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Why Gene Editors Like CRISPR/Cas May Be a Game-Changer for Neuroweapons

Diane DiEuliis and James Giordano

This year marks the Eighth Review Conference (RevCon) of the Biological Toxins and Weapons Convention (BWC). At the same time, ongoing international efforts to further and more deeply investigate the brain's complex neuronal circuitry are creating unprecedented capabilities to both understand and control neurological processes of thought, emotion, and behavior. These advances have tremendous promise for human health, but the potential for their misuse has also been noted, with most discussions centering on research and development of agents that are addressed by existing BWC and Chemical Weapons Convention (CWC) proscriptions. In this article, we discuss the dual-use possibilities fostered by employing emergent biotechnologic techniques and tools—specifically, novel gene editors like clustered regular interspaced short palindromic repeats (CRISPR)—to produce neuroweapons. Based on our analyses, we posit the strong likelihood that development of genetically modified or created neurotropic substances will advance apace with other gene-based therapeutics, and we assert that this represents a novel—and realizable—path to creating potential neuroweapons. In light of this, we propose that it will be important to re-address current categorizations of weaponizable tools and substances, so as to better inform and generate tractable policy to enable improved surveillance and governance of novel neuroweapons.

Keywords: CRISPR, Gene editing, Neuroweapon, Neurotherapeutic pathways, Dual-use neuroscience, Biosecurity policy

This year marks the Eighth Review Conference (RevCon) of the Biological Toxins and Weapons Convention (BWC), the purpose of which is to ensure that the convened parties' directives continue to be relevant to and viable for prohibiting the development, production, and stockpiling of biological weapons in the face of newly emerging scientific advancements and biotechnologies. Apropos of issues raised at previous RevCons and elsewhere, there are growing concerns about current and future weaponization of neurobiological agents and tools (ie, "neuroweapons"[1-6]). Indeed, the past decade's advances in brain science (eg, neuroimaging, functional neurogenomics, novel neuro-psychotropic agents and pharmaceutical delivery preparations and systems, transcranial and intracerebral neuromodulatory technologies) and ongoing international efforts to further and more deeply investigate

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the brain's complex neuronal circuitry[7,8] are creating unprecedented capabilities to both understand and control neurological processes of thought, emotion, and behavior.

While these advances have tremendous promise for human health, the potential for misuse has also been suggested.[2,9] Most prior discussions have typically centered on research and development of neuropharmacological and/or toxicological agents that are addressed by existing BWC and Chemical Weapons Convention (CWC) proscriptions.[10] However, it is important to note that prohibitions are not absolute, and such agents can be—and are—employed in research and development (R&D) programs that focus on defense against biochemical warfare. Moreover, many chemical agents and toxins are routinely used in neuroscientific research that is (at least explicitly) claimed to be for medically diagnostic or therapeutic purposes, and a "spillover" effect (ie, transferring medical R&D into security and defense agendas[11,12]) that can enable viable dual use of both developed (and/or stockpiled) agents and the empirical outcomes of such studies has not gone unheeded.[3,6,9,13]

Commentary on the Eighth RevCon of the BWC recognized that emerging biotechnological tools are rapidly changing traditional views of what and how extant biological agents can be weaponized.[14] For example, genetic modifications of some microbes (eg, bacteria, viruses) and cells are now more readily possible through the use of clustered regularly interspaced short palindromic repeats (CRISPR/Cas). While traditional agents may be modified genetically using prior (existing) gene-editing tools, such as TALENs or zinc fingers, these approaches necessitate significant knowledge and expertise in their design and implementation because they require a new nuclease pair for each and every genomic target. Such impediments may be circumvented by employing CRISPR/Cas techniques. However, like any technique, CRISPR/Cas–based approaches are not without limitations or difficulty. A notable drawback is that, evolutionarily, microbes do not possess robust nonhomologous end joining (NHEJ), and this may restrict the efficiency of CRISPR/Cas. However, this can be delimited, at least to some extent, through phage-based "recombineering" tools, such as those produced by AddGene, that engineer bacterial DNA.[15]

Such CRISPR/Cas adaptors and modifiers enable facile use of the technique in a variety of gene editing applications. Given this ease of use, surveillance and oversight of dual-use applications of CRISPR-based modifications of biological materials can be a daunting task, as has been noted in a number of prospective analyses.[16] Further complicating this scenario is recognition that current policy frameworks to protect against misuse of CRISPR and related techniques and methods may be inadequate.

