PRENATAL ULTRASOUND: IT'S NOT JUST A PHOTOGRAPH

BY EMILY L. WILLIAMS AND MANUEL F. CASANOVA, MD

Given the deregulation of prenatal ultrasound intensity and the technology's growing use since the 1970s, as well as the rising diagnostic rates of autism, concerns have been raised about ultrasound's safety during pregnancy. Here, we review research showing how ultrasound can affect growth rates of stem cell populations, raising questions as to whether neural stem cells are vulnerable targets to ultrasonic waves during the prenatal period. The increase in total neuron number in autism is indicative of increased proliferation of neural stem cells, and therefore any agent that can ultimately lead to such an outcome is suspect as a risk factor for development of the condition. Further research is needed to confirm whether ultrasound is one such agent.

INTRODUCTION

Prenatal ultrasound has been in use for several decades and has continued to increase in popularity over the years. Ultrasound machines aim sound waves at a target tissue (in this case, a developing baby) and whatever sound waves bounce back are then measured and converted into an image. Contrary to many people's impressions, ultrasound is not harmless. Studies performed in the 1970s and 1980s confirmed that ultrasound can cause damage to tissues by heat production as well as by something called "cavitation" (Sikov, 1986). Noninertial cavitation is when air trapped in a liquid is moved by sheer force, in this instance, ultrasonic waves.

In the case of cavitation within tissues, gases trapped in the liquid surrounding a cell can collide with the cell and damage it. Moreover, even if the gas bubble does not collide directly with the cell, it can spin beside the cell, creating a force in the surrounding liquid that can affect cellular functions (Wu & Nyborg, 2008). Usually, heat production and higher levels of cavitation will cause necrosis, a process in which the cell dies by bursting open and spilling its contents into the surround. This can then trigger inflammation and damage to neighboring cells. (The alternate form of cell death, called "apoptosis," is a safer, programmed death where the cell packs itself up into neat little compartments and commits suicide, preventing its components from spilling out and damaging other cells.) Even when the gas bubble does not overtly damage the cell, lower levels of cavitation can still affect a cell's internal functions and signaling mechanisms (Wu & Nyborg, 2008). Realizing that there are many molecules on a cell's surface performing different functions, it is clear that the sheer force of a spinning bubble immediate to a cell's membrane can have a measurable and possibly even lasting effect on that cell.

DETERMINING ULTRASOUND SAFETY

Originally, researchers determined "safe" intensity levels of ultrasound for humans by looking at studies of the levels at which ultrasound causes necrosis (Stolzenberg et al., 1980). In the 1990s, however, regulations over ultrasound intensity were loosened due to changing healthcare needs. Because the resolution of the image is determined by the intensity of the ultrasound beam and the

What does ultrasound do to a cell, and are any of those changes long-lasting? Sadly, while we continue to subject women and their babies to ultrasound after ultrasound, we don't really know the answers to those questions.

amount of tissue it has to pass through to get to its target, in order to meet the demands of new techniques such as echocardiography and the accommodation of a wider range of patient physiques, the FDA decided to loosen regulations to allow practitioners to apply the intensity necessary to improve image resolution (Meltzer, 1996; Miller et al., 1998).

Ultrasound machines come equipped with two acoustic indicators. The first indicates whether the ultrasound is generating too much heat in the tissue, while the second is a mechanical index gauging levels of cavitation. Both indices are intended to act as "danger signals" to the technician. The machine uses a general formula to estimate when dangerous thermal and cavitational levels have been reached; it then either notifies the technician (via the indicators) or automatically shuts off. However, professionals generally are ignorant of the thermal and physical mechanics that underlie the risks to their patients, lacking a full understanding of the meaning of the thermal indicator and of the theory behind cavitational tissue damage (Sheiner et al., 2007). While practitioners are therefore dependent upon the machines' capabilities to estimate when exposure is reaching potentially harmful levels, one study found that an alarming 80% of end users (nurses, technicians, and other practitioners who apply ultrasound in their practices) were unaware of even the location of the acoustic indices (Sheiner et al., 2007)! We must emphasize that practitioners are not being careless, nor are they to blame for the current situation, which instead is a reflection of inadequate research, poor regulations, and unproven assumptions. By allowing ultrasound, in its many uses, to be widely integrated into daily treatment and diagnostics without ensuring that safety is maintained at all levels, we have put the cart before the horse.

