Problem: ***Neuroscience research is limited by the blood brain barrier:*** The study of various neuropathologies, ranging from substance use disorder (SUD) to dementia, is currently hindered by an inability to deliver drugs to the brain. Our goal is to accelerate research progress in the field of neurobiology by providing researchers with a tool to deliver molecules to the brain with greater fidelity. Investigation of brain-based targets is restricted by the blood brain barrier (BBB), a boundary that protects the brain from pathogenic and neurotoxic substances in circulating blood. The BBB prevents access to the brain for the vast majority of small molecule (~98%) and nearly all large-molecule drugs [1]. This hurdle not only affects disease treatment, but also discovery-based research. For example, siRNA is a common technique for suppressing a specific protein (i.e. neurotransmitter) that relies upon delivery of large molecules to the cells of interest [2].

Solution*: Brain-targeted drug delivery vehicles:* Exosomes allow cells to communicate with one another by collecting molecules (i.e. proteins, RNA, etc.) inside of small compartments within the cell, and ejecting these packages outwards where they may be absorbed by neighboring cells. The study of exosomes is relatively new, but has exploded recently due to their potential for diagnosing diseases and delivering therapeutic molecules. Exosomes have an innate ability to cross the BBB [3, 4] that has been significantly enhanced by attaching proteins cells to the exosomal surface that specifically target the brain [5]. One such protein that was applied to great success is the same molecule that is responsible for the neuro-invasiveness of the rabies virus [6]. By attaching the active portion of the rabies viral glycoprotein (RVG) to exosomes, independent labs have demonstrated enhanced delivery of exosome contents to the brain [7, 8]. For example, Liu *et. al*, applied this technology to prevent morphine relapse in live mice by delivering siRNA that diminished the number of opioid receptors expressed [8]. This application of the technology demonstrates the potential for SUD researchers to effectively deliver molecules to a desired location within the central nervous system.

It is important to note the difference between an exosome and the more established drug delivery vehicle, liposomes. Liposomes are structurally similar to exosomes, consisting of an outer lipid membrane that surrounds the molecules for delivery; however, liposomes are fully synthetic. The biologically-sourced exosomes contain intrinsic processes for cellular uptake and immune system avoidance that are not present in liposomes [9]. However, the maintenance of primary cell lines can be expensive. The synthetic nature of liposomes makes this form of drug delivery vehicle more cost-effective for production, and they can also be tagged with targeting proteins. Liposomes tagged with the RVG peptide demonstrated a similar ability to cross the BBB as was detected by Liu with RVG-tagged exosomes [10]. The efficacy of RVG-tagged liposomes has not been directly compared to that of the RVG-tagged exosomes. Although more than a dozen liposomal nanomedicines have been approved [9], no effort has been made to sell brain-targeted liposomes/exosomes to scientists for basic research.

We aim to produce brain-targeted delivery vehicles that are custom loaded with the consumer's choice of molecules (i.e. drugs, siRNA, etc.). The process of creating brain-targeted delivery vehicles is complex and intimidating for those unfamiliar with the technique, leading promising hypotheses related to neurotransmitter function to be relegated to theories. Commercialization of a technique to deliver molecules across the BBB would transform SUD research, facilitate progress in neuroscience, and create a currently untapped market.

APPROACH

The overall strategy that we propose (Fig. 1) describes the production of brain-targeted delivery vehicles for distribution to neuroscientists.

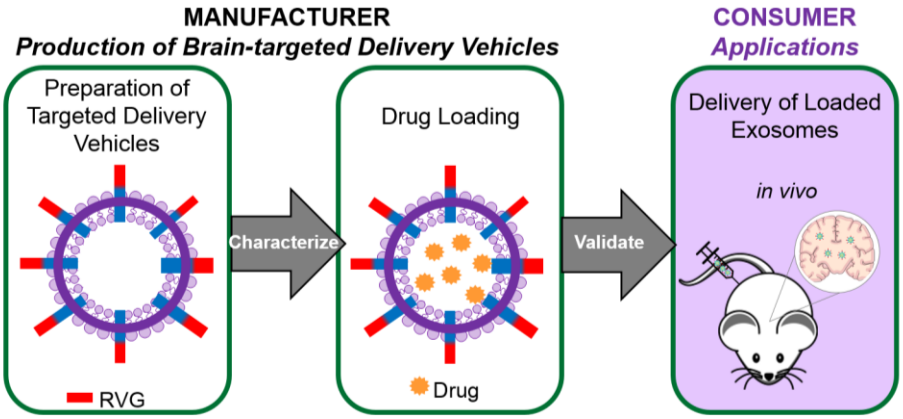

Figure 1. *Development and application of brain-targeted drug delivery vehicles.*

Manufacturer

Production of Brain-targeted Delivery Vehicles

Two types of biological delivery vehicles will be compared for cost, efficacy, and feasibility--exosomes and liposomes (Table 1). These molecules will be targeted for delivery to the brain through the addition of the rabies virus glycoprotein-derived peptide (RVG). Targeted exosomes and liposomes using RVG as a brain-targeting strategy have been successfully generated and implemented for gene delivery [7, 10], and our goal is to expand upon and commercialize this technology. Each RVG-tagged delivery vehicle (exosome or liposome) represents a minimum viable proof (MVP); however, only one will be selected for commercialization based on the outcomes of this study.

