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Advancing Understanding of Autism Spectrum Disorder's Possible Causes and Treatments
Researchers identify novel ways to reduce social impairments associated with autism

SAN DIEGO — Studies released today reveal new understanding of autism spectrum disorder, outlining potential causes and mechanisms behind the disease and highlighting novel treatment strategies promoting social behavior. The findings were presented at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

In the United States, the prevalence of autism spectrum disorder has been increasing; 1 in 68 children were diagnosed with the disorder in 2010, and the prevalence remained the same for 2012. It's characterized by problems in two core domains: social interactions and repetitive behavior. However, no treatments improve these core impairments.

Today's new findings show that:

- Rats exposed to commonly used pesticides mirror symptoms seen in autism, suggesting a possible link between pesticides and neurodevelopmental disorders (Jill Silverman, abstract 776.02, see attached summary).
- Giving the hormone oxytocin to pregnant prairie voles just before they give birth promotes maternal bonding and increases sociability of the offspring (William Kenkel, abstract 387.05 see attached summary).
- A new therapy increases the level of the social hormone oxytocin and reduces the social impairments observed in a mouse model of autism (Elena Minakova, abstract 572.09, see attached summary).
- Transplanting embryonic stem cells into the adult brain of a rat model of autism improves symptoms by increasing inhibition in the brain, providing a possible treatment strategy (Daniel Lodge, abstract 47.13, see attached summary).
- Children with autism may overproduce synaptic connections in the amygdala, a region involved in sociability and emotion, suggesting a possible neurobiological explanation for the impairments associated with the disease (Cynthia Schumann, abstract 31.18, see attached summary).

"Today's findings provide a number of new therapeutic possibilities for addressing autism," said Emanuel DiCiccio-Bloom, MD, of Rutgers University, an expert on neurodevelopmental disorders. "By better understanding potential environmental and neurological causes of autism, we can develop more effective treatments for addressing the core symptoms."

The research was supported by national funding agencies such as the National Institutes of Health, as well as other public, private, and philanthropic organizations worldwide. Find out more about autism spectrum disorder at BrainFacts.org.

Related Neuroscience 2016 Presentation
Dialogues Between Neuroscience and Society
Global Mental Health and Neuroscience: Challenges and Opportunities
Saturday, Nov. 12, 11 a.m.-1 p.m., SDCC Ballroom 20

Abstract 776.02 Summary

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Exposure to Low Dose of Common Pesticide Disrupts Neurological Development in Rats *Compound appears to cause social impairments and repetitive behaviors reminiscent of autism*

Early-life exposure to common pesticides may lead to neurodevelopmental disorders, like autism, attention deficit hyperactivity disorder, and intellectual delay, according to new animal research released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. The results corroborate findings from human studies and reveal that even low doses of the pesticides may have significant effects.

Organophosphate pesticides are some of the most commonly used pesticides throughout the world, and epidemiological studies link their use to autism, attention deficit hyperactivity disorder, developmental delay, and other neurodevelopmental disorders. Animal studies clearly reveal toxic effects at very high doses; however, scientists haven't tested the consequences of early-life exposure to very low doses that may better represent what humans may experience.

This study tests the effects of chlorpyrifos, a widely used organophosphate, on the neurodevelopment of rats. Newborn rats received a low dose, a high dose, or a control material. The team then counted the ultrasonic vocalization calls the rat pups made when separated from their mothers. Pups receiving the lower chlorpyrifos dose made fewer vocalizations, an effect that was strongest in female pups. Once the rats became juveniles, the researchers performed sociability tests to assess autism-like behaviors. In general, rats will approach and investigate an unknown rat more than a familiar rat. But rats exposed to a low dose of chlorpyrifos did not distinguish between known and unknown animals, suggesting a deficit in social recognition. Rough-and-tumble social playing was decreased in females receiving the low dose compared with control females, while low-dosed males chased and sniffed other rats less during social play than control males. Exposed animals of both sexes engaged in more self-grooming, reminiscent of the repetitive behaviors seen in autism.

The mechanism behind the sex differences and lack of stronger effects of the higher dose remain unknown. "Our characterization suggests that developmental exposure to a low dose of organophosphate pesticide disrupts neurological development and complex behaviors," said senior author Jill Silverman, PhD, of the School of Medicine at the University of California, Davis. "This informs us that we need better understanding and regulation of the pesticides that go onto food products so that, rather than trying to diagnosis and develop treatments for many developmental disorders down the line, problems that may be caused by this chemical can be prevented."