On the face of it, ultrasound does not appear to have negative effects on a baby's development. When used as directed, babies exposed to ultrasound do not seem to have gross bodily defects, lower birth weights, or other atypical outcomes that can be viewed with the naked eye (Newnham et al., 1993; Stark et al., 1984). However, science still poorly understands the physics of ultrasonic waves and how those waves can affect different ranges of target tissues. What we as researchers, practitioners, and parents do not know (but take for granted in the absence of conclusive research) is whether ultrasound is truly safe when no obvious deformities are present. What does ultrasound do to a cell, and are any of those changes long-lasting? Sadly, while we continue to subject women and their babies to ultrasound after ultrasound, we don't really know the answers to those questions.

AUTISM AND ULTRASOUND

Whereas gross physical defects do not occur with typical ultrasound exposure, researchers have not fully delved into possible microscopic effects. Most previous research has centered on the tissue damage (i.e., cell death) of which ultrasound is capable. More recently, investigators have become interested in effects that alter how cells function and express genes, and have postulated that these changes may ultimately affect a cell's developmental trajectory. This area of research has raised the concern that ultrasound may alter development of vulnerable systems such as the brain (Ang et al., 2006).

In some of our earlier work, we found that a key underlying feature in the neuroanatomy of autism is an increase in the total number of something called a "minicolumn" (Casanova et al., 2002). In the cortex of the brain, excitatory cells are lined up in vertical columns (minicolumns), and in autism there appears to be a greater number of these columns. One way to create a greater number of minicolumns is to increase the proliferation of neural stem cells, which supply neurons for these columns (Rakic, 2000). Ultimately, this can lead to a greater cranial volume, and this is precisely what is seen in autism (Redcay & Courchesne, 2005).

The layers of epithelial tissue that underlie the developing cortex are called the paraventricular zone, which is essentially a germinal zone for neural stem cells. From this area, different types of neural stem cells arise, which later produce neurons and glia. This germinal tissue is highly vascularized, so that it has a lot of blood vessels running through it feeding it (Shen et al., 2008). One reason for this is because stem cells divide a lot; cells that are frequently dividing need a good blood supply to support their growth and division.

Most of the cells in our body are considered "differentiated," meaning that, for most intents and purposes, they cannot change into another type of cell. However, stem cells are a peculiar breed of cell, with the potential to develop into many different kinds of cells (something called "pluripotency" or "multipotency"). A neural stem cell can develop into a pyramidal cell, an interneuron, a glial cell, and so forth. When it comes to ultrasound, stem cells seem to have a unique reaction to its effects: they start to grow and divide. This has been known for several decades. Ultrasound's ability to induce more rapid healing has been used to treat certain types of bone breaks (Dyson & Brookes, 1983) and to induce growth and healing in ulcerative tissue for the treatment of some types of ulcers. Ultrasound also seems to have a unique effect on the vascular system, causing blood vessels to dilate (Corretti et al., 2002) and to produce nitric oxide (NO) and growth factors such as FGF2, VEGF, and IL-8 (Doan et al., 1999; Reher et al., 1999; Reher et al., 2002). Because the varying intensities of ultrasound have different effects on cellular functions, low intensity ultrasound (which seems to improve the permeability of a given tissue) has been used in some types of pharmacological administration (Monti et al., 2001).

