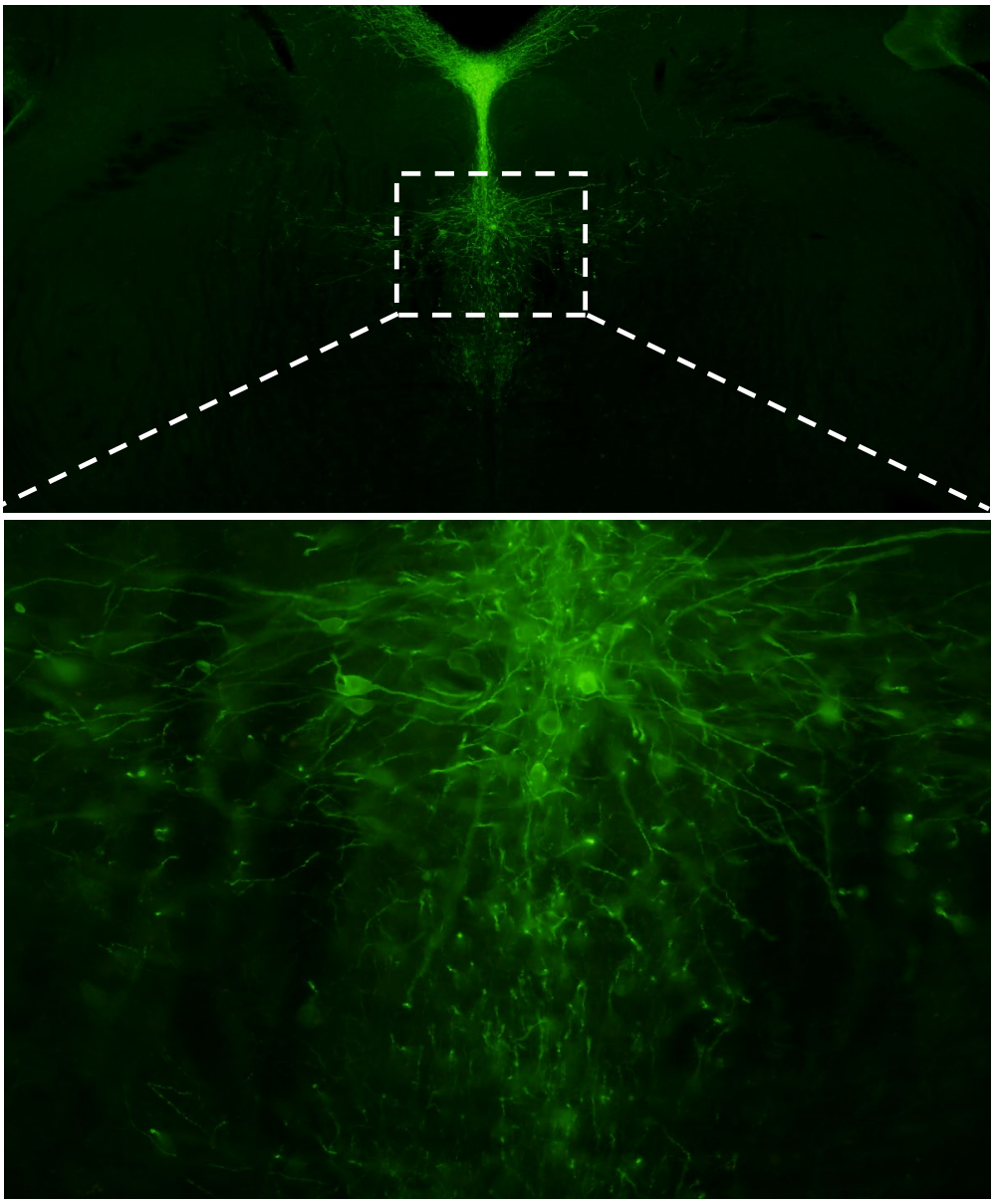
XJENZA ONLINE

VOLUME 5 ISSUE 1, 2017



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The Journal of the Malta Chamber of Scientists

Editor-in-Chief: Giuseppe Di Giovanni

FRONT COVER CAPTION

Optogenetic EYFP-ChR2 expression in the dorsal raphe nucleus of a Tph2-ChR2(H134R)-EYFP mouse. Top: Low magnification epifluorescent image of a coronal brainstem section. The boxed area is shown underneath. Bottom: Example recording of the electrical activity of a neocortical neuron recorded in the motor cortex of an anesthetized mouse. 5-HT optogenetic photostimulation (indicated by the horizontal blue bar) results in a rapid and prominent suppression of action potential firing. The inset shows the superimposed spikes, red trace is the averaged spike waveform. The blue bar marks the 5-HT photostimulation (train of 10 ms pulses at 10 Hz). From: Targeting the 5-HT system to control seizures by Crunelli et al. Bottom

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Chronological List of Past and Present Editors of Xjenza The Journal of the Malta Chamber of Scientists

