Montag-Sallaz et al., 2002), as well as increased neuronal activity in projections to the dentate gyrus (Morellini, Lepsveridze, Kähler, & Dityatev, 2007), abnormal thalamo-cortical projections (Demyanenko et al., 2004) and olfactory axons (Montag-Sallaz et al., 2002). Many of these changes are consistent with observed neuroanatomical changes in people with schizophrenia (Freedman, Hall, Adler, & Leonard, 1995; Goldsmith & Joyce, 1995; Hufner, Frajo-Apor, & Hofer, 2015; Johnstone et al., 1989; Jou, Hardan, & Keshavan, 2005; Moberg et al., 1999; Schlosser et al., 2003). Additionally, CHL1 deficient mice show larger latencies to explore novel stimuli (Morellini, Lepsveridze, Kahler, Dityatev, & Schachner, 2007), impaired timing (Buhusi, Scripa, Williams, & Buhusi, 2013) and impaired prepulse inhibition (Irintchev, Koch, Needham, Maness, & Schachner, 2004). All of these are behavioral measures which show some concordance with behaviors which have been noted in schizophrenics (Parwani et al., 2000; Penney, Meck, Roberts, Gibbon, & Erlenmeyer-Kimling, 2005; Weike, Bauer, & Hamm, 2000). Latent Inhibition (LI) is an additional behavioral measure which has been shown to be attenuated in schizophrenics (Martins Serra, Jones, Toone, & Gray, 2001). LI in CHL1 deficient mice has not yet been evaluated.

LI is the loss of future associability by a stimulus that has been repeatedly presented without consequence (Lubow & Moore, 1959). The loss of associability results in slower learning of a new conditioned stimulus – unconditioned stimulus relationship if the preexposed stimulus is presented with consequence in the future. In addition to being attenuated in chronic schizophrenics (Martins Serra et al., 2001), LI has been shown to be reinstated by administration of both typical and atypical neuroleptics (Leumann, Feldon, Vollenweider, & Ludewig, 2002). In

animals LI has been shown to be disrupted by reduced expression of parvalbumin neurons (Lodge et al., 2009), by the administration of amphetamine (Weiner, Lubow, & Feldon, 1988) as well as by stress (Shalev, Feldon, & Weiner, 1998a, 1998b; Shalev & Weiner, 2001). Additionally, LI has been shown to be reinstated following amphetamine administration by the administration of typical and atypical neuroleptics (Russig, Kovacevic, Murphy, & Feldon, 2003; Russig, Murphy, & Feldon, 2002; Warburton, Joseph, Feldon, Weiner, & Gray, 1994). Finally, LI can be used to model both the positive and the negative symptoms of schizophrenia through the attenuation and the potentiation of LI (Shadach, Gaisler, Schiller, & Weiner, 2000; Weiner, Shadach, Tarrasch, Kidron, & Feldon, 1996). As such, LI provides a behavioral model for studying schizophrenia.

In this experiment, the relationship between CHL1 deficiency, chronic mild stress (CMS) and schizophrenia-like symptoms (lack of LI) was explored within the context of a double hit hypothesis. It was found that neither CHL1 deficiency nor stress alone was sufficient to disrupt LI. When combined, CHL1 deficiency and stress resulted in disrupted LI lending support to the double hit hypothesis of schizophrenia.