The fact that the application of ultrasound causes dilation of blood vessels and stimulates the production and release of growth factors like FGF2 and VEGF is highly relevant to autism. Both of these growth factors are shared by the vasculature and neural stem cells, meaning that both types of cells produce them and use them to promote growth (Raab & Plate, 2007). If ultrasound can increase the local production of growth factors surrounding the paraventricular zone, it is likely to create more neural stem cells and, thus, more neurons later on. In other words, it is theoretically plausible that ultrasound could trigger the overproliferation of neural stem cells that, in turn, leads to the increase in minicolumns (neurons) noted as characteristic in autism. Although other genetic or environmental agents could have a similar effect in activating the molecular pathways in neural stem cells that promote increased proliferation, the high rates of exposure to ultrasound (and the lack of research surrounding its impact) are an argument in favor of placing ultrasound towards the top of the list of environmental concerns.

One way to create a greater number of minicolumns is to increase the proliferation of neural stem cells, which supply neurons for these columns. Ultimately, this can lead to a greater cranial volume, and this is precisely what is seen in autism.

Unfortunately, concerns about ultrasound's use within healthcare must now be extended to its increased use in and marketing by the private sector. Not only are parents able to have 3D and 4D keepsake images taken of their baby, promoted as a great way to "start the family album early," but they can also purchase their own ultrasound equipment for home use, such as the Doppler heart rate monitor (Baby Pictures, 2009; Rados, 2004). As illustrated by an online shopping reviewer (Online 3 Shopping, 2011), most people are completely unaware of any potential danger from unnecessary exposures:

> Goodness I love my fetal Doppler. [. . .] I have not had a day where I could not hear the heartbeat from week 8 of pregnancy when I got it! The heart rate isn't exact but I can hear my little one at any time of the day and as clear now (16 weeks) as when I got it! [. . .] My doctor thinks I got an amazing deal!

DOES THIS MEAN WE SHOULD STOP USING PRENATAL ULTRASOUND?

Ultrasound is an extremely useful tool, and we are not advocating cessation of its use. Ultrasound can be compared to amniocentesis, which also has a place in clinical decision-making while conferring some risks to the baby (American Pregnancy Association, 2006). The risks of amniocentesis do not mean that we never use it; rather, we use amniocentesis when it is truly needed and when the benefits outweigh the risks, just as we would do with any other kind of diagnostic method or treatment.

With this comparison in mind, we have four recommendations regarding the use of ultrasound:

1. Greater caution and moderation in the application of ultrasound in healthcare settings, particularly in regards to unwarranted early and/or multiple applications of ultrasound;
2. Cessation of use of ultrasound by businesses or in the home for unnecessary monitoring or imaging;
3. Better education of both healthcare professionals and the public as to its potential dangers; and
4. More research to investigate how ultrasound really works and ascertain the technology's effects on development, including that of autism.

Our concerns about the inappropriate use of ultrasound are not intended to incite parents to boycott ultrasound, but rather to advocate for more caution in decisions about when and how it is used. This means that parents who are worried about early or multiple ultrasound exposures should discuss their concerns with their OB/GYN, coming up with a plan of action on which both can agree. Although ultrasound can make an important contribution to the health of one's baby, when the method is used unwisely or to excess, the risks can outweigh the benefits. At the same time, healthcare professionals who are involved in the prescription or application of ultrasound should cultivate greater awareness of ultrasound research. Some of the most telling evidence as to its biological effects is not found in epidemiological studies investigating the method's safety, but rather in fields such as osteopathic and cardiovascular research, which use the method for healing, vasodilation, and more efficacious delivery of medications to target tissues.

Given the weak regulations surrounding ultrasound safety, research is needed to elucidate the ways in which ultrasound may alter development. The increased diagnostic rates of autism over the last two decades and the research to date on ultrasound's effects on stem cell populations underscore the need for further non-epidemiological investigations to examine the potential relationship between ultrasound exposure and the development of autism. As we cannot stress enough, ultrasound is not just a photograph.

REFERENCES

American Pregnancy Association. *Amniocentesis*. 2006. Taken on 01/09/2011 from: http://www.americanpregnancy.org/prenataltesting/amniocentesis.html

Ang Jr. ESBC, Gluncic V, Duque A, Schafer ME, Rakic P. Prenatal exposure to ultrasound waves impacts neuronal migration in mice. *Proceedings of the National Academy of Sciences*. USA, 2006; 103: 12903-10.