2013–
Editor: Giuseppe Di Giovanni
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Xjenza Online Vol. 4 Iss. 2 - December 2016
Xjenza Online Vol. 4 Iss. 1 - July 2016
Xjenza Online Vol. 3 Iss. 2 - December 2015
Associate Editors: *David Magri, Ian Thornton, Ian Cassar, Philip Farrugia, Sebastiano D'Amico, Nicholas Sammut, Joseph Galea, David Mifsud, Sandro Lanfranco, Mario Valentino, Godfrey Baldacchino, Liberato Camilleri*
Xjenza Online Vol. 3 Iss. 1 - August 2015
Xjenza Online Vol. 2 Iss. 2 - October 2014
Xjenza Online Vol. 2 Iss. 1 - March 2014
Xjenza Online Vol. 1 Iss. 2 - October 2013
Xjenza Online Vol. 1 Iss. 1 - March 2013

2003–2007
Editors: Joseph N. Grima and Richard Muscat
Xjenza Vol. 12 - 2007
Xjenza Vol. 11 - 2006
Xjenza Vol. 10 - 2005
Xjenza Vol. 9 - 2004
Xjenza Vol. 8 - 2003

1996–2002
Editor: Angela Xuereb
Associate Editor: *Richard Muscat*
Xjenza Vol. 7 - 2002
Xjenza Vol. 6 - 2001
Associate Editors: *Martin Ebejer and Richard Muscat*
Xjenza Vol. 5 - 2000
Xjenza Vol. 4 Iss. 2 - 1999
Xjenza Vol. 4 Iss. 1 - 1999
Associate Editors: *Martin Ebejer, Richard Muscat, and Christian A. Scerri*
Xjenza Vol. 3 Iss. 2 - 1998
Xjenza Vol. 3 Iss. 1 - 1998
Associate Editors: *Martin Ebejer, Richard Muscat, Christian A. Scerri and Emmanuel Sinagra*
Xjenza Vol. 2 Iss. 2 - 1997
Xjenza Vol. 2 Iss. 1 - 1997
Xjenza Vol. 1 Iss. 2 - 1996
Xjenza Vol. 1 Iss. 1 - 1996

Scope of Journal

Xjenza is the Journal of the Malta Chamber of Scientists and is published in an electronic format. Xjenza is a peer-reviewed, open access international journal. The scope of the journal encompasses research articles, original research reports, reviews, short communications and scientific commentaries in the fields of: mathematics, statistics, geology, engineering, computer science, social sciences, natural and earth sciences, technological sciences, linguistics, industrial, nanotechnology, biology, chemistry, physics, zoology, medical studies, electronics and all other applied and theoretical aspect of science.

The first issue of the journal was published in 1996 and the last (No. 12) in 2007. The new editorial board has been formed with internationally recognised scientists, we are planning to restart publication of Xjenza, with two issues being produced every year. One of the aims of Xjenza, besides highlighting the exciting research being performed nationally and internationally by Maltese scholars, is to provide insight to a wide scope of potential authors, including students and young researchers, into scientific publishing in a peer-reviewed environment.

Instructions for Authors

Xjenza is the journal of the Malta Chamber of Scientists and is published by the Chamber in electronic format on the website: http://www.mcs.org.mt/index.php/xjenza. Xjenza will consider manuscripts for publication on a wide variety of scientific topics in the following categories

1. Communications
2. Research Articles
3. Research Reports
4. Reviews
5. Notes
6. News and Views
7. Autobiography

Communications are short peer-reviewed research articles (limited to three journal pages) that describe new important results meriting urgent publication. These are often followed by a full Research Article.

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Review Articles describe work of interest to the wide readership characteristic of Xjenza. They should provide an in-depth understanding of significant topics in the sciences and a critical discussion of the existing state of knowledge on a topic based on primary literature. Review Articles should not normally exceed 6000 words.Authors are strongly advised to contact the Editorial Board before writing a Review.

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Article Structure

A manuscript for publication in Xjenza will ordinarily consist of the following: Title page with contact information, Abstract, Highlights, Keywords, Abbreviations, Introduction, Materials and Methods, Results, Discussion, Conclusion, Appendices and References.

The manuscript will be divided into clearly defined numbered sections. Each numbered subsection should be given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by the section number as opposed to simply 'the text'.

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- Title should be concise yet informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after each author's name and in front of the appropriate address. Provide full postal address of each affiliation, including the country name and, if available, the e-mail address.
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Abstract A concise and factual abstract is required of up to about 250 words. The abstract should state briefly the background and purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references and non-standard abbreviations should be avoided. If essential, these must be defined at first mention in the abstract itself.

Abbreviations Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention as well as in the footnote and should be used consistenly throughout the text.

Introduction State the objectives of the work and provide an adequate background, avoid a detailed literature survey or a summary of the results.

Materials and Methods Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results Results should be clear and concise. Numbered/tabulated information and/or figures should also be included.

Discussion This should explore the significance of the results of the work, yet not repeat them. Avoid extensive citations and discussion of published literature. A combined section of Results and Discussion is often appropriate.