Research was supported with funds from the MIND Institute and the National Institute of Child Health and Human Development.

Scientific Presentation: Wednesday, Nov. 16, 2-3 p.m., Halls B-H

Abstract 17368, Early life exposure to the organophosphorous pesticide chlorpyrifos produces behavioral phenotypes relevant to neurodevelopmental disorders
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TECHNICAL ABSTRACT: Organophosphorus pesticides (OPs) are among the most widely used pesticides in the world. OPs have been implicated in the etiology of neurodevelopmental disorders including developmental delay and autism spectrum disorder (ASD) (Shelton et al., 2014; Shelton et al., 2012). Exposure to chlorpyrifos (CPF), one of the most widely used OPs, has been linked to mental and motor delays as well as increased rates of attentional issues, specifically attention deficit/hyperactivity disorder (ADHD), in children (Rauh et al., 2006). The present study examined sophisticated social and cognitive behaviors in a novel rat model of CPF exposure. Newborn Sprague-Dawley rats were administered either peanut oil vehicle, 1.0 mg/kg CPF, or 3.0 mg/kg CPF via daily s.c. injections from postnatal day (PND) 1 to PND 4. Isolation-induced ultrasonic vocalizations (USVs) were collected from pups on PND 5, 8, and 11. Sociability and repetitive self-grooming were assessed at the juvenile age using the ASD-relevant behavioral assays of three-chambered social approach and dyadic reciprocal social interaction. Locomotion was evaluated via an open field exploration assay and cognitive testing was carried out using pairwise visual discrimination and reversal learning in an operant touchscreen chamber. Pups exposed to 1.0 mg/kg CPF emitted fewer USVs than did pups treated with vehicle or 3.0 mg/kg CPF, and this effect was markedly higher in females. CPF did not affect the sociability phase of three-chambered approach; however, performance in the social novelty portion suggests that CPF disrupts recognition of previously acquired social cues. Relative to vehicle controls, CPF altered juvenile reciprocal social interactions on several key social parameters in a sexually dimorphic manner. CPF-treated females but not males displayed decreased rough and tumble playing while CPF-treated males exhibited increased chasing and anogenital sniffing, behaviors which were unaffected by CPF exposure in females. Moreover, self-grooming was elevated in both sexes at different doses. Similar exploratory behavior in a novel open arena was observed across treatment groups, eliminating hypo- and/or hyperactivity as confounding behaviors. Performance in the touchscreen learning task did not differ between CPF-treated rats and vehicle controls. Several pieces of evidence point to deficits in sex-dependent and/or species typical sociability in juvenile rodents. Impairments were noted in a range of behavioral assays which, taken together with existing literature, suggests that developmental OP exposures disrupt neurological development and neural correlates that underlie complex behaviors.

Abstract 387.05 Summary

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Treating Pregnant Prairie Voles With Oxytocin Influences Offspring's Social Development *Hormone promotes social interaction and bonding even when not administered directly to pups*

Oxytocin given to mothers during labor may have lifelong effects on the sociability of their offspring, according to new animal research released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Used to induce labor and promote contractions in about a quarter of births in the United States, the hormone oxytocin also affects the brain, where it promotes social interactions and the bond between mother and child. However, little is known about how oxytocin given to mothers might affect the social development of their offspring.

In this study, researchers gave pregnant prairie voles either oxytocin or saline (as a control) on the day they were to deliver their pups. Pups born to oxytocin-treated mothers made more distress cries when separated from their mothers than pups born to control-treated mothers. As adults, prairie voles with oxytocin-treated mothers demonstrated more caring behavior toward other pups and spent more time huddling with others and less time alone, compared with prairie voles of saline-treated mothers. These results suggest oxytocin promotes social interactions and maternal bonding even when given to the mothers, rather than directly to the pups.

“Behavioral differences resulting from oxytocin treatment at birth persisted into adulthood and produced a gregarious pattern of behaviors characterized by heightened sociability,” said lead author William Kenkel, PhD, of Indiana University Bloomington. “The results from our study support earlier findings that exposure to oxytocin in early life can have long-lasting consequences.”

Research was supported with funds from the National Institute of Child Health and Human Development.