Baby Pictures. Ultrasound services. 2009. Taken on 01/08/2011 from: http://www.babypicturesultrasound.com/services.html

Casanova MF, Buxhoeveden DP, Switala AE, Roy E. Minicolumnar pathology in autism. *Neurology*. 2002;58: 428-32.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R, International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology*. 2002;39: 257-65.

Doan N, Reher P, Meghji S, Harris M. In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. *Journal of Oral and Maxillofacial Surgery*. 1999;57(4): 409-19.

Dyson M, Brookes M. Stimulation of bone repair by ultrasound. *Ultrasound in Medicine & Biology*. 1983;101(Suppl 2):61-6.

Meltzer RS. Food and Drug Administration ultrasound device regulation: The output display standard, the "mechanical index," and ultrasound safety. *Journal of the American Society of Echocardiography*. 1996;9(2):216-220.

Miller MW, Brayman AA, Abramowicz JS. Obstetric ultrasonography: A biophysical consideration of patient safety-- The "rules" have changed. *American Journal of Obstetrics and Gynecology*. 1998;179: 241-54.

Monti D, Giannelli R, Chetoni P, Burgalassi S. Comparison of the effect of ultrasound and of chemical enhancers on transdermal permeation of caffeine and morphine through hairless mouse skin in vitro. *International Journal of Pharmaceutics*. 2001;229(1-2):131-7.

Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI. Effects of frequent ultrasound during pregnancy: A randomized controlled trial. *The Lancet*. 1993;342(8876):887-891.

Online 3 Shopping. *Sonotrax -B, Ultrasound Fetal Doppler, LCD Display with Back Light*. 2011. Retrieved on 01/09/2011 from: http://www.online3shopping.info/sonotrax-b-ultrasound-fetal-doppler-lcd-display-with-back-light/

Raab S, Plate KH. Different networks, common growth factors: Shared growth factors and receptors of the vascular and the nervous system. *Acta Neuropathologica*. 2007;113(6): 607-26.

Rados C. FDA cautions against ultrasound 'keepsake' images. *FDA Consumer*. 2004;38: 12-16.

Rakic P. Radial unit hypothesis of neocortical expansion. *Novartis Foundation Symposium*. 2000;228: 30-42.

Redcay E, Courchesne E. When is the brain enlarged in autism? A meta-analysis of all brain size reports. *Biological Psychiatry*. 2005;58(1): 1-9.

Reher P, Doan N, Bradnock B, Meghji S, Harris M. Effect of ultrasound on production of IL-8, basis FGF and VEGF. *Cytokine*. 1999;11: 416-23.

Reher P, Harris M, Whiteman M, Hai HK, Meghji S. Ultrasound stimulates nitric oxide and prostaglandin E2 production by human osteoblasts. *Bone*. 2002;31: 236-41.

Sheiner E, Shoham-Vardi I, Abramowicz JS. What do clinical users know regarding safety of ultrasound during pregnancy. *Journal of Ultrasound in Medicine*. 2007;26: 319-25.

Shen Q, Wang Y, Kokovay E, Lin G, Chuang SM, Goderie SK, Roysam B, Temple S. Adult SVZ stem cells lie in a vascular niche: A quantitative analysis of niche cell-cell interactions. *Cell: Stem Cell*. 2008;3: 289-300.

Sikov MR. Effect of ultrasound on development: Part 2: Studies in mammalian species and overview. *Journal of Ultrasound in Medicine*. 1986;5(11): 651-61.

Stark CR, Orleans M, Haverkamp AD, Murphy J. Short- and long-term risks after exposure to diagnostic ultrasound in utero. *Obstetrics & Gynecology*. 1984;63: 194-200.

Stolzenberg SJ, Torbit CA, Edmonds PD, Taenzer JC. Effects of ultrasound on the mouse exposed at different stages of gestation: Acute studies. *Radiation and Environmental Biophysics*. 1980;17: 245-70.

Wu J, Nyborg WL. Ultrasound, cavitation bubbles and their interaction with cells. *Advanced Drug Delivery Reviews*. 2008;60: 1103-16.