In this article, we address issues and approaches for developing improved understanding of and insight into the dual-use possibilities fostered by employing emergent biotechnologic tools to produce neuroweapons. Based on our analyses, we posit the strong likelihood that development of gene-based neurotropic substances will advance apace with other gene-based therapeutics, and we assert that this represents a novel—and realizable—path to creating potential neuroweapons. In light of this, we propose that it will be important to generate tractable policy to enable surveillance and governance of such potentially weaponizable substances. Toward this end, we believe that a crucial first step toward this goal will be the revision—and reconception or characterization—of viable genetically modified neuroactive substances (eg, microbes, cells, toxins, modified or enhanced neuromodulators) and the ways that the gene-editing techniques and their products could be used to create novel weapons.

Background

In the 5 years since the last RevCon, biotechnology has seen important advances in gene-editing techniques and advanced gene sequencing and synthesis. While the ability to edit genes is not new, tools such as CRISPR/Cas have made sequence-specific gene editing much easier and have enabled genetic manipulations that to date have been challenging if not impossible to achieve.[17,18] Paired with custom "guiding" RNA sequences, these editors can be targeted to a variety of genetic sequences. In fact, thousands of guide RNAs have already been created and are currently available to the research community. With this expanded ability to target and study DNA, observe epigenetic controls, "toggle" genes on and off, and engineer entire biological circuits in simple organisms, new understanding of, and capabilities to alter, genetic codes and biological cells, products, and organisms are being realized.[19] Indeed, CRISPR/Cas has enabled development of genetic models of disease and is advancing gene drive—the long-envisioned but heretofore unachievable ability to durably change genomes within a single generation.[20] Anticipation of improvements to human health has been important to escalating interest in and applications of research employing CRISPR/Cas tools and techniques.[21] However, such work and its possible manifestations have also prompted concerns about genetic manipulation of humans and human embryos[22] and the effects of CRISPR-modified cells and organisms on human biology and society.[23]

Apropos of these concerns are security implications of both the unintended and purposeful misuse of CRISPR and related technologies. In the 2016 annual threat report, US Director of National Intelligence James Clapper categorized gene editing as a potential vector or element of weapons of mass destruction (WMDs).[24] This was mirrored by speculations in the November 2016 report of the President's Council of Advisors on Science and Technology (PCAST) about the risks posed by the sole reliance on extant definitions of WMDs and biological weapons, given emerging capabilities to genetically engineer novel infectious

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pathogens, organic toxins, and insect and plant vectors of environmental and economic disruption.[25] As well, the PCAST report has led to the initiation of technical assessments, including those of a group from the National Academies, to determine the plausibility, possibility, and likelihood of such risks.[16] Despite such expressed concerns, the implications of gene editing for neuroscience research have not yet been fully assessed,[3,5,6,9,26] and others have drawn specific attention to dual- and direct-use risks of weaponizing neuroscientific techniques and technologies, including potential inadequacies of current policies for addressing any such risks. In this light, we further explore technical aspects of the application of novel gene-editing approaches to neural systems and discuss the potential national security ramifications generated by the use of such techniques.

Technical Possibilities and Problems

Biological toxins that specifically act on the nervous system and chemical "nerve agents" have been well described.[27] In an attempt to establish a deeper insight into and broader understanding of the variety of possible neuroweapons, Giordano and Wurzman[2] and Giordano[9] created an updated and more comprehensive categorization of approaches capable of being weaponized to include neuropsychotropic drugs and technologies, as well as the possibility of genetically modified organic neurotoxins and neuromicrobiological agents. Neuroactive approaches are best characterized by the nature of their effector pathways: By acting through particular mechanisms, they can modify cognition, sleep, alertness, emotional state, motor control, and pain perception, which represent dimensions of potential use that are beyond traditional infectious agents' ability to incur illness and lethality (although those dimensions must be included for infectious agents that target the nervous system). For example, in earlier work,[2,28] we discuss examples of paralytic neurotoxins produced in living organisms such as dinoflagellate algae, a class of organism that has already been commercially leveraged through synthetic biology and gene editing to engineer particular molecular products.[29] It then is not difficult to imagine a CRISPR-modified paralytic agent that has greater potency, stability, or scaled-up production that renders it more viable and durable as a neuroweapon.

