Piglets in mazes provide insights into human cognitive development

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Events that take place early in life almost certainly have consequences for later cognitive development. Establishing the connections is difficult, however, because human infants cannot be used as laboratory subjects.

Rodney Johnson and his collaborators have developed an alternative model for studying infant brain development. "Assistant professor Ryan Dilger and I became interested in establishing the neonatal piglet as a model of human brain and cognitive development 3 or 4 years ago," he said.

The idea came to Johnson when a former student, who was working for an infant formula company, asked about finding ways to determine differences in cognitive development between breast-fed infants and infants fed on formula.

"Human breast milk is the gold standard, but not every infant can be breast fed. A major goal for many infant formula companies is to improve the formulation to capture all of the benefits of breast milk," he explained.

Johnson and his group had been working with rodent models to study learning and memory; they also had done some research looking at infectious disease in pigs. They wondered if it would be possible to develop tests to look at learning and memory using neonatal piglets.

It seemed like a reasonable idea because the growth and development of

the piglet brain is similar to that of the human brain. The brain growth spurt is a perinatal event in both humans and pigs. At birth, the human brain is about 25 percent of adult size. In the first 2 years of life, it reaches 85 to 90 percent of adult size. The piglet brain grows in a similar way in a shorter time.

Johnson's team first developed structural MRI methods for quantifying brain volume in the neonatal piglet. They then used these techniques to determine total brain and brain region volumes in a cohort of male and female domestic pigs, taking repeated measurements every 4 weeks starting at 2 weeks of age and finishing at near sexual maturity at 24 weeks of age.

They found that at 4 weeks, the piglet brain had grown to approximately 50 percent of its maximum volume, and it continued to grow rapidly for the next 8 weeks. Human infant brains grow in a similar way in the postnatal period. The results suggested that environmental insults during this period could affect brain structure and function.

The researchers' next task was to develop a test to assess the piglets' learning and memory, using a T-maze. They thought that this would be easy. They were wrong.

"It actually turned out to be very complicated because there were a lot of things that went wrong that we didn't predict," said Johnson. "For example, when we first started these studies, we used things like Skittles and apple slices as a reward because that's what people using older pigs had done."

The piglets, which were being fed on infant formula, had no interest in solid food, nor were they motivated to perform the tasks if the reward was the same as their regular food. They were, however, very willing to work for chocolate milk, specifically Nesquik™.

They did not receive the Nesquik™ anywhere else. "The idea is that piglets see chocolate milk only during the test and then it's back to the standard formula," said Johnson. "It helps increase their precision because it's something they look forward to."

Tests were conducted in a plus-shaped maze with one arm blocked off to leave a 'T' shape. Piglets were trained to locate the milk reward in a constant place in space as well as direction, using visual cues from outside the maze. When they learned to perform the task correctly the reward location was reversed, and the piglets were retested to assess learning and working memory. Correct choices decreased in the reversal phase but improved over time.

The results demonstrate that the T-maze can be used to measure cognitive abilities. Johnson and his collaborators will use these new tests to examine how stressors such as nutrient deficiencies and infections affect the human brain during this time of early, rapid growth.

"We want to know if this will alter the trajectory of normal development in a way that makes them more susceptible to behavioral disorders that occur later in life, such as autism and depression," Johnson said. "Exposure to environmental insults early in life may also reduce stress resilience," he added.

"There is a lot of interest in the concept of programming, the notion that things that occur early in life set that individual up for problems that occur many years later," he continued. "Because the pig brain grows so much like a human brain, we thought this could be a very attractive model."

The researchers have used the piglet model to demonstrate that an iron-deficient diet causes iron depletion in specific brain areas and is accompanied by cognitive deficits. They are using structural MRI and

the T-maze task to study how viral pneumonia in the early neonatal period affects brain and cognitive development.

These data are complemented by measures of neuroinflammation, neurogenesis, and neuron morphology, procedures Johnson's group has established in the neonatal piglet. Ryan Dilger is developing novel MRI procedures to measure biochemicals in the brain and to reveal how neural connections develop.

They are hoping to receive NIH funding to look at maternal viral infections. "We will infect pregnant gilts at the beginning of what would be the third trimester in humans and then study brain and cognitive development in the offspring," Johnson said. "This is the type of interesting question we can pursue now that the piglet model is in place."

Johnson said that it would be difficult to do this kind of research anywhere else.

"The Beckman Institute has been wonderful. We're not MRI experts, nor do we want to be. However, we had a hypothesis and thought MRI technology could help us address it," he said. "The support staff at Beckman has been really critical to helping develop the protocols."

Johnson said that the other major advantages of the U of I are that the College of Agricultural, Consumer and Environmental Sciences has a unique research facility for housing pigs, and the Department of Animal Sciences maintains several swine farms that produce piglets on a regular basis.

More information: The most recent research has been published online in *Developmental Neuroscience*.

The research is described in more detail in the following articles:

Conrad, M.S., R.N. Dilger and R.W. Johnson. 2012. "Brain growth of the Domestic Pig (Sus scrofa) from 2 to 24 weeks of Age: A Longitudinal MRI Study." Developmental Neuroscience (in press, online first).

Elmore, M.R.P., R.N. Dilger, R.W. Johnson. 2012. "Place and Direction Learning in a Spatial T-maze test." Animal Cognition 15:667-676.

Conrad, M.S., R.N. Dilger, A. Nickolls, and R.W. Johnson. 2012. "Magnetic Resonance Imaging of the Neonatal Piglet Brain." Pediatric Research 71:179.