Max Planck Institute of Psychiatry
German Research Institute of Psychiatry

External Examiner's Report

PhD Candidate

Urszula Skupio
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PhD Thesis title

Role of astroglial glucocorticoid receptor in the mechanism of opioid action

Having studied the thesis submitted by Urszula Skupio, I am satisfied that the candidate reaches, and in many respects exceeds, the international standards required for the award of the PhD degree.

The subject chosen for research is original and important for understanding the neurobiological mechanisms that underlie the development of addiction to opioids and other drugs of abuse. Stress, acting through glucocorticoids which, in turn, activate of repress gene transcription, is known to modulate susceptibility to addictive behaviour; stress is also thought to determine the course of addictive behaviour by influencing cognitive, emotional and motivational behaviours, the assessment and processing of rewarding stimuli, as well as the expression of drug dependence (withdrawal) as seen at the time of abstinence. To date, the assumption has been that glucocorticoids, acting via glucocorticoid receptors (GR), modulate addictive behaviours by acting on neurons, in particular those comprising the so-called mesocortico-limbic reward system. Based on new evidence that glial cells (especially astrocytes) contribute importantly to neuroadaptations and brain functions, the research in this thesis focused on the role of GR in astrocytes in various components of the reward pathway in the development and response to a prototypic abused opiate, morphine. Given the conceptual novelty, complexity and multiplicity of macro- and molecular elements involved, the candidate and her supervisor, Professor Ryszard Przewlocki, deserve to be commended for their courage in undertaking and accomplishing such a challenging project.

The thesis demonstrates a judicious choice of experimental paradigms and analytical approaches, exposing the candidate to a broad range of modern biomedical research techniques (molecular, pharmacological, behavioural, anatomical, bioinformatic). The script indicates that the candidate is competent in all these methods and sufficiently conversant with them to interpret and discuss the outcome of her experiments using these techniques.
Overall, the text is well-structured and written and can form the basis for 2-3 publishable reports in high-ranking journals. Those publications will undoubtedly transform current understanding of how stress hormones impact on addictive behaviour; more importantly, they will stimulate new questions as well as basic and translational research.

The dissertation opens with an Abstract that conveys a clear picture of the background, main approaches, findings and interpretation of the research. This is followed by an Introduction (Chapter 1) that displays a thorough knowledge of the background literature and rationale for the research; this section is written in very comprehensible manner. Chapter 2 summarizes the specific questions addressed in the research, and Chapter 3 provides a description of the spectrum of research methodologies applied. The results of the research are described in Chapter 4 and these are discussed in context of the existing body of knowledge in Chapter 5; the latter chapter also notes some of the limitations of the present work (and field in general). Lastly, conclusions from the experimental work performed are presented in Chapter 6 which also includes a plausible model of how astrocytic GR may influence opioid actions in the nucleus accumbens.

Below follows a short list of issues that would benefit from greater clarity or elaboration. However, these MINOR ISSUES DO NOT reduce my high rating of the work.

**Introduction (Chapter 1)**

p. 10 a short description of the distribution of μ opioid receptors would complement those given for δ and κ opioid receptors;

p.12 do the morphine effects on locomotor activity depend on the time of day at which the drug is given?

p.21 does MAPK signalling not also affect gene expression (indirectly)?

p.22 what are the arguments for/against the idea that “membrane GR” are identical to intracellular GR? Would one expect a “nuclear protein” to easily incorporate into the membrane and retain its signalling functions?

p.27 although very few astrocytes in the NAc express GR, GR-GFAP immunohistochemistry was used to demonstrate efficient GR knockout (p. 57, p. 90); a comment on this would be appropriate

p.42 could it mean that drugs of abuse induce gliosis (reactive astrocytes with upregulated GFAP expression)?

**Materials & Methods (Chapter 3)**

It is hard for the reader to pick exactly what dose of morphine was examine at what time and for how long; such information could have been incorporated into Fig. 5 or 6;

p.45 (and elsewhere) saccharine is a good reward (hedonic/taste properties), but it is not a “natural” reward and it lacks energy, a key factor that motivates consumption of sugars such as sucrose; perhaps this deserves explanation (energetic value of sucrose could confound results);
It appears that some of the scores of withdrawal behaviour were semi-quantitative? Scoring (subjective/objective, e.g. 71) deserves better explanation or a standard reference.

Results (Chapter 4)
Given the interest in GR (ligand-dependent transcription factor), monitoring of corticosterone during end of morphine self-administration would be appropriate — as it is, there seems to be an assumption (based on literature?) that the opiate would elevate corticosterone levels and increase GR activity.

In a related manner, and although fully understandable (practical reasons etc), it should also be kept in mind that exogenous glucocorticoids (e.g. dexamethasone) do not mimic “stress”, but rather reduce the secretion of endogenous hormones (corticosterone). In light of the interest in stress, GR and addiction, this issue should be addressed in future extensions of the present research.

Fig. 6 would benefit from a better indication of time (even one that is not-to-scale) — how many weeks/months did each of the “phases” extend over?

Is Fig. 22 incomplete? On p. 101, reference is made to corticosterone levels in dexamethasone-treated mice (Figure 22!!).

(and elsewhere) It appears that GR knockout in astrocytes alter some, but all many responses to morphine. Is that correct. It would help the reader (and interpretation of the data) if a table summarizing the exact behaviours affected was presented.

the tests of depression were not performed in mice that had experience chronic stress (the best-known trigger of depression-like behaviour); is this because it is assumed that chronic morphine would elevate glucocorticoid secretion (causally-related to such behaviour and an effect that only mimics an important physiological response to stress)?

Discussion and Summary and Conclusions (Chapters 5 and 6)
The Discussion represents an excellent integration of background, results from different sets of experiments using a variety of experimental approaches and a justified interpretation of the findings in the context of the available knowledge. The model presented in the last chapter will serve to guide further studies, especially with respect to the reasonable hypothesis that astrocytic GR regulate the metabolic support available for NAc spiny neurons. The only criticism that may be made is the apparent assumption that the experiments and results reflect the role of stress in the development of addiction, whereas the studies in fact establish that astrocytic GR contribute to the neural processes that result in addictive behaviour (as is correctly reflected in the title of the thesis).

For the oral examination, I would propose that the candidate is asked three general questions:
Can she explain (or speculate)
1. whether her results with respect to morphine may be generalized to other drugs/substances of abuse?
2. how the vicious cycle between stress/elevated glucocorticoid secretion and opioid dependence is driven/maintained, and what might be the underlying structural and neurochemical adaptations?

3. how her findings on the importance of astrocytic GR may help prevent or treat drug addiction and withdrawal symptoms?

Taken together, the work presented in this dissertation represents a large amount of work and use of a variety of contemporary, but highly appropriate, approaches. The experiments were carefully designed and executed, properly analysed and interpreted; moreover, the results are robust, add important new knowledge, are publishable, and are suitable for developing new testable hypotheses. Briefly, the candidate has demonstrated the ability to successfully conceive, develop, execute and report a biomedical research project. Overall, the quality of the work is of very high standard and this dissertation fulfils an important part of the requirements for the award of the PhD degree and, in my view, can be accepted without changes or corrections.

I hereby recommend that the High Scientific Council of the Institute of Pharmacology of the Polish Academy of Sciences allows Ms. Ursula Skupio to proceed through the further formal steps towards obtaining the doctoral degree. I also propose that the Council grants this thesis a note of “distinction”.

Osborne Almeida, PhD