Scientific Presentation: Monday, Nov. 14, 2016, 2-2:15 p.m., SDCC 5B

Abstract 17430, Developmental consequences in offspring following maternal oxytocin treatment at birth
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TECHNICAL ABSTRACT: Oxytocin is the most widely used hormone to induce and/or augment labor and is routinely administered in at least 23% of births in the U.S. However, oxytocin is also a centrally active neuropeptide with long-lasting developmental consequences on the neonatal brain. Here, we report several changes in oxytocin-regulated behaviors observed in the offspring of female prairie voles treated with oxytocin (0.25 mg/kg) on the day of delivery as a model for human labor induction. In the neonatal period, we examined ultrasonic vocalizations as a broad measure of response to separation and found that pups born to oxytocin-treated dams vocalized more than saline-treated control offspring. In the adolescent period, we examined anxiety-related behavior in an open field and found no treatment effect. In adulthood, we examined alloparental responsiveness and observed increased caregiving behavior in the offspring of oxytocin-treated dams. Offspring born to oxytocin treated dams were also more gregarious during a test of partner preference formation, spending more time huddling in side by side contact and less time in a solitary cage. In each of these domains, we have replicated these effects and have shown via cross-fostering that they are not due to changes in maternal behavior. We are currently investigating the epigenetic mechanisms for these long-lasting effects of early life oxytocin exposure. Such translational findings have direct implications to human health and behavior.

Abstract 572.09 Summary

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Medication Reduces Social Deficits in Mouse Model of Autism *Compound increases levels of hormone that promotes social bonding*

A new drug amends the social impairments seen in a mouse model of autism, even when administered to adult mice, according to new research released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Previous research links decreased levels of oxytocin, a hormone that promotes social bonding, with autism spectrum disorder, which is characterized by impaired social interactions and trouble communicating. Efforts to treat autism by boosting oxytocin have failed because oxytocin crosses the blood-brain barrier only poorly. However, a new drug called Melanotan-II (MT-II) crosses that barrier and triggers oxytocin release.

In this study, researchers employed a mouse model of autism, in which the mice produce less oxytocin in their brains compared to wild-type animals. As adults, wild-type mice and autism model mice received either MT-II or a placebo continuously for seven days. Autism model mice that received the placebo displayed social deficits, choosing to spend as much time with a toy as with another mouse, while autism model mice treated with MT-II chose to spend more time with the mouse, exhibiting social behavior similar to wild-type mice. The autism model mice treated with MT-II also made more ultrasonic vocalizations — calls to other mice — than autism model mice that received the placebo.

“This approach appears to help with social deficits in adult animals in which the neurocircuitry is much harder to change, unlike earlier time points in development when the brain is more plastic,” said lead author Elena Minakova, MD, of the University of California, Los Angeles. “The findings are promising for application of medical therapy to improve social deficits associated with autism by targeting the endogenous release of oxytocin.”

Research was supported with funds from the UCLA Children's Discovery and Innovation Institute Friedman Fellowship Award.

Scientific Presentation: Tuesday, Nov. 15, 3-3:15 p.m., SDCC 23A

Abstract 17370, Melanotan-II reverses autistic features in the environmental mouse model of autism
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TECHNICAL ABSTRACT: Autism spectrum disorder is a complex neurodevelopmental disorder characterized by impaired social interactions and difficulty with communication. The etiology of autism involves an interplay between genetic and environmental variables resulting in dysregulation of neurotransmitter expression, aberrant synaptogenesis, neuronal apoptosis, and decreased levels of the pro-social neuropeptide, oxytocin. Central release of oxytocin has neuromodulatory effects on the limbic system and can promote social bonding. Recent studies have shown that Melanotan-II (MT-II), a melanocortin-receptor agonist with blood-brain-barrier permeability, can promote central oxytocin release in the brain. Objective: To test the hypothesis that MT-II treatment improves social deficits in a validated environmental mouse model of autism. Methods: To generate the environmental mouse model, a pro-inflammatory state in the fetal mouse brain was produced through cytokine injection of IL-6 in pregnant C57BL/6 females. Male mice underwent social behavioral testing using a validated three-chamber apparatus prior to treatment and post-treatment. Baseline ultrasound vocalizations (USVs) were also recorded to assess communication. Mice were treated by surgical placement of a subcutaneous pump with an intraventricular catheter allowing for continuous MT-II infusion over 7 days. Control mice were implanted with isotonic saline-filled pumps. Immunohistochemistry was done to assess for oxytocin and vasopressin expression in the paraventricular nucleus (PVN) Results: Following MT-II administration, IL-6 autistic mice showed significant improvement in their sociability and social novelty index scores ($p < 0.0001$ and $p < 0.05$, respectively) with levels found comparable to wild-type C57BL/6 mice. Control saline-administered IL-6 autistic mice continued to exhibit low social behavioral scores. MT-II-treated IL-6 autistic mice also demonstrated increased USVs compared to saline-administered controls. PVN staining of untreated adult IL-6 mice showed a significant reduction of oxytocin and vasopressin expression compared to wild-type mice with an increase in both neuropeptides following MT-II administration. Conclusions: MT-II administration to IL-6 autistic mice corrected social deficits with normalization of behavior metrics to levels found in wild-type mice. This change in behavior is likely due to central release of oxytocin elicited by MT-II. The affected autistic IL-6 mice exhibited abnormally low oxytocin and vasopressin level in the PVN suggesting a novel role for the cytokine to regulate expression patterns of both neuropeptides.

Abstract 47.13 Summary

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Stem Cell Therapy Improves Symptoms of Autism in Rats *Injecting inhibitory cells appears to regulate overactive prefrontal cortex*

Transplanting embryonic stem cells that release the inhibitory neurotransmitter GABA into the brains of a rat model of autism reduced the animals' characteristic social impairments and cognitive deficits, according to new research released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Autism spectrum disorder is characterized by disruptions in two core domains: social communication and interaction and repetitive patterns of behavior or thought. These symptoms may result from a disruption of the brain's major inhibitory neurotransmitter, GABA. People with autism exhibit a loss of parvalbumin-positive (PV) interneurons — a type of neuron that releases GABA — and increased excitation in the prefrontal cortex. The researchers hypothesized that restoring PV interneurons in a rat model of autism would help regulate activity in the prefrontal cortex and reduce symptoms.

In this study, the researchers triggered mouse embryonic stem cells, which can differentiate into any cell type, to become PV cells. Thirty days after transplanting these cells into the adult prefrontal cortex of the autism model and wild-type rats, the researchers tested the rats for social interaction, ultrasonic vocalizations, and cognitive flexibility. The PV transplants increased the time that autism model rats spent with other rats and improved their ability to adapt to changes. The researchers also found that the transplanted cells reduced activity in the prefrontal cortex to normal levels.

“We demonstrated that PV cell transplants were able to alleviate deficits in social interaction and cognitive function, suggesting that this may be a novel and effective treatment strategy to reduce the core symptoms of autism,” said senior author Daniel Lodge, PhD, of the University of Texas. “Such a finding is exciting because it offers the potential for an urgently needed treatment strategy for autism.”

Research was supported with funds from the Owens Foundation and the National Institute of Mental Health.

Scientific Presentation: Saturday, Nov. 12, 1-2 p.m., Halls B-H

Abstract 17369, Embryonic stem cell transplants as a therapeutic strategy in a rodent model of autism

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TECHNICAL ABSTRACT: Autism is a neurodevelopmental disorder characterized by disruptions in three core behavioral domains: deficits in social interaction, impairments in communication, and repetitive and stereotyped patterns of behavior or thought. There are currently no drugs available for the treatment of the core symptoms of ASD and drugs that target comorbid symptoms often have serious adverse side effects, suggesting an urgent need for new therapeutic strategies. The neurobiology of autism is complex, but converging evidence suggests that ASD involves disruptions in the inhibitory GABAergic neurotransmitter system. Autistic patients show reductions in GABA itself, in the GABA synthesizing enzymes, GAD65 and GAD67, and in subunits of the GABA_A receptor. Post mortem studies demonstrate that these reductions may be due to an actual loss of GABAergic interneurons, specifically the parvalbumin (PV)-positive subtype. PV-positive interneurons play a critical role in regulating pyramidal cell excitability and synchronizing coordinated network activity. Autistic patients show hyperexcitability and reduced coordinated activity in the prefrontal cortex (PFC) and this dysregulated PFC activity has been associated with autism-like behaviors, including reduced social interaction and cognitive inflexibility. Therefore, we hypothesize that restoring PV interneuron function will normalize aberrant cortical activity and ultimately abolish the behavioral deficits associated with autism. To induce an autism-like phenotype, pregnant rats were injected with the viral mimetic Poly I:C (7.5 mg/kg) on gestational day 12. To restore interneuron function, we used a mouse embryonic stem cell line containing dual reporters (Lhx6::GFP and Nkx2.1::mCherry) to grow PV-positive interneurons. Cells were sorted using flow cytometry, then injected into the mPFC of Poly I:C- or saline-treated offspring. After a 30 day recovery period, during which time the transplanted cells migrate and integrate into the mPFC circuitry, we measured social interaction, ultrasonic vocalizations, attentional set-shifting, and pyramidal cell activity in the mPFC. PV-positive transplants into the mPFC of Poly I:C-treated rats increased social interaction time, improved cognitive flexibility, and normalized pyramidal cell function, suggesting that targeting PV-positive interneurons in the mPFC may be a novel and effective treatment strategy to reduce the core symptoms of autism.

Abstract 31.18 Summary

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Autistic Brains Have Excessive Neuronal Connections in Childhood

Differences seen in brain region important for social communication, emotional regulation

The brains of children with autism spectrum disorder may fail to control the process of forming and removing connections between neurons, according to new research released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

MRI studies show children with autism spectrum disorder (ASD) have larger amygdala — a region involved in social communication and emotional regulation — compared with typically developing (TD) children. Researchers hypothesized this might be due to a difference in the creation and removal of dendritic spines. Dendrites are the branch-like projections from neuronal cell bodies, and they are covered in little bumps known as dendritic spines. Each dendritic spine represents a contact point with another neuron. During normal development, there is an overproduction of dendritic spines, but the spines that are unused are later removed in a process called synaptic pruning. If this system is dysregulated in an area of the brain, it may cause differences in that region's overall size and function.

To test whether the size differences between ASD and TD brains are due to variances in the density of dendritic spines, the researchers collected post-mortem brain tissue from 13 TD and 14 ASD cases. Researchers stained a section of brain containing the amygdala to reveal the dendrites and then counted the spines. People with ASD that were younger than 18 years old had greater spine density in the amygdala compared to TD children. However, with age, the density of the spines in ASD cases decreased, and by adulthood, there was no observable difference in spine density compared with the TD cases. This supports previous work demonstrating that the difference in amygdala size in autistic brains disappears by adulthood.

“These findings suggest a possible underlying difference in innervation or synaptic pruning mechanisms in the amygdala of children with ASD that may contribute to the altered developmental trajectory as well as the social and emotional impairments,” said senior author Cynthia Schumann, PhD, from the University of California, Davis MIND Institute.

Research was supported with funds from the National Institute of Mental Health and National Institute of Child Health and Human Development.

Scientific Presentation: Saturday, Nov. 12, 2016, 2-3 p.m., Halls B-H

Abstract 17371, Greater amygdala spine density in young ASD brains
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TECHNICAL ABSTRACT: The amygdala, a medial temporal lobe structure implicated in social and emotional regulation, undergoes an aberrant growth trajectory in individuals with autism spectrum disorder (ASD), which includes precocious growth early in childhood followed by cell loss in adulthood. The goal of this study is to determine if the aberrant amygdala growth in children with ASD may be attributable to alterations in neuronal dendritic morphology. Tissue from 34 postmortem human brains (age range 4-46) was assembled from the NIH NeuroBioBank at the University of Maryland, the BEARS program at UC Davis MIND Institute, Autism BrainNet and the Harvard Brain Tissue Resource Center. Blocks of amygdala tissue approximately 1.5x1.5x0.5cm were excised from the temporal lobe and neurons were visualized using a modified Golgi-kopsch staining technique. After dehydration and embedding in parlodion, 150µm sections were cut on a sliding microtome. 10 lateral nucleus neurons per case were selected (fully impregnated, central within the slice and free from obscurities) to be traced using NeuroLucida software (MBF Biosciences). Spine counts were collected from one dendrite per neuron. Measures of dendrite morphology such as total dendritic length, segment count and spine density were analyzed using NeuroLucida Explorer and statistical analyses conducted in SPSS. The success of Golgi-kopsch staining in post-mortem human tissue was in line with previous studies (Rosoklija et al., 2014) allowing complete data collection from 13 TD and 14 ASD cases. There was a positive correlation between total dendritic arborization and age in both TD and ASD amygdala. There was no difference in dendritic arborization between diagnostic groups. However, spine density was significantly different between diagnostic groups across age ($F_{(3,26)}=4.36$, $P=0.014$). Younger cases (≤ 16 years old) of ASD have a greater spine density than TD controls. While spine density remains relatively constant in the amygdala into adulthood in TD, there is a significance decrease in spine density as people with ASD age. This study shows a clear difference in spine density between young ASD and TD cases, which possibly represents an underlying difference in amygdala innervation or pruning mechanisms in ASD brains that may contribute to social and emotional impairments into adulthood.