Materials and Methods

Subjects: The subjects were 96 male mice in a C57BL/6J background subdivided by genotype as follows: deficient in the CHL1 gene (KO, n = 24), heterozygous for the CHL1 gene (HET, n = 38), and wild-type littermate controls (WT, n = 34), of which three mice (1 KO, 2 HET) were removed for behavior which differed by more than 2 standard deviations from that of the other mice. Mouse genotype was confirmed by PCR genotyping from a tail biopsy sample. The mice were housed in pairs of three or four in a climate-controlled room under a 12-h light-dark cycle. The mice were further divided into Stress (S, n = 54) and No Stress (NS, n=34) groups. The stress group received 6 weeks of chronic mild stress (CMS) beginning at 6 weeks of age, while the no stress group was kept in standard laboratory conditions. Behavioral testing for all groups occurred at 12 weeks of age. Water was given ad libitum, while weight was maintained at 85% of the ad libitum weight by restricting food access (Harlan Laboratories, Indianapolis, IN) during the week of behavioral training and testing. Mice were tested during the light period of the cycle. All experimental procedures were conducted in accordance with the standards for the ethical treatment of animals as outlined in the Guide for the Care and Use of the Laboratory Animals (2011).

Chronic Mild Stress: The CMS regimen consisted of three different stressors each day, with each stressor lasting a minimum of 2 hours. The different stressors were: 1) the water bottle removed from the home cage; 2) mice changed into a cage that had housed other mice; 3) mice given a new, clean home cage; 4) food removed from the home cage; 5) bedding in the home cage was wet; 6) mice placed into a small plastic box; 7) home cages placed at a 45-degree angle; 8) rat bedding spread evenly throughout the home cage; 9) light on during what normally would have been the 12-h dark period for the mice. Due to light-dark cycle manipulations, stress mice were housed in a different colony room from the control (no stress) mice, but using standard laboratory conditions except when the stress condition dictated otherwise (Matthews, Forbes, & Reid, 1995; Pochwat et al., 2014; Raineki et al., 2014).

Apparatus: The apparatus consisted in 8 standard mouse operant chambers (Med Associates, St. Albans, VT) equipped with a house light, a fan, three nose pokes (two on the front wall and one on the back wall), a programmable audio generator, a shocker/scrambler module, a lever, and a standard mouse 20-mg pellet feeder. Mouse operant chambers were housed inside sound attenuating cubicles (Med Associates, St. Albans, VT). The tone and click were produced using a programmable audio generator (ANL-926, Med Associates, St. Albans, VT), which was set such that the tone and click were generated at 80dB, as measured using a Realistic Sound level meter (Radio Shack) positioned in the center of the operant chamber. The foot shock was generated using a shocker/scrambler module (ENV-414, Med Associates, St. Albans, VT), which was set such that the current was generated at 0.5 mA, as measured using a digital multimeter (Radio Shack).

Acclimation: Mice were given four days of acclimation in the operant chambers. Acclimation consisted of one day of free food, and three days of fixed ratio 1 (FR1) training. The mice were in the operant chambers for a total duration of 4.5 hours during the acclimation phase.

Preexposure: Mice were assigned in a counterbalanced manner to either a tone (n=47) or click (n=41) preexposure group. Mice were given one session of preexposure training lasting for 60 minutes. Preexposure occurred in the same context in which all other training occurred. Mice received 40 30s presentations of the preexposed stimulus separated by a 60s inter-stimulus interval (ISI). The total duration of stimulus preexposure was 20 minutes. During the preexposure phase mice were rewarded for nose poking on a variable interval schedule.

Conditioning: The conditioning trial occurred in the same context as all other phases of training. During the conditioning trial, both the preexposed stimulus and the nonpreexposed stimulus were preexposed twice prior to their pairing with foot shock. Each stimulus presentation lasted 30s and was separated by a 240s ISI. After receiving four stimulus presentations, the fifth stimulus presentation was the preexposed stimulus paired with a 1s, 0.5mA foot shock that occurred during the last second of the stimulus presentation. This preexposed stimulus foot shock pairing was then followed (after the ISI had elapsed) by the presentation of a nonpreexposed stimulus paired with foot shock in the manner described previously. During the conditioning trial, mice were rewarded for nose poking behavior on a variable interval schedule. The conditioning trial lasted for 35 minutes.

Rebaseline: On the day following conditioning mice were allowed one day for their behavior to return to baseline behavior. On this day nose poking behavior was rewarded on an FR1 schedule, with the session lasting one hour.