Table 1. *Manufacturing process for production of targeted exosomes and liposomes.*

		Exosomes [7]	***Liposomes [10]***
I.	***Preparation*** Isolation and RVG- tagging	Harvest from cell culture	Formulation and extrusion
II.	***Characterization*** Size and quality assessment	Nanoparticle tracking and immunoblot analysis	Transmission electron microscopy
III.	***Drug Loading*** Encapsulation of requested cargo (i.e. small molecule drug)	Co-incubation of cargo and RVG-exosomes followed by sucrose gradient centrifugation [11]	N/A, encapsulation occurs during liposomal preparation (Step I)
IV.	***Validation*** Evaluation encapsulated drug concentration	Quantitation using liquid chromatography coupled to mass spectrometry	Quantitation using liquid chromatography coupled to mass spectrometry

Consumer

Application of Brain-targeted Delivery Vehicles

The preliminary results obtained in the comparative study described above will determine the selection of either liposomes or exosomes as the MVP. The final prototype available to consumers will consist of a brain-targeted delivery vehicle that is custom loaded with the consumer's choice of cargo (drug, siRNA, etc.). Our technology is primarily designed for researchers to utilize in live animal studies *(in vivo)*; however, it will also be available for *in vitro* use. The customization process for encapsulating the consumers' choice of cargo will require initial consumer involvement.

COMMERCIALIZATION

For neuroscientists who seek to conduct exploratory and therapeutic research, our startup would provide brain-targeted delivery vehicles that can be exploited for a broad spectrum of scientific research. This biotech company is the first to offer scientists convenient access to a customized brain-targeting delivery solution.

Customer Identification: The target audience that would benefit from our product consists of neuroscientists that evaluate live animal models within their research. Given the nature of neuropathologies, the majority of scientists in these fields rely on the use of biochemical, molecular, and behavioral experimental techniques; however, encapsulation technologies are currently inaccessible for neuroscientists. The NIH and NIDA have already demonstrated interest in advancing the implementation of brain-targeted delivery vehicles, as demonstrated by the SBIR and STTR solicitations entitled "Extracellular Vesicle Tools, Technologies, and Products for Neuroscience Research" (RFA-DA-17-008/009). In order to determine the level of need by neuroscientists, as well as establish an appropriate pricing model, we will conduct surveys with neuroscientists. Our team has been accepted into an accelerator program at the University of Georgia (UGA) that consists of four weeks dedicated to customer discovery, two weeks for financial literacy, and a final two weeks regarding investor readiness. Initially, we will poll the neuroscience departments at local universities (i.e. UGA, Georgia Institute for Technology, and Emory University). If we receive encouraging feedback, we will hire a consulting firm such as Stratistics (Strategy MRC) to conduct a national poll to solidify customer preferences.

Product-to-Market Trajectory: Upon successful generation of an MVP, it will be necessary to obtain experimental validation by an unbiased party who demonstrates the capabilities of the technology and publishes the data in a peer-reviewed journal. In order to expand the customer base, research-relevant professional societies and scientific conferences will be targeted as platforms for product promotion. As consumer interest increases, product-related workflow parameters may need to be optimized to scale-up production, distribution, and customer support to ensure commercial success. Partnering with larger, established companies or third-party service providers is an attractive and popular route for biotech startup companies that may be considered to support certain activities or establish regional operations in new markets.

Intellectual Property (IP): The only conflicting IP that we are aware of belongs to Oxford University, and specifically refers to the loading of exosomes with genetic material. We are considering the potential for in-licensing this technology, and expect a favorable negotiation since we do not intend on employing the technology in humans. If negotiations are unsuccessful, non-genetic molecules (i.e. proteins and drugs) may be loaded as they lie outside of the scope of the patent. Furthermore, our initial studies may demonstrate that liposomes are as efficient as exosomes. We do not currently maintain any IP, but there is a potential for developing novel targeting peptides in the future. For example, the West Nile Virus is also highly neuroinvasive due to a single protein on the viral surface. This protein has yet to be tested as a targeting molecule for exosomes/liposomes. Considering our current lack of IP, one concern is that other established companies will become direct competitors once they become aware of the customer base. Our company's advantage lies in the identification of an untapped market. A successful marketing and branding campaign can offer significant advantages for those who are first-to-market. However, it should be noted that even if our company fails due to over-competition, we will have created a new market and thereby succeeded in our mission to accelerate neuroscience research.

BIOSKETCH

Our team consists of two individuals, both of which are currently enrolled in life sciences programs at the University of Georgia (UGA) located in Athens, GA.

Sumitra Pati is a Ph.D. candidate in the Department of Pharmaceutical and Biomedical Sciences at UGA. She has a B.S. in Biochemistry and Chemistry and a M.S. in Biotechnology. She is equipped with a scientific skillset that includes mass spectrometry-based lipidomics, with studies of addictive behaviors, obesity, and Alzheimer's disease, and NMR-based metabolomics.

David F. Thieker is a Ph.D. candidate in the Department of Biochemistry and Molecular Biology at UGA. He has a B.S. in Biochemistry and B.A. Chemistry. He has unique scientific expertise in molecular modeling, protein engineering, and kinetic characterization methods. David has also received training in biotech management at the Keck Graduate Institute located in Claremont, CA.

Our team has a long history with 10+ years of partnership, collaboration, and compatibility. We share South Carolinian roots, alma maters, and a passion for advancing biomedical research given by our personal and educational pursuit of a doctoral education in the life sciences. In 2014, our team was a winner at the Forge Healthcare Hackathon (Atlanta, GA) where we were awarded a U.S. provisional patent for our medical device that was designed to detect retained surgical instruments within patients. We continued to work to obtain proof of concept for six months; however, another group's filing of a non-provisional patent superseded our work. This project was a challenging but rewarding experience that provided us with experience in entrepreneurship and intellectual property management.

We each possess the ability to design and manage multiple scientific projects under pressure, a skill developed during our graduate training. We are currently in a unique position because we are both in our final year of a Ph.D. and at a time of transition. Our next position can either be to pursue the proposed work via a biotech startup full-time or to gain additional post-doctoral training with regards to brain-targeted drug delivery.

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