This is important when considering applications of CRISPR/Cas or other gene-editing technologies, and it is the focus of our assessment. Gene-editing tools or synthetic biology could be used to enhance (in vivo or in vitro) production of traditional or novel neurotoxins or infectious agents or to modify existing agents that are known to act on the nervous system and brain. Significantly, CRISPR-type gene editors could directly act on genes in the brain to alter neural phenotypes that influence cognition, emotion, and behavior. Such tools may not be adequately addressed in or covered by existing policy and regulations.

Several genes have been implicated in cognition and behavior,[30,31] and a number of research programs are currently employing gene-editing methods to study genetic function and epigenetic regulation in neurons and glia[32] and to produce in vivo animal and stem cell–derived in vitro models of neurological diseases. While the use of CRISPR-type techniques in humans was thought to represent a future possibility, the administration of CRISPR-modified cells to human patients in China[33] has created a potential new timetable for human applications. As with other biotechnological methods, it appears not to be a question of if or, given the aforementioned human use, even when,[3] but rather at what pace and to what extent will these and other human uses be implemented. Recommended[12] and robust[34] research programs are already being initiated to determine the genetic capabilities that could enhance human performance. Such developments add to the urgency of the discourse, actionable scrutiny, and the need to readdress and revise guidelines and governance policies.

Yet, even with the accelerated pace of gene-editing technology and techniques, there remain a number of challenges to these types of approaches. Gene editing can produce off-target effects, and these can be difficult to assess in vivo. More important, while gene editing molecules can be used with relative ease in cells and tissues in vitro (which could then be surgically transplanted in vivo), it is much more difficult to deliver molecular editors into living organisms. Genetically active molecules need to be viable in the body, whether injected intramuscularly, infused into the vasculature, inhaled, or absorbed through digestion. Because these molecules work directly on DNA, they also need to successfully cross cell membranes, traverse the cell body, enter the nucleus, and access DNA inside tightly packed chromosomes.

An additional challenge is that gene editing molecules that target the brain must cross the blood-brain barrier. For decades, genetic viral vectors have been pursued as delivery vehicles for neurotherapeutics[35] and as means to manipulate genes of neurons (for review, see reference 36). More recently, customized vectors have successfully been created for use in the nervous system of mice via bloodstream access.[37] But these challenges represent opportunities for research and discovery: At present, gene therapy and nanotechnologic "cassettes" are being studied as means to deliver active DNA editing molecules to a variety of target tissues,[38,39] and thus it is likely that in the near future, genetic techniques will be developed that enable delivery of small, bioactive molecules specifically to nervous tissue.

As these problems are resolved, CRISPR/Cas-type gene editors can be more widely employed to further advance genetic manipulation in the brain. To be sure, such interventions would be of great benefit in the treatment or prevention of a number of neurologic (and perhaps psychiatric) disorders. For instance, monogenetic pathologies, such as Huntington's disease,[40] have long been pursued as

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realistic targets, while more complex, heterogenetic conditions present more difficulty. Novel gene editors such as CRISPR/Cas will change this paradigm, as they are highly specific and enable modification of single nucleotide polymorphism (SNP)[41] as well as multiplex genetic changes.[42]

An important caveat, however, is that we still do not fully understand brain function to an extent sufficient to predict with certainty the brain's response to any genetic manipulation, whether simple or complex. Plasticity, the ability to modify cellular structure and function in response to a variety of stimuli, is a hallmark characteristic of brain tissues, and such plastic responses could both ameliorate purposeful changes and lead to unexpected outcomes over time. Given the novelty and uniqueness of genetic modification, and the possibility for incurred "downstream" transcriptional and/or translational effects, it is unknown if and how such modifications could be manifested on the cellular, network, and whole brain levels; equally unknown are the trajectory, valence, and extent of such effects, and if and what alterations of cognition, emotion, or behavior could result.

It is known that CRISPR/Cas can incur off-target effects. In terms of therapeutic use, this could be problematic. However, off-target effects may not be of concern in the design of a weaponized CRISPR/Cas–based agent, as long as damage or manipulations to cellular DNA is of an extent that results in illness, disability, degradation, or lethality, and such effects are constrained (or constrainable) to the individual(s) or population(s) intended (see Wurzman and Giordano[43] for an overview).