Conclusion The main conclusions based on results of the study may be presented in a short Conclusions section. This may stand alone or form a subsection of a Discussion or Results and Discussion section.

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Acknowledgements Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided assistance during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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Citations and References Reference and citation styles for manuscripts submitted to Xjenza should be in accordance to the APA v6 style.

Citation in text References to cited literature in the text should be given in the form of an author's surname and the year of publication of the paper with the addition of a letter for references to several publications of the author in the same year. For further information regarding multiple authors consult the APA v6 guidelines. Citations may be made directly

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The reference section Every reference cited in the text should also be present in the reference list (and vice versa). The reference list should also be supplied as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTeX (*.bib) file. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

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Agree, E. M. and Freedman, V. A. (2011). A Quality-of-Life Scale for Assistive Technology: Results of a Pilot Study of Aging and Technology. *Phys. Ther.*, 91(12):1780–1788.

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Fisk, M. J. (2003). *Social Alarms to Telecare: Older People's Services in Transition.* Policy Press, Bristol, 1st edition.

Reference to a Chapter in an Edited Book:

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Journal Abbreviations Journal names should be abbreviated according to:

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  - Full postal address.
  - Telephone and fax numbers.
- All necessary files have been sent, and contain:
  - All figures are given separately in PDF, SVG, JPEG of PNG format.
  - Caption for figures is included at the end of the text.
  - All tables (including title, description, footnotes) are included in the text and large tables have been given separately as CSV.
  - The reference list has been given in XML, RIS, zotero.splite or BIB file format.
- Further considerations
  - Abstract does not exceed about 250 words.
  - Manuscript has been 'spell-checked' and 'grammar-checked'.

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Di Giovanni, G. (2017). *Xjenza Online*, 5:1–2.

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Editorial

Giuseppe Di Giovanni*
Department of Physiology and Biochemistry, University of Malta, Msida, Malta

Dear readers of Xjenza Online, what an incredible start to the year for Xjenza!

A Special Issue of Xjenza Online was published in June as the Proceedings of the 6th Mediterranean Neuroscience Society Meeting (MNS2017) held in Malta from 13 to 15 of June at the Radisson Blue Hotel in Saint Julian's. More than 500 hundred copies were printed, one for each attendee at the conference which turned out to be a truly fantastic international event. Just think that one copy of our journal has arrived home in 41 countries from Armenia to the USA! I am really satisfied, as the editor of a journal that is proudly local - even though we have started to and persisted in publishing articles from international scholars - that we have achieved such an unthinkable international distribution.

Xjenza Online was a major supporter of MNS2017, apart from publishing a SI on the Proceedings of the Conference (Xjenza Online Vol (SI), 2017), we also gave a prize for the best Maltese posters presented. Our prize, "Best Poster Prize Xjenza/RIDT" was among those given by Elsevier/Journal Neuroscience Methods-Neuropharmacology and Springer Nature/The Receptors. What an honour to appear with Elsevier and Springer/Nature. Moreover, we organized a Workshop on how to publish with Elsevier, one of the major publishing houses. Presentations were given by Shamus O'Reilly (Senior Publisher Elsevier), Natalie Farra, (Senior Acquisitions Editor, Neuroscience) Ryan Scicluna (University of Malta Library), Vincenzo Crunelli (Editor Journal of Neuroscience Methods), Bruno Frenguelli (Editor Neuropharmacology), Giuseppe Di Giovanni and David Magri (Editors of Xjenza Online) on various aspects of publishing a research paper.

This issue also recalls the MNS2017 meeting, in that you can find the speech given by HE Marie Louise Coleiro Preca, President of Malta who opened the Conference. There are also commentaries by Raphael Mechoulam, from Israel, who in the 60s, discovered and isolated the THC molecule, the main active principle of cannabis, and by Ilana Gozes from Israel too on autism, schizophrenia and Alzheimer's disease and the common thread between neuropeptides and brain regulating genes.

This issue starts with a review on serotonin and epilepsy authored by international scholars Giuseppe Di Giovanni, Vincenzo Crunelli and Magor Lorincz from Malta, UK and Hungary, respectively, reporting the latest fascinating findings of an MCST founded project called EPILEFREE aiming at stopping seizures in animal models of absence epilepsy which has clear translational applications.

Raymond Mangion talks us through *"Legislation as a means of Legal Entrenchment and Enforcement of Medical and Surgical Practice in Malta from 1801–1901"*. This is an interesting study full of fascinating details about the first 100 years of British rule during which the foundations of the present-day medical and surgical system were prepared and laid. Nicola Ballarini and Ian Thornton with their research article *"Biological Motion across Viewpoints"* report that the recognition of human bodies depicted as biological motion stimuli is viewpoint-dependent, as with many other types of object.

Beppe Aquilina and Ruben Cauchi's research on Gilles de la Tourette syndrome using the fruit fly shows that histamine deficiency is not associated with an increase in spontaneous repetitive grooming behaviour but rather a decrease in Drosophila melanogaster. Therefore, the grooming behaviour seen in *Drosophila hdc* knockouts is not a translationally relevant TS phenotype.