Test: One day following the rebaseline session the mice were tested for latent inhibition. The test session lasted 35 minutes, with the preexposed stimulus being presented for 3 minutes after 12 minutes had elapsed from the beginning of the session. 12 minutes following offset of the preexposed stimulus, the nonpreexposed stimulus was presented for 3 minutes. Mice were rewarded on a variable interval schedule for nose poking throughout the trial. Mouse behavior was video recorded using cameras (Hauppauge Computer Works Inc., Hauppauge, NY) mounted inside the sound attenuating cubicles, using iSpy software (iSpy Connect, Margaret River, Australia) on an IBM-compatible PC.

Behavioral analysis: Recordings from the test session were submitted to behavioral analysis of freezing behavior using FreezeScan software (CleverSys Inc., Reston, VA). Freezing was defined as the absence of movement with the duration thereof being calculated automatically by the FreezeScan software (Hsiao, Chen, Chen, & Gean, 2011).

Data analysis: The duration of freezing behavior in the first 30s of the presentation of both the preexposed and nonpreexposed stimuli in the test session were submitted to a mixed ANOVA with between-subjects variables stress (S and NS) and gene deficiency (KO, HET, and WT) and within variable preexposure (PE and NPE) followed by planned comparisons. All statistical analyses were conducted at an alpha level 0.05.

Results

The average freezing duration during the presentation of the preexposed (PE) and non-preexposed (NPE) stimuli in the stress (S) and non-stress groups is shown in Figure 1. The analysis indicated a stress x freezing interaction, suggesting that reliably less LI was expressed under stress irrespective of genotype ($F(1, 87)=160.51$, $p<0.01$). The analysis also indicated a stress x genotype x freezing interaction, suggesting that this attenuated expression of LI was particularly pronounced in the KO-S group, relative to all other stress groups ($F(2,87)=73.08$, $p<0.05$). Indeed, KO mice showed LI under the no-stress condition ($F(1,87)=26.97$, $p<0.01$) but failed to express LI under stress ($F(1,87)=0.58$, $p>0.05$). Instead, WT and HET mice showed LI irrespective of stress condition (all $Fs(1,87)>9.78$, $p<0.01$). These results provide support for a double hit hypothesis of schizophrenia under which genetic factors combine with environmental factors to bring about the onset of schizophrenia like symptoms (lack of latent inhibition).

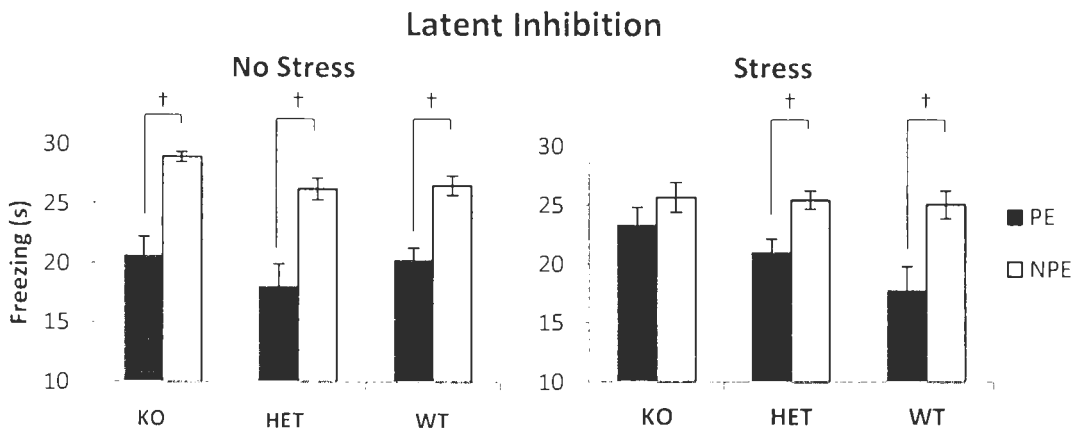

Figure 1 | Latent inhibition (LI) by stress and genotype (average freezing duration ± SEM). Irrespective of stress, both CHL1 heterozygotes (HET) and wild type littermate controls (WT) show robust latent inhibition, i.e. significantly less freezing to the preexposed (PE) stimulus than to the nonpreexposed (NPE) stimulus. In contrast CHL1 knock out (KO) mice are sensitive to stress: they show LI under no stress but fail to show LI under stress. * = $p<0.05$; † = $p<0.01$.