Democratization, Diffusion of Science

A particularly sentinel feature of novel gene editor techniques such as CRISPR/Cas is their fairly broad and facile accessibility. Unlike other weaponizable elements (eg, materials in the nuclear realm, or scheduled chemicals on lists of toxins), CRISPR/Cas tools are comparatively inexpensive and available to a wide variety of actors. Customizable kits are commercially available, and companies (eg, AddGene) make sequences accessible to any user. Security concerns arise from the notion that state actors could reengineer traditional bioweaponry agents with new gene-editing tools, including those that affect the nervous system. A wider group of actors wishing to take advantage of editing technology to create bioagents could be included in the risk space for neuroagents, as described above.[2,3]

In this latter category, it is vital to acknowledge the existence of a robust "do-it-yourself" (DIY) community that already exists in biology, with both open community laboratories that are active around the country (eg, DIYBio, BioCurious, HiveBio, GenSpace, etc) and more directed and organized amateur biology occurring at venues such as the international Genetically Engineered Machines (iGEM) competition.[44] DIY "biohackers" are engaged in a wide variety of pursuits,[45] and rather sophisticated microbiological projects presented at iGEM have prompted further concerns that an amateur could (accidentally or purposefully) create a harmful entity using CRISPR/Cas techniques.[46]

We posit that 3 problems arise in and from the use of gene-editing approaches to develop neurotropic agents: First, as mentioned previously, is that genetically engineering molecules for specific use in the brain will be complex endeavors in terms of delivery challenges, targeting, and dosing and thus may pose difficulties for an amateur biologist. But, as previously noted, this generates effort(s) toward overcoming or compensating for such technical problems. This fosters the second problem—namely, that it is likely that attempts at neuro-hacking will increase in both number and sophistication. It may be, for example, that more indirect means of hacking the brain will be concomitantly pursued, which involve manipulation of microbiome elements that have been shown to both engage bidirectional signaling with the central nervous system and affect cognition and emotion[47,48] and/or the generation of novel (but indirect) pharmacological or microbiological activators/effectors of neural function. Moreover, as we have noted, a neuroweapon need not be infectious to neuronal or glial cells to have impact; by definition, a neuroweapon may act by affecting behavior in a variety of ways and be used for strategic purposes other than lethality. Thus, the third problem is that it becomes difficult to assess and monitor each and all of those genetic modifications that could be leveraged in these ways.

Implications and Recommendations

The ability to accurately target specific genes in the brain could enable both specific and generalized modulation of key aspects of physiological function, cognition, and behavior. Novel gene-editing technologies and techniques will allow further elucidation of functional genetic and epigenetic mechanisms that can be used to develop potential neurotherapeutics and enable creation of new, or alteration of existing, biosynthetic pathways that are involved in neurological functions. But as we and others have noted, there is frequent and relatively easy "spillover" from the medical silo to dual- and direct-use arenas of weapon development and production.[9,13,28] In that regard, we note encouraging work on potential governance models for general dual-use concerns of emerging technology that emphasize risk assessments,[49] responsible conduct in the global arena,[50] and a thoughtful precautionary approach.[51] Our analysis underscores the importance of considering agents that are not lethal, but which could be used to manipulate physiology, cognition, and/or behavior for strategic means. Further, we suggest that given the heterogeneity of gene effectors that are possible, any list-based mechanism for identifying agents of concern, such as the

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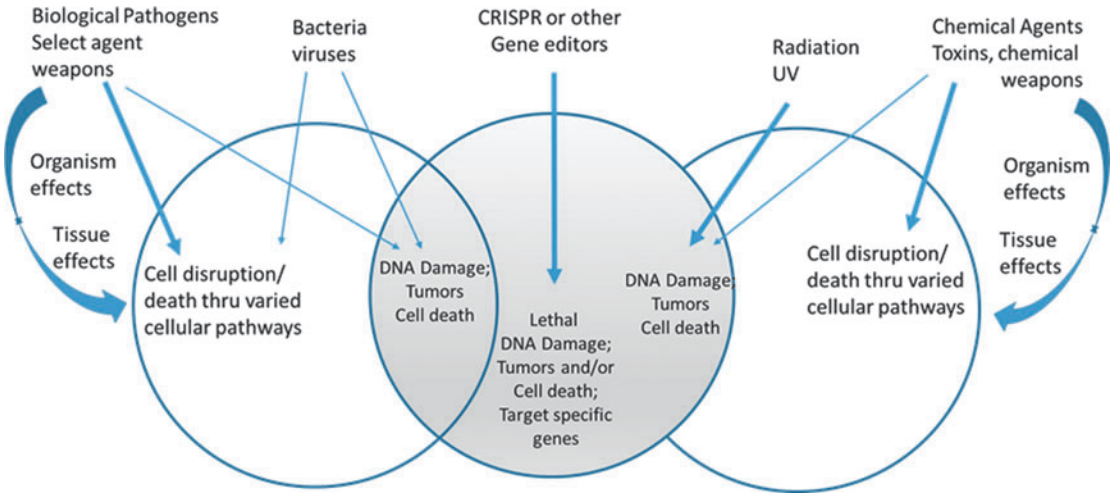