In the following research article Stephen Mifsud reports a preliminary survey and taxonomy of wild roses (*Rosa* spp.) occurring on the Maltese islands. *Rosa* spp. are neglected and understudied as Maltese flora as

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only three species have been reported yet. Mifsud in his research described 27 populations of wild or naturally occurring roses, of which twelve species, hybrids or cultivars have been recorded, eight of which are new for the Maltese flora.

The last research article by Cutajar and colleagues presents a case study concerning the development of a BioMEMS device for a deep brain stimulation (DBS) system integrated with a drug delivery system (DDS) for application in humans. The qualitative feedback received from the identified stakeholders, together with the quality of the case study employed, namely, an integrated DBS and DDS solution, indicate sufficient evidence to suggest that the model provides a sound basis in this direction.

The last Commentary, written by Samantha Austen, takes a brief look at the evolving field of native language transfer research in second language acquisition and the possible implications for language teaching.

We conclude as usual with two news items, the first on the workshop: How to Publish, by David Magri, organized by Xjenza Online at the MNS2017 and the last one by Colleen Bower on *Science in the Citadel* that was held in Gozo's iconic medieval Citadel on the 22nd of April. It provided an atmospheric venue for the first science festival on the island. The aim of the festival was to engage the public with science, to inspire the next generation towards scientific endeavour, and to showcase Gozo as a potential future hub for scientific discussion and development.

I would like to conclude by thanking, as always, the editorial board of Xjenza Online, the new board members, the referees and all the authors who have contributed to this issue, which I believe to be one of the best.

I hope you enjoy this issue, until next time!

Giuseppe Di Giovanni
Editor in Chief

Crunelli, V., Lörincz, M., Furdan, S., Orban, G., Colangeli, R., Delicata, F., Deidda, G., Attard Trevisan, A., Pierucci, M., and Di Giovanni, G. (2017). *Xjenza Online*, 5:3–14.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.01

Review Article

Targeting the Serotonin (5-HT) System to Control Seizures

Vincenzo Crunelli[1], Magor L. Lörincz[2], Szabina Furdan[2,3], Gergely Orban[1], Roberto Colangeli[1], Francis Delicata[1], Gabriele Deidda[1], Adrian Attard Trevisan[3], Massimo Pierucci[1] and Giuseppe Di Giovanni[1*]

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Abstract. Compelling animal and human evidence suggests that serotonin plays an important role in the pathophysiology of epilepsy as it is involved in iperexcitability, epileptogenesis, seizure generation, depression and psychiatric disorders comorbid with epilepsy. Serotonin involvement in epilepsy is complex; the reasons are twofold i) epilepsy is in reality a spectrum disorder, and ii) serotonin effects vary from one form of epilepsy to another, due also to the different serotonin receptors involved. Here, we will focus on the role of serotonin and its 5-HT_2 receptors in absence epilepsy. Our recent pharmacological experimental evidence in GAERS will be reviewed together with our preliminary optogenetic results. 5-HT_{2C} receptor agonists may represent a new approach to interfere with seizure generation and seizure management. Our optogenetic experiments also indicate that by modulating rhythmic cortical activity, optogenetic stimulation of the serotonergic system may provide seizure control without the adverse effects induced by pharmacological activation of 5-HT_{2C} receptors. Thus, targeting the serotonergic system could provide novel insights into the pathophysiological mechanisms of seizure generation and lead to potentially novel treatments.

Keywords: Serotonin receptors, epilepsy, epileptogenesis, antiepileptic drugs, optogenetics, closed-loop control

1 Introduction

1.1 Serotonin systems

The dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) of the midbrain raphe nuclei, send serotonin (5-HT) widespread innervation to all brain areas (Azmitia & Segal, 1978; van der Kooy & Hattori, 1980; Steinbusch, Nieuwenhuys, Verhofstad & der Kooy, 1981; Steinbusch, 1984; Van Bockstaele, Biswas & Pickel, 1993). There is some specificity regarding the two raphe nuclei. The DRN, which is composed of approximately 50% of 5-HT neurons, is mainly responsible for the 5-HT innervation of the mammalian medial prefrontal cortex (mPFC) and neostriatum (Bobillier et al., 1976; Azmitia & Segal, 1978; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). On the other hand, MRN projections containing 5-HT innervate the hippocampus (HIP) and septum.

