The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Susanne Schmid**
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Location of experimental work to be carried out :

Building :	**DSB**	Room(s):	**5000A, 5000D, 5000F/G**
Building :	**MSB**	Room(s):	**473**
Building :		Room(s):	

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **NSERC, OMHF, CIHR**
GRANT TITLE(S): **Mechanisms underlying cogntive function**
UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Cleusa DeOliveira	**Cleusa.deOliveira@schulich.uwo.ca**	**2009**
Marei Typlt	**mtyplt@uwo.ca**	**2010**
Erin Azzopardi	**eazzopar@uwo.ca**	**2010**
Jordan Robinson	**jrobin73@uwo.ca**	**2011**
Magdalena Mirkowski	**mmirkows@gmail.com**	**2011**

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

please see attached document

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

The sole input into our brain is sensory information. This information is processed, stored, and integrated in order to generate the brain's output – a behavioural response. Our five senses are constantly active, bombarding the brain with sensory information. In order to cope with this vast amount of information, different mechanisms are in place that filter out unimportant information and enable the brain to protect its resources for the processing of salient and potentially important input. One of the sensory filtering mechanisms is called sensory gating. Sensory gating takes place in the central nervous system sensory pathways at a pre-attentive stage of sensory information processing. Sensory gating disruptions are associated with several neurological disorders, including schizophrenia, autism, Alzheimer's and Parkinson's disease. Two different mechanisms underlying sensory gating have been described: 1) a homosynaptic mechanism that takes place when the same (innocuous) stimulus is repeatedly presented and leads to synaptic depression within the activated pathway; and 2) the activation of an inhibitory system by the processing of a salient stimulus that attenuates other incoming sensory signals. Both processes are short-lived and help to protect neural resources.

Both sensory gating mechanisms can be observed using the acoustic startle response as a tool. The startle response is mediated by a short and well described pathway. Homosynaptic depression in the sensory part of this pathway by repeated activation directly affects the amplitude of the motor response. It can be measured as short-term habituation of the startle response in both humans and animals. If a salient sensory stimulus directly precedes an acoustic startle stimulus, the activation of inhibitory mechanisms by this stimulus attenuates the following startle stimulus signal and hence the motor response. This is measured as pre-pulse inhibition of startle. Additionally, long-term habituation contributes to sensory gating, but nothing is known about the underlying mechanism to date.

Our goal is to further unravel the mechanisms underlying sensory gating in order to better understand sensory information processing in the brain and its disruptions associated with disease. We use habituation and prepulse inhibition of startle as models, since they are easy to observe and primary pathways are well described. Our objectives are:

1) To further establish the neurocircuitry involved in startle and sensory gating, especially in cross-modal settings

2) To test the hypothesis that midbrain cholinergic neurons are crucial for sensory gating (pre-pulse inhibition and long-term habituation)

3) To describe synaptic mechanisms mediating sensory gating and its disruptions in disease models.

We address the objectives using immunohistochemistry, including analysis of immediate early gene activation, behavioural studies combined with lesions of neuronal structures or specific cell subtypes implicated in sensory gating, behavioural studies combined with stereotaxic injections of receptor agonists or antagonists, in vitro (patch clamp) and in vivo electrophysiology, and optogentic activation of cholinergic neurons in vivo. All methods except the new optogenetic approach are well established in the lab.

This research will give valuable insights of fundamental mechanisms of early stage sensory information processing in the brain and its disruption in disease. This understanding will enable the identification of new drug targets and may also be useful for developing technical systems with an ability to filter information based on their salience rather than their physical properties.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☐ YES ☒ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☐ NO

If YES, please give the name of the species ____________________

What is the origin of the microorganism(s)? ____________________

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ____________________

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☐ YES ☒ NO
(If NO, please proceed to Section 3.0)

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☐ No		Not applicable
Rodent	☐ Yes ☐ No		
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☐ Yes ☐ No			
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ No			

Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☐ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: _____

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☐ YES ☒ NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☐ YES ☒ NO If **NO**, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ☐ YES, complete table below ☐ NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☐ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☐ NO ☒ YES, specify **Urotensin II fusion protein**

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

6.2 If YES, please specify which biological agent will be used:
Please attach a full description of the biological agent.

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

6.5 Please give the Health Care Facility where the clinical trial will be conducted:

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **rats, mice**

7.3 AUS protocol # **2008-006, 2008-010, 2011-077**

7.4 List the location(s) for the animal experimentation and housing. **5000 suite, DSB**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☐ YES, specify:

7.6 Will the agent(s) be shed by the animal:
☐ YES ☐ NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☒ YES ☐ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☒ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

- Pound source dogs ☐ YES ☐ NO
- Pound source cats ☐ YES ☐ NO
- Cattle, sheep or goats ☐ YES, species ☐ NO
- Non-human primates ☐ YES, species ☐ NO
- Wild caught animals ☐ YES, species & colony # ☐ NO
- Birds ☐ YES, species ☐ NO
- Others (wild or domestic) ☒ YES, specify **laboratory rats and mice** ☐ NO

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☒ YES ☐ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **UrotensinII fusion protein**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone **n/a**

9.4 How much of the toxin or hormone is handled at one time*? **1-3 microgram**

9.5 How much of the toxin or hormone is stored*? **0.2 milligram**

9.6 Will any biological toxins or hormones be used in live animals? ☒ YES ☐ NO
If **YES**, Please provide details: **stereotaxic microinjection into the brain (< 1 microgram)**

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: **The urotensin II fusion protein contains a fraction of a diphtheria toxin sequence.**

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

10.2 If YES, please give the name of the species.

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

10.6 Please describe the risk (if any) of escape and how this will be mitigated:

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree...)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☐ NO

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Susanne Schmid ***Date:*** 30. 07. 2012

14.0 Containment Levels

14.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☒ 1 ☐ 2 ☐ 2+ ☐ 3

14.2 Has the facility been certified by OHS for this level of containment?
- ☐ YES, location and date of most recent biosafety inspection:
- ☐ NO, please certify
- ☒ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants):

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO

If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:

All safety precautions will be followed. The protein is in a solution, stored at -20C. It will be handled with saftey glasses, gloves and lab coat. It will not be injected with a sharp needle, but througfh a blunt cannula. In case of spills the fusion protein will be disarmed with bleach and picked up with a paper towel. The paper towel will be disposed of as biohazard waste

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Susanne Schmid ***Date:*** 30. 07. 2012

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Diphteria Fusion Toxin

Construction of Toxin (Steward Clark, University of California Irvine)
Toxin construction was done by genetically replacing the targeting domain of Dtx with an alanine and glycine ether (similar in sequence to the N-terminal of the carp UII orthologue) linked to the last eight amino acids of the rat UII sequence (Dtx-UII). One form of the fusion toxin was constructed with a disrupted disulphide bond, dismantling the cyclic nature of UII, which is known to be essential for receptor binding (Itoh et al. 1988). This construct was used as an untargeted control. Targeting domain constructs were obtained from Sigma-Aldrich (St Louis, MO, USA) as complementary oligonucleotides with appropriate sticky splice sites available (list below), and these were then phosphorylated and annealed. The resulting double stranded DNA was then ligated into pETJV127 DAB389-IL-2 (gift from Drs J. Van der Spek and J. Murphy, University Hospital, Boston, MA, USA) which had the sequence for IL-2 removed by excision with SphI and HindIII (NOPE: we have never requested, nor have ever had, the cDNA of the natural form of Dtx which includes that naturally occurring targeting domain). Plasmids were grown in DH5ƒÑ bacteria and verification of the sequence of single clones was done by automated DNA sequencing (Laragen, Los Angeles, CA, USA) with the primer: 5'-CGGGTATCGGTAGCGTAATGG- 3', which anneals to the Dtx sequence.

B) Dtx-CUK, SphI-His6-UII-HindIII

A		H	H		H	H	H	H		K	K	A	A	G	G	G	A	E	C	F	W	K	Y	C	I	.
	ca	cat	cat	cat	cat	cat	cac	aaa	aaa	gcg	gcg	ggc	ggc	ggc	gcg	gaa	tgc	ttc	tgg	aaa	tac	tgc	att	ta		
3'	gta	cgt	gta	gta	gta	gta	gtg	gtg	ttt	ttt	cgc	cgc	ccg	ccg	ccg	cgc	ctt	acg	aag	acc	ttt	atg	acg	taa	att	cga	5'

Material Safety
The urotensin II receptor is known to be expressed in the brain, vasculature, liver and kidney. These organs will be particularly vulnerable to toxin exposure. Therefore, any instances of puncture wounds with contaminated materials should be treated seriously. The effected individual should seek medical attention. Splashes on to skin should be treated by through washing of the affected area. Inadvertent splashes into eyes or other mucosal membranes should be flushed, and medical attention should be sought. The isolated toxin is **NOT** a self-replicating / pathogenic agent. Therefore, much like a chemical toxin, exposure to the toxin will have acute effects. Also, it has been shown in cell culture toxicity assays that the toxin is 10 000 times less toxic to cells which do not express the UII receptor than those that do. Therefore, a superficial exposure which is dealt with quickly should not result in any deleterious consequences.

Handling at UWO
We will use the toxin for stereotaxic injections into a specific area of the rat brain in order to lesion a specific cholinergic cell group. We will receive a total of 1mL, that we will reconstitute and aliquot. We will be using a ~3% solution for our injections with injection volumes being in the submicrolitre range. Once the toxin has been stereotaxically injected into the brain of the animal, and the wound sutured closed, there should be no exposure risk to laboratory or animal care staff. The amounts that are injected have been shown not produce detectable toxicity outside a 5mm3 within the brain, so the risk of this toxin being spread outside the cranium is very low. The toxin is proteiacous in nature and so normal proteiase activity within the brain will deactivate the toxin by cleaving the protein.

During surgery and microinjection laboratory personnel Dry contaminated materials will be collected carefully into biohazard bags (red colour), sealed and autoclaved before disposed as trash. Non-autoclaved disposable glassware will be kept in a separate, clear marked container, containing liquid bleach for 24 hours soaking before it is disposed as regular glassware. Liquid waste will be aspirated directly into collecting bottle which contains 10% bleach solution. Decontamination solution will be disposed as directed for liquid as described below. Solid waste will be placed in a plastic bag and disposed as biohazard waste. Liquids: All toxin solution must be diluted 1:1 with the decontamination solution for one-half hour or more. After decontamination aqueous wastes can be disposed of down the drain.