Figure 1. Pathway Spectrum Across Potential Weapon Agents. This diagram provides a schematic of traditional weapons pathways, with a new distinction realized through gene editing in the central shaded sphere.

current list of select agents and toxins defined by the BWC and CWC, will be inadequate for a genuinely heightened awareness of any potential risks and threats. As could be noted from the recent deliberations of the BWC RevCon, there is little consensus regarding what constitutes a better intersessional process to address emerging technology.[52] But lack of consensus should not be taken as lack of concern. Indeed, we are encouraged by those urging continued dialogue (as mentioned in our introduction), as well as the type of discourse exemplified by the specific acknowledgment of neuroweapons by the Australia Group to the CWC.[53] In this light, we offer that a more reasoned approach will be to characterize neuroactive agents according to their biological pathways of effect (see Figures 1 and 2).

Creation of any such neuroweapons would still pose considerable technological challenge, and, thus, scientific and technological capability, expertise, and tacit knowledge would be required to develop advanced delivery and specific targeting mechanisms to fully realize an agent that could be directed for use against others. But the speed and extent of current developments are such that these kinds of neuroagents can be readily envisioned (if not soon created), and keen acknowledgment of these possibilities is therefore necessary and important.

In the interim, it is likely that more indirect means of manipulating the brain and behavior will be developed. "Neurohacking" will increase, and biotechnology, such as CRISPR/Cas and novel gene editors, will provide tools to realize production of novel neuroagents with dual-use potential. Simple acknowledgment of these facts, however, is insufficient. It will be essential to pursue and obtain a deeper and fuller understanding of the ways that genetic

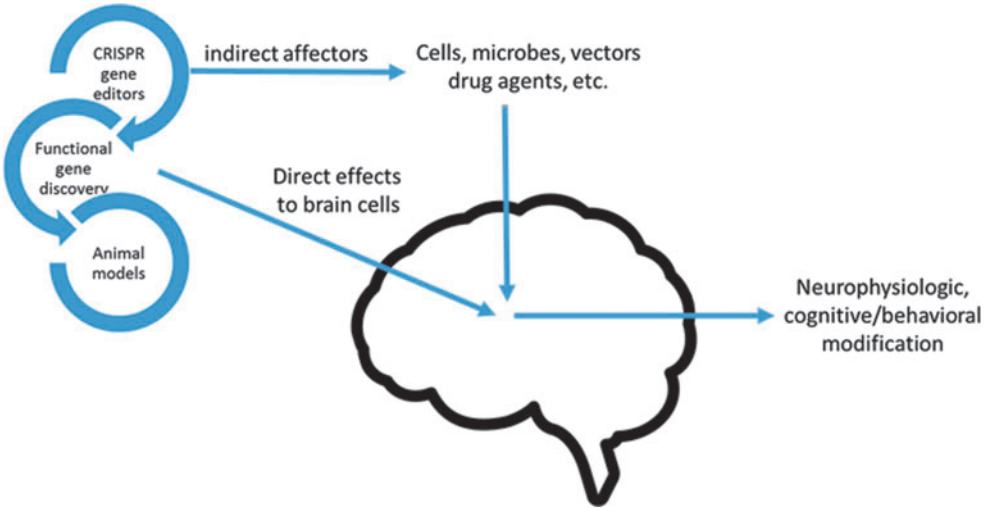

Figure 2. Gene Editing's Potential Impacts on the Brain. Gene editing technologies will enable both modification of entities which can affect brain function (indirect affectors) and also lead to functional gene discovery and modeling that can create entities that alter structure and function of brain cells (eg, neurons and glia). In either case, the goals will be to alter neurophysiologic, cognitive-emotional, and/or behavioral states. These approaches can be employed to engender novel neurotherapeutics and/or neuroweapons.

pathways to human cognitive and behavioral modification can be engaged for dual and direct use as neuroweapons, to formulate policies based on this level of understanding, and to engage surveillance of the use of these technologies in various silos of development and application, so as to afford both preventive and more preparatory capabilities.

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