The DRN contains 8,000 to 91,000 5-HT neurons in mice and humans, respectively. It represents 30–56% of 5-HT neurons in the Central Nervous System (CNS) depending on the species (Jacobs & Azmitia, 1992; Thevenot et al., 2003). It is worth noting that 5-HT neurons may co-express and release other neurotransmitters, such as glutamate, nitric oxide and GABA, corticotropin-releasing factor (Jacobs & Azmitia, 1992; Trudeau, 2004; Hioki et al., 2010; Lu, Simpson, Weaver & Lin, 2010; Monti, 2010). The 5-HT neurons of the

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DRN are also heterogeneous due to the expression or lack of some differentiation factors such as Lmx1b (Ding et al., 2003) or pet-1 (Kiyasova et al., 2011; Gaspar & Lillesaar, 2012; Smidt & van Hooft, 2013). Six parts of the DRN have been described, based on their anatomy and functional topography (Hale & Lowry, 2011). Dorsal parts of the DRN send projections to the central and basolateral nuclei of the amygdala, the dorsal hypothalamic area and the mPFC (Lowry et al., 2008). The ventral part of the DRN innervates the sensorimotor cortex and the caudate putamen. The lateral part projects mainly to subcortical regions, including the lateral hypothalamus and the superior colliculus. The interfascicularis part projects to the HIP and the medial septum. The rostral parts send projections to the caudate putamen and the substantia nigra (SN), whilst the caudal part sends projections to the amygdala, ventral HIP and thalamic nuclei. The DRN also receives several afferents from the lateral habenula, the preoptic area, the lateral dorsal and posterior hypothalamic nuclei, basal telencephalon, bed nucleus of the terminalis stria amygdala, cingulate cortex and PFC. In central and caudal levels, the DRN receives projections from the SN, the reticulate formation, the periaqueductal grey matter, and the parabrachial nucleus (Lowry et al., 2008).

The distribution of 5-HT terminals in the brain has been studied through autoradiography, using the binding of the two 5-HT uptake sites (serotonin transporter, SERT) ligands [^{3}H]-imipramine and [^{3}H]-citalopram (D'Amato, Largent, Snowman & Snyder, 1987; Hrdina, Foy, Hepner & Summers, 1990; Dewar, Reader, Grondin & Descarries, 1991), immunohistochemistry using antibodies directed against SERT (Hrdina et al., 1990) and 5-HT (Steinbusch et al., 1981; Steinbusch, 1984), or by measuring tryptophan hydroxylase (TPH) activity (Saavedra, 1977).

The organization of 5-HT contacts on other neural elements lacks specificity. Axon terminals of 5-HT neurons are in contact with a variety of structures, including axon terminals, dendritic spines and shafts, but rarely neuronal somata. This organization of 5-HT varicosities and synapses in the brain lends support to the hypothesis that diffusion processes or volume transmission are the main features of 5-HT transmission (Descarries, Seguela & Wakins, 1991; Umbriaco, Garcia, Beaulieu & Descarries, 1995; Descarries & Mechawar, 2000).

1.2 Serotonin and Epilepsy

Compelling animal and human studies have shown 5-HT involvement in many psychiatric and neurological diseases, including epilepsy. It is well known that 5-HT controls, directly or indirectly, neuron excitability by modulating various ion channels, controlling the release of other neurotransmitters and the activation of intracellular pathways via the activation of its plethora (fourteen) receptor subtypes (Barnes & Sharp, 1999; D'Adamo et al., 2013). Therefore, 5-HT is logically involved in the cascade of events that can change a normal neuronal network into a hyperexcitable one (Bagdy, Kecskemeti, Riba & Jakus, 2007; Jakus & Bagdy, 2011; Ghanbari, El Mansari & Blier, 2012). 5-HT is likely to play a role in the initiation, propagation and maintenance of seizure activity, apart from the epileptogenesis. Here, we will focus on the evidence of a 5-HT$_2$R control of epilepsy. 5-HT$_{2A}$Rs, along with 5-HT$_{2B}$ and 5-HT$_{2C}$, belong to the 5-HT$_2$ subfamily that consists of three Gq/G11-coupled receptor. 5-HT$_{2A/2C}$Rs in general mediate excitatory effects of 5-HT on CNS neurons (Di Giovanni, Di Matteo, Pierucci, Benigno & Esposito, 2006; Millan, Marin, Bockaert & la Cour, 2008).

Classically, epilepsy syndromes are classified into two distinct types, focal and generalized, according to the brain circuitry that sustain the oscillations that lead to seizures, site of seizure onset, electroencephalographic and behavioural characteristics (Berg et al., 2010). Generalized and focal epilepsy also differ in the nature of the pathological and neurochemical imbalance between glutamate and GABA function. Indeed, drugs that increase extracellular GABA levels and/or GABA transmission are first choice in focal/generalized convulsive epilepsy, whereas they exacerbate generalized nonconvulsive seizures. As matter of fact, a structural GABA analogue, gabapentin, which increases GABA synthesis, exacerbates generalized nonconvulsive absence seizures (ASs) and is not indicated in non-convulsive epilepsies (Manning, Richards & Bowery, 2003). Consistently, we have shown that an increase of tonic GABA inhibition is a *conditio sine qua non* for the generation of absence seizure in rat and mouse models of the this form of epilepsy (Cope et al., 2009; Errington, Gibson, Crunelli & Cope, 2011, 2014).