Discussion

Using a within-subjects LI procedure the results demonstrate that C57BL/6J mice show LI, consistent with previous findings (Gould & Wehner, 1999). Additionally, the results show that

CHL1 HET and KO mice show LI under standard conditions. When mice receive CMS in adolescence or young adulthood KO mice failed to show LI while WT and HET mice both continued to show LI. The finding that stress failed to disrupt LI in WT and HET mice is not surprising despite previous studies that found attenuated LI as a result of acute stress in humans (Braunstein-Bercovitz, Dimentman-Ashkenazi, & Lubow, 2001), as studies in rats have shown that some stressors such as tail-pinch are insufficient to disrupt LI on their own (Hellman, Crider, & Solomon, 1983). Additionally, it has been found that in some cases stress can even potentiate the LI effect (Melo, Ferrari, Teixeira, & Sandner, 2003) suggesting that there may be a number of factors which affect how stressors effect the expression of LI.

Stress in CHL1 deficient mice abolished LI. Stress has been shown to increase levels of tyrosine hydroxylase in the mesolimbic system (Ortiz, Fitzgerald, Lane, & Terwilliger, 1996) and to increase DA release in the NAc, PFC, striatum (Abercrombie & Keefe, 1989) and the BLA (Inglis & Moghaddam, 1999). It has been observed that the increased dopamine release triggered by amphetamine administration (Weiner et al., 1988), stress (Shalev et al., 1998a) and corticosterone administration (Shalev et al., 1998b; Shalev & Weiner, 2001) have all been sufficient to reduce LI. This suggests a strong link between not only behavioral stress and attenuated LI but also between the biological correlated of stress and attenuated LI. One possible explanation for this is that as dopaminergic activation increases in the mesolimbic system latent inhibition decreases. Evidence for this theory is found in cases where haloperidol and clozapine restore LI following amphetamine administration (Russig et al., 2003; Russig et al., 2002; Warburton et al., 1994). Thus increased neuronal activation due to stress may be part of the reason why LI was abolished in the CHL1 deficient mice that experienced chronic mild stress.

Conclusion

Under conditions that produce LI in unstressed WT mice, LI was not abolished by either stress or a genetic predisposition to schizophrenia (CHL1 deficiency) alone. LI was abolished in mice that both experienced environmental stressors and possessed a genetic predisposition to schizophrenia. These results provide strong evidence for a double hit hypothesis of schizophrenia within the existing framework of LI as a model for schizophrenia. It is possible that the neural dysfunction causing the loss of LI is the result of interactions between increased neuronal activation due to both stress and a genetic predisposition to heightened neural excitability. An alternative explanation for this neural dysfunction could be that parvalbumin interneurons in CHL1 deficient mice are more susceptible to oxidative stress and as such, their expression is reduced as a result of the CMS resulting in the loss of the expression of LI.

Reflections

When I first became involved in this project, I was really excited. I had a working knowledge of what latent inhibition (LI) was, and I knew that it was a behavioral model that could be used to study schizophrenia as well as learning and attentional processes. I was really excited because the idea of using an animal model of a disease was really interesting to me. For this reason I was really happy when the opportunity presented itself for me to get involved in this project. One of the early challenges was figuring out how the programs that ran the operant chambers worked. Luckily, they were well documented with the computer code written in a clear manner. As a result I was able to understand how the programs were working with guidance from my mentor. From there I began to run mice through the procedure and record their behaviors. Additionally I was ensuring that the mice in the stress group were receiving the appropriate stress every day. I had the opportunity to craft protocols, with guidance, that would determine how the project was carried out. It was very exciting. I feel like the easiest part of the project was the behavioral testing for LI. The harder part came later; sectioning the brains, and then staining them for the c-Fos activation. I got to do a lot of sectioning which was challenging but exciting in its own way because I became much more familiar with brain anatomy as a result of having sectioned so many mouse brains. Additionally I had the opportunity to observe Bret stain the brains for c-Fos activation, and I even got to help him with the staining a little bit at times. This involved a lot of micropipette work. After having finished staining the brains, it was time to take pictures of the brain regions and to count them. I took the pictures of the nucleus accumbens core and shell, and then I helped to count those two regions using a light microscope. It was exciting as I got to see the preliminary results, and it appeared that there were differences in the different groups. Ultimately, with guidance from my mentor I learned how to use Statistica software to perform the desired analyses. After having performed the analyses, I was able to start working on preparing graphs. One of the most important things that I learned is to make sure that all of the data is final before starting to make the graphs. The graphs are time consuming, and it is nice if having to spend the time to remake them can be avoided. Also, it is worth the time and effort to go through several versions of a figure to find the one that will be most intuitive and allow for the easiest communication of the information. If a figure is well made, it will serve as a much needed visual to help people understand what you are explaining whether at a conference or as a student preparing to apply for graduate school or medical school. Finally, after preparing the figures it is time to start on the actual text. One of the more important things I learned is the value of starting to read early and continuing to read. I don't think that it is possible to have read too much or to be too prepared to write a paper. Additionally, reviews are useful tools. Reviews are a great way to get an idea of what has already been researched within a given field, and it also gives an idea of what the most important research topics are moving forward. They will help to focus future reading and to give structure to it. I think that it is very important to consider where the research one is conducting fits within the larger body of research being conducted by other researchers. Finally, I think that I would recommend starting as early as possible on actually writing the paper. Writing is hard, and it will become necessary to find more papers to read because you will realize that there are other aspects of the research that need to be considered even though you actually hadn't considered those perspectives previously. This made writing the thesis a lot harder for me because I would have to go find more papers, but it also made it a lot more rewarding because I was able to learn a lot more than I would have any other way. Ultimately, I think that actually writing the thesis is the hardest part because there is so

much information that has been gleaned, and there are so many different directions that I thought I might end up exploring in the paper, but ultimately I realized that I needed to focus on just a few things to be able to write a more cohesive paper. I think that this was by far the most rewarding experience of my undergraduate career as I had more opportunities to make mistakes and to grow from those mistakes. Also it was the first time that I had really been allowed to take what I was learning in a textbook and apply it to real life, meaning to actually get hands on experience doing what I was learning about in my classes. My advice to future students would be to get involved in something beyond just going to class as soon as possible so that they can identify what they are passionate about. After having done that I think that they ought to pursue what they are passionate about, and have fun doing it. Finally, they should get started on their thesis as soon as possible. The sooner they start into it the better it will turn out.

Author Bio

J. Daniel Obray received his Bachelor's of Science degree in Psychology from Utah State University. He graduated with a minor in Computer Science as well as a minor in Chemistry. He received a SURCO and an URCO fellowship while at Utah State University and he was given the opportunity to attend the annual meeting of the Society for Neuroscience twice, and to present there each time. He was involved in Psi Chi and helped to found the Neuroscience Club at USU. Upon graduation Daniel decided to pursue a Ph.D in Psychology with an emphasis in Cognitive and Behavioral Neuroscience at Brigham Young University. He will be working with Dr. Scott Steffensen researching the neural mechanism that underlies addiction as it pertains to dopamine release in the ventral tegmental area and the nucleus accumbens.

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