PROJECT FINAL REPORT

Grant Agreement number: **PIEF-GA-2011-303313**

Project acronym: **ObInNSC1**

Project title: **The Physiological Control of Stem Cells: Obesity, Insulin, and Neural Stem Cell Dynamics**

Funding Scheme: **FP7-PEOPLE-2011-IEF**

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SUMMARY

Summary

Environmental factors that affect an organism's *milieu intérieur*, such as diet, infection, or societal stress, can greatly impact stem cell activity, organ function, and health.
This Intra-European Fellowship has allowed a young researcher of proven ability to investigate this phenomenon in an internationally renowned laboratory.
New findings have been made, and avenues of research opened, in our understanding of how a high fat diet and obesity can impact stem cells in the brain.

Whilst it had been observed that the diet-induced obesity caused by long term exposure of mice to a high-fat diet correlated with a decrease in adult neurogenesis, we have found that this is preceded by a period of increased neurogenesis. This is an extremely interesting result; it suggests that a high fat diet can affect neural stem cells prior to the onset of obesity, and that the resulting increase in proliferation then leads to stem cell exhaustion. This has led us to expand our study to other strains of laboratory mice that do not develop diet-induced obesity, in order to understand whether this is a direct universal effect of a high-fat diet.
The study had initially planned to have a focus on the Insulin/IGF signalling pathway. However after failing to find the hallmarks of this pathway's activity, we have taken a new research direction. In order to identify the molecular mechanisms responsible for the interaction of high-fat diet/obesity with adult neurogenesis, we are taking an unbiased approach based on RNA sequencing. We have used Laser Capture Microdissection to isolate stem cells, allowing us to isolate their RNA and compare gene expression between our experimental groups.

During the course of the fellowship the fellow has been able to learn and develop a number of skills important for career development and skill transfer. These include: attending international conferences; teaching undergraduates; mentoring PhD students; participating in the peer review process; presenting and sharing his work with his peers; gaining qualifications in animal husbandry and surgery; advancing his interest and skills in advanced light microscopy; and improving his grasp of the Swedish language.
The Karolinska Institute's 'visiting researcher' framework has helped the fellow fully integrate into Swedish society.

The research performed gives us new insight into how dietary factors can affect the body at the level of the stem cell, and may provide new tools for researchers aiming to develop stem cell therapies or treat diseases caused in part by stem cell malfunction. The work performed thanks to this fellowship will form the core of a scientific publication that other researchers will be able to build on.

Context & Objectives

Stem cell populations are essential to the maintenance, repair, and function of the tissues in which they reside. In keeping with their importance, stem cells are subject to a myriad of regulatory signals and interactions that tightly control their proliferation and differentiation.
One mode of stem cell regulation is via systemic endocrine cues. In this way, environmental factors that affect an organism's *milieu intérieur*, such as diet, infection, or societal stress, can greatly impact stem cell function.
Understanding how altered physiological states affect the dynamics of stem cell populations will provide a platform from which we may be able to therapeutically address aspects of illness and disease that are derived from altered stem cell function. Further, an increased understanding of how stem cell proliferation and differentiation are controlled may provide new tools for researchers trying to develop stem cell-based therapies.
Neural stem cells (NSCs) in the adult hippocampus serve as a powerful paradigm for the study of environment-stem cell interactions; the rate at which they divide and generate new neurons is acutely sensitive to environmental factors such as exercise and stress. The environmental variable we are interested in is diet: specifically, the effect of diet-induced obesity on adult hippocampal NSCs.
Obesity has become widespread in Europe, and the rest of the world. The World Health Organisation (WHO), based on the latest estimates from EU countries, reports that obesity affects between 10 and 30% of adults. Further, the WHO estimates that within the European Region nearly 10% of school age children are obese, which represents approximately 3.75 million children.
Obesity has been linked to poor performance in cognitive tests involving memory, the development of dementia in the old, and, though conflicting reports exist, depression. Strikingly, these conditions have also been linked to impaired hippocampal neurogenesis and function. In recent years a number of short reports have been published that suggest a high-fat diet, and resulting obesity, impairs hippocampal neurogenesis in rodents. However, the mechanism by which obesity can alter NSC behaviour and fate remains unknown. This project aims to marry advanced genetic manipulation and microscopy with the established C57BL/6J mouse model of diet-induced obesity to precisely define the effects of a high-fat diet on NSCs, and elucidate the molecular mechanisms responsible.
In order to elucidate the mechanisms by which a high-fat diet and obesity regulate neural stem cell behaviour our initial immediate objectives were:

- To use immunohistochemistry to define the changes in NSC behaviour, and signalling pathway activity, that occur during the development of obesity.
- To optimise tamoxifen dosage for, and perform genetic lineage tracing of hippocampal NSCs.
- To develop conditional knock-out models of the Insulin and Insulin-like Growth Factor Receptors for use in adult hippocampal NSCs.

Results & Foreground

We have analysed hippocampal NSCs at the population level with respect to proliferation and neuron production and how these parameters are altered in response to a high fat diet and obesity. Mice are shifted from a control to a high-fat diet at 6 weeks of age. At 22 weeks of age, following 16 weeks on a high-fat diet, obesity and hyperinsulinemia are fully developed. Proliferation and neuronal production were assayed, via immunofluorescence with antibodies against Ki67 and Doublecortin respectively, at various time points during these 16 weeks. After 16 weeks, as previously reported, we found a reduction in proliferation and neurogenesis of approximately 20%. Earlier time points provided a novel and very interesting result. We found that the reduction in neurogenesis in obese mice was preceded by an increased rate of neurogenesis. This result suggests that a high-fat diet acts to increase NSC proliferation, which then results in NSC "exhaustion" and the reduced levels of neurogenesis seen in obese mice after 16 weeks. We are now assaying total NSC numbers using an antibody against Nestin (a NSC marker) in combination with antibodies against Sox2 and GFAP, in order to determine the nature of any exhaustion.
That the change in NSC proliferation occurs prior to the development of obesity also suggests that dietary fat may have a more direct effect on stem cells than was previously thought i.e. it may not act via its effects on fat tissue. Accordingly, we are now investigating the effects of high-fat diet on NSCs in other mouse strains that are not susceptible to diet-induced obesity. It may be that regardless of weight gain and appearance, simply eating a high-fat diet is enough to impair neurogenesis and brain function.

Whilst it has proven relatively straight forward to reduce tamoxifen dosage to a level where recombination is rare enough to allow a clonal analysis approach, transferring these protocols into an obesity model can be challenging. We have found that transgenes often have uncharacterised physiological phenotypes i.e. they do not respond to dietary fat in the same way as the C57BL/6J strain. It has subsequently been reported that such metabolic/physiological phenotypes are frequently present in transgenic animals but remain largely uncharacterised. Optimisation of lineage tracing protocols within the diet-induced obesity paradigm is ongoing. Whilst not allowing genetic labelling of a single stem cell and its progeny, we have promising data from a *nestin*-GFP transgenic mouse line in our high-fat diet paradigm. *nestin*-GFP allows powerful population level analysis of NSCs (and their lineages to a limited extent) in the high-fat diet paradigm, and could also be extremely useful for the transcriptional analysis described below as it allows unambiguous identification of individual stem cells.

The prime candidate for a link between high-fat diet, obesity, and adult neurogenesis was Insulin/IGF signalling. However, analysis of pathway activation via phosphorylated pathway effectors such as AKT/PKB, S6K and 4EBP has suggested that Insulin/IGF signalling does not significantly change in response to high-fat diet in the proliferative zone of the hippocampus. Given the lack of evidence for the direct involvement of Insulin/IGF signalling, at the NSC level, in the regulation of proliferation and neurogenesis by dietary fat it was

decided to postpone the genetic manipulation of these pathways and instead focus on an unbiased exploratory approach to identify the signalling mechanisms involved.
We have used laser capture microdissection (LCM) to isolate the subgranular zone –the small region of the hippocampus that houses the neural stem cells. High quality RNA can be extracted from these samples, enabling subsequent gene expression analysis. We are currently optimising an immunostaining protocol in combination with LCM to allow the identification and isolation of individual progenitor cells, allowing a more precise single cell analysis. RNA sequencing can now be performed in a well-established lab pipeline. Comparison of the transcriptional snapshots generated between control mice and those on a high-fat diet will generate candidate pathways that might mediate the interaction between dietary fat and stem cell behaviour.

It is expected that the final results will identify the molecular mechanism by which a high fat diet influences NSC behaviour. This should happen in two steps: Firstly the identification of candidate pathways via transcriptional analysis of the hippocampal NSCs and subsequent immunohistochemistry; Secondly, the genetic and pharmacological interrogation of candidate pathways to identify the factors that are necessary and sufficient to mediate the effect of a high-fat diet.

Impact, Implications & Dissemination

The knowledge gained about how high-fat diet regulates this NSC population may well be applicable to stem cell populations in other tissues, and provide a platform from which we may be able to therapeutically address aspects of illness and disease that are derived from altered stem cell function. Our initial findings already suggest that a high-fat diet may exert adverse effects on stem cell population dynamics and tissue function prior to, and perhaps independent from, weight gain; thus stressing the universal importance of a well-balanced diet.
The completion of this project will allow people to understand how the dietary choices they make can directly affect aspects of brain function derived from an adult stem cell population.
The data generated thus far will form the core of a scientific publication that the research community will be able to build on. At the Karolinska Institute significant publications are announced and explained on the home website as well as through the print, radio and television media. Further, a regular open public lecture series is held where researchers explain their published research and its implications.

ETHICAL & SOCIAL IMPLICATIONS

The Karolinska Institute enforces some of the highest animal welfare and ethical standards in Europe –going beyond minimum legal requirements. We worked within these high standards in regard to animal housing, maintenance, health monitoring, anaesthesia, and euthanasia.
We always sought to minimise any suffering and use as few animals as possible. No animals experienced significant suffering during this project (at most an animal received a single intraperitoneal injection).

The completion of this project will allow people to understand how the dietary choices they make can directly affect aspects of brain function derived from an adult stem cell population.

Any molecular mechanisms discovered may provide a platform from which we might therapeutically address aspects of illness and disease that are derived from altered stem cell function. Further, an increased understanding of how stem cell proliferation and differentiation are controlled may provide new tools for researchers trying to develop stem cell-based therapies.

The data generated thus far will form the core of a scientific publication that the research community will be able to build on. The work will be presented at international conferences. And, importantly, in order to disseminate the work to the general public we can utilise the Karolinska Institute framework that interacts with print, radio, and television media outlets; and also offers a public lecture platform.

4.3 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	**PIEF-GA-2011-303313**
Title of Project:	**The Physiological Control of Stem Cells: Obesity, Insulin, and Neural Stem Cell Dynamics**
Name and Title of Coordinator:	**Professor Jonas Frisen**

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***YES***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	
• Did the project involve Human data collection?	
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	X
• Were those animals transgenic small laboratory animals?	X
• Were those animals transgenic farm animals?	

• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	**NO**
• Research having direct military use	
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator		1
Work package leaders		
Experienced researchers (i.e. PhD holders)		1
PhD Students		
Other		

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**0**
Of which, indicate the number of men:	

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

○ Yes
X No

6. Which of the following actions did you carry out and how effective were they?

	Not at all effective				Very effective
❑ Design and implement an equal opportunity policy	○	○	○	○	○
❑ Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑ Organise conferences and workshops on gender	○	○	○	○	○
❑ Actions to improve work-life balance	○	○	○	○	○

○ Other:

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

○ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

X No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

X No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

1,5 Main discipline[21]:

○ Associated discipline[21]:

○ Associated discipline[21]:

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*

○ Yes
☒ No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No
○ Yes- in determining what research should be performed
○ Yes - in implementing the research
○ Yes, in communicating /disseminating / using the results of the project

[21] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**0**
To how many of these is open access[22] provided?	
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[23]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		**0**

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑ Increase in employment, or	❑ In small & medium-sized enterprises
❑ Safeguard employment, or	❑ In large companies
❑ Decrease in employment,	❑ None of the above / not relevant to the project
❑ Difficult to estimate / not possible to quantify	

[22] Open Access is defined as free of charge access for anyone via Internet.
[23] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
Difficult to estimate / not possible to quantify	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes X No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

X Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

❑	Press Release	❑	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
❑	Brochures /posters / flyers	❑	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

X	Language of the coordinator	X	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES
1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]
1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)
1.3 Chemical sciences (chemistry, other allied subjects)
1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)
1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY
2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]