1.3 Absence Seizures

A typical AS consists of a sudden and relatively short period of a lack of consciousness, which is invariably accompanied by a stereotypical EEG activity of synchronous and generalized spike and wave discharges (SWDs). ASs are present in various idiopathic generalized epilepsies (IGEs), while they are the only phenotype in childhood absence epilepsy (CAE). In CAE, the average age in which ASs start is 3 to 8 years and they are neither induced or generated by either visual or other sensory stimuli. The majority ($\sim$ 60%) of children suffering from CAE show spontaneous remission often around adolescence, although, in approximately a third of cases, absences continue later in life. This benign outcome of CAE concords with a lack of metabolic and neuropathological signs in this epilepsy. Nevertheless, in up to 90% of CAE sufferers for whom ASs persist during adulthood, there is the occurrence of generalized tonic clonic seizures (GTCSs) (Crunelli & Leresche, 2002).

The annual incidence rate of CAE is 2–8 per 100,000 children under 15 years of age, and its prevalence is 2–10% among children with any type of epilepsy. CAE is genetically determined, with a 16–45% positive family history. Although penetrance is incomplete, a concordance of 70–85% and 33% has been reported in monozygotic twins and first-degree relatives, respectively. Thus, CAE is commonly described as a familial disease with a complex genotype, and evidence exists that it may represent a channelopathy. Indeed, the emerging picture from the vast majority of genetic studies of AS cohorts preferentially points to abnormalities in genes encoding either calcium channels and/or GABA receptors, though it needs to be stressed that in many of these studies, ASs were not the only epileptic phenotype (Crunelli & Leresche, 2002).

As far as the pathophysiological mechanisms of ASs are concerned, invasive experimental work (Williams, 1953) and more recent non-invasive imaging studies in humans (Holmes, Brown & Tucker, 2004; Hamandi et al., 2006; Bai et al., 2010) have indicated that these seizures are generated by paroxysmal electrical activity of cortical and thalamic networks. In particular, studies in mouse and rat genetic absence models have shown that SWDs initiate in somatosensory cortex, from where they rapidly spread to other cortical areas and to the thalamus. The presence of a cortical "initiation site" for SWDs of ASs has now been conclusively demonstrated in CAE and other patients with ASs, challenging the classical view of a SWD as a fully generalized EEG paroxysm, at least at its onset.

The main activity of layer V/VI cortical neurons during SWDs are rhythmic depolarizations that occur in phase with the EEG spike, and whose waveform is drastically different from the classical paroxysmal depolarizing shifts of convulsive epilepsies. Possible candidates for cortical abnormalities underlying the expression of this firing pattern may include an increased NMDA-mediated excitation in deep layers, a decreased GABAergic inhibition in layer II/III and/or abnormalities in HCN channels. In NRT neurons *in vivo*, the enhanced and more synchronous cortical volley of SWDs, together with the convergence of the corticothalamic input, results in bursts of EPSPs, that at times generate a T-type Ca^{2+} channel dependent high frequency burst of action potentials in correspondence to each spike of the SWD. Alterations in GABA-A $\gamma 2$ subunits, T-type Ca^{2+} channels, gap-junction coupling and/or excitatory and inhibitory synaptic strengths have been suggested to occur in the NRT of genetic absence models. The strong and prolonged inhibitory output of the NRT, coupled to the deficient GABA transporter-1, lead TC neurons to the presence of rhythmic sequences of 4–6 $GABA_A$ IPSPs and a marked increase in tonic $GABA_A$ inhibition. Thus, the firing rate of these thalamic neurons during ASs decreases and only occasional action potentials are observed in synchrony with the spike component of SWDs. Notwithstanding, there is always a synchronized output from thalamus to cortex during ASs (Crunelli & Leresche, 2002).

1.4 Role of 5-HT in Generalized Epilepsy: Pharmacological Evidence

Bonnycastle, Giarman and Paasonen (1957) proposed the implication of 5-HT in epilepsy for the first time in the late fifties. Successively, a large body of evidence has confirmed a direct relationship between 5-HT impairment and epilepsy, both in generalized and focal epilepsy (see Svob Strac et al., 2016). For instance, an increase in 5-HT CNS concentration, by using 5-HT transporter (SERT) blockers or increasing its metabolism by introducing more tryptophan to the diet, has been associated with an antiepileptic activity, while a decrease in 5-HT brain concentration leads to a diminished threshold for various types of convulsive seizures (see Bagdy et al., 2007). Moreover, an increase of the firing rate of DRN neurons contextually to ASs (Lörincz, Olah, Baracskay, Szilagyi & Juhasz, 2007), a decrease in 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) level in the thalamus and a significant negative correlation between the severity of epilepsy (as time spent in ASs) and the thalamic levels of 5-HT (Midzyanovskaya et al., 2006) have been observed in WAG/Rij (Wistar Albino Glaxo/Rijswijk) rat model of absence epilepsy. Many anti-epileptic drugs (AEDs) also act by elevating brain extracellular 5-HT, and many SSRIs show an antiepileptic effect (Bagdy et al., 2007). Moreover, a common serotonin dysfunction may underlie both epilepsy and comorbid depression, seen in epileptic patients (Kanner et al., 2012; Guiard & Di Giovanni, 2015; Svob Strac et al., 2016). There is a clear association between the ASs and behavioural states, with clinical seizures occurring preferentially in states of 'decreased or fluctuating vigilance', and very few during deep stages of non-REM sleep. This behavioral state-dependence implies that, as it is the case for other oscillations generated by the thalamocortical loop, the expression of SWDs is controlled by various neuromodulatory systems (Steriade & McCarley, 2005; Saper, Fuller, Pedersen, Lu & Scammell, 2010). These include, among others, monoaminergic systems in the brainstem (Lörincz & Adamantidis, 2017), cholinergic systems in the basal forebrain and brainstem (Steriade & McCarley, 2005), and serotoninergic system originating in the raphe nuclei. The activity of neurons in these neuromodulatory neurons is highly brain state-dependent (Lörincz & Adamantidis, 2017) and their selective stimulation or inhibition leads to brain state changes (Carter et al., 2010; Xu et al., 2015; Eban-Rothschild, Rothschild, Giardino, Jones &

de Lecea, 2016). Given the behavioural-state dependence of ASs and the prominent effects of neuromodulators on brain states, external changes in the neuromodulator systems could in principle affect the expression of ASs. This novel therapeutic approach would be beneficial as monotherapy with gold-standard anti-absence drugs is only effective in 50% of CAE patients (Glauser et al., 2010). Indeed, it has been shown that drugs that affect the tone of the 5-HT system can modulate, and in some cases abolish ASs when injected systemically.

Nevertheless, none of the common AEDs target the 5-HT system, at least as main mechanism. Selective 5-HT ligands may indeed induce dangerous off-target effects. Therefore, the challenge for pharmaceutical research is to develop new 5-HT compounds with better side effects profile, potentially efficacious for both epilepsy and its comorbid depression (see our recent review Svob Strac et al., 2016).

Of the plethora of 5-HTRs, the 5-HT_{2C}R seems to be the most promising since it has been shown to be involved in generalized convulsive epilepsy, seizure generation and network excitability (Isaac, 2005; Jakus & Bagdy, 2011). Moreover, 5-HT_{2C} knock down (KO) mice show spontaneous, occasionally lethal, tonic-clonic seizures (Tecott et al., 1995). Furthermore, in 5-HT_{2C} KO mice the threshold for electrical (kindling, electroshock), audiogenic and chemical-induced (i.e., by pentylenetetrazol; PTZ) seizures was decreased (Applegate & Tecott, 1998; Heisler, Chu & Tecott, 1998). Consistently, treatments with 5-HT_{2C}R agonists increased the threshold for PTZ and electroshock-induced seizures in mice (Upton, Stean, Middlemiss, Blackburn & Kennett, 1998). On the other hand, 5-HT_{2C}Rs do not seem to affect focal epilepsy. For instance, metachlorophenylpiperazine, lorcaserin, but not RO60-0175, 5-HT_{2C} agonists with different pharmacological profiles (Fletcher & Higgins, 2011; Higgins et al., 2013), were able to stop the hippocampal maximal dentate gyrus activation (MDA) in a rat temporal lobe epilepsy (TLE) model (Orban et al., 2014). m-CPP and lorcaserin antiepileptic effects were not blocked by SB 242084 pretreatment, a selective 5-HT_{2C}R antagonist, but rather potentiated (Orban et al., 2014). Therefore, our findings suggest that 5-HT_{2C}Rs are pro-epileptic and the m-CPP and lorcaserin activate other 5-HTRs, most likely 5-HT_{1A}Rs (Orban et al., 2013). The fact that RO60-0175 was not effective in blocking the MDA elongation further supports the evidence that this compound is far from being a specific 5-HT_{2C}R tool (Damjanoska et al., 2003; Navailles, Lagiere, Le Moine & De Deurwaerdere, 2013; Orban et al., 2014).

On the other hand, the involvement of 5-HT_{2C}Rs in non-convulsive generalized seizures is more compelling compared to focal epilepsy. Hitherto, the results have been hampered by the lack of selectivity of the 5-HT_{2C}R available drugs (Bagdy et al., 2007; Guiard & Di Giovanni, 2015).

mCPP decreased the duration of SWDs via the activation of 5-HT_{2C}Rs in WAG/Rij rats since its effect was blocked by SB 242084 (Jakus et al., 2003). On the other hand, 5-HT_{2C}Rs seem not to play a role in basal modulation of ASs (Jakus et al., 2003; Jakus & Bagdy, 2011). Similarly, DOI, a 5-$HT_{2A/2C}$ mixed agonist and the two 5-HT reuptake inhibitors fluoxetine and clomipramine, reduced the time spent in seizure in groggy model of ASs (Tokuda et al., 2007). DOI-elicited decrease in ASs was blocked by ritanserin, a non-selective 5-HT_2 antagonist, which had no effect on its own (Ohno et al., 2010), further confirming that phasic 5-HT activation of 5-HT_2Rs does not modulate the occurrence of SWDs. Moreover, mCPP had no effect in modulating absence seizures, while DOI reduced the total time spent in seizure in the AY-9944 model of atypical AS (Bercovici, Cortez, Wang & Snead, 2006).

We have recently investigated the effects of pharmacological manipulation of 5-HT_2Rs in the expression of absence seizures of in GAERS (Genetic Absence Epilepsy Rat from Strasbourg), another polygenic model of ASs using selective 5-HT_{2C} drugs (Venzi et al., 2016). Since early results obtained with unselective 5-HTR ligands (Marescaux, Vergnes & Depaulis, 1992b, 1992a) (see Danober, Deransart, Depaulis, Vergnes & Marescaux, 1998) did not show any significant 5-HT modulation of AS in GAERS, we used lorcaserin and CP809, 101, the most selective 5-HT_{2C} agonist available (Siuciak et al., 2007) and the selective 5-HT_{2C} antagonist SB 242084. 5-HT_{2C}Rs activation reduced total time spent in seizures in GAERS. Moreover, we observed an overexpression of 5-HT_{2C}Rs in the ventrobasal (VB) thalamus in GAERS compared to non-epileptic control (NEC) rats (unpublished observations). Therefore, a dysfunction of 5-HT_{2C}Rs might be involved in the pathogenesis of ASs and selective agonists at these receptors may be potential targets for new anti-absence drugs.

5-HT_{2C}Rs are widely expressed in the CNS, including key areas involved in the pathogenesis of the ASs, such as the cortex and thalamus (Crunelli & Leresche, 2002) or areas known to modulate SWDs i.e., striatum, nucleus accumbens and substantia nigra pars reticulata (Depaulis, David & Charpier, 2016). Therefore, since we administered the drugs intraperitoneally, it is difficult to rule out which brain area is involved in the anti-absence effect of 5-HT_{2C}R agonists.

5-HT_{2C}Rs are expressed in thalamocortical (TC) neurons in the dorsal lateral geniculate nucleus (dLGN) (Coulon et al., 2010). GABAergic interneurons of the dLGN contain 5-HT_{2C}R mRNA, and their activation induces an increase of phasic $GABA_A$R inhibition in

dLGN TC neurons in mice (Munsch, Freichel, Flockerzi & Pape, 2003). The intracellular pathways that couple the 5-HT_2Rs to the Ca^{2+}-influx mechanism depend on the PLC system and the transient receptor potential (TRP) protein TRPC4 (Munsch et al., 2003). We instead showed that mCPP (ineffective in mice; Munsch et al., 2003) decreased phasic inhibition as well as tonic $GABA_A$R current in dLGN neurons in rats, an effect blocked by pretreatment with SB 242084 (Crunelli & Di Giovanni, 2015). 5-HT_{2C}R GABAergic modulation is not limited to the dLGN but is also present at the level of the somatosensory VB thalamus, where RO 60-0175 decreased both tonic and phasic inhibition GABAA (unpublished observations). The control of tonic inhibition seems to be phasic in nature, since SB 242084 did not have any effects on its own, but blocked RO 60-0175 effect in wistar rats. RO 60-0175 produces a normalization of the increased $GABA_A$ tonic current in GAERS, thought to be a necessary mechanism for the development of ASs (Cope et al., 2009).

5-HT in the thalamus induces depolarization of TC neurons and change in their firing pattern from burst to single spike activity (McCormick, 1992). 5-HT induces membrane depolarization by inhibition of a leak K^+ conductance (Meuth et al., 2006) and hyperpolarization-activated non-selective cation current (Ih) (Pape & McCormick, 1989; Chapin & Andrade, 2001). 5-HT_{2C}R agonist CP809,101 and 5-HT produce similar depolarization effects activating Gq protein-coupled receptors (Coulon et al., 2010). Ketanserin, a 5-$HT_{2A/2C}$R antagonist, was capable of blocking 5-HT-induced switch in the NRT neuronal pattern activity. Therefore, 5-HT modulation of sleep-waking activity might depend on GABAergic neurons of the NRT (McCormick & Pape, 1990; McCormick & Wang, 1991).

5-HT promotes waking and suppress REM sleep but on the other hand 5-HT_{2C}R KO mice have an increase of waking and a reduction in NREM sleep. Different results come from pharmacological experiments where selective 5-HT_{2C}R antagonists and nonselective 5-$HT_{2A/2C}$R antagonists increase SWS (slow wave sleep) and reducing REM sleep respectively (Popa et al., 2005). On the other hand, nonselective 5-$HT_{2A/2C}$R agonists and selective 5-HT_{2C}R agonists increased waking and reduced SWS and REM sleep.

During ictal activity recorded in animal models of absence epilepsy, TC neurons are generally silent (Pinault et al., 1998; Polack et al., 2007) due to an increased corticothalamic excitatory inputs into NRT neurons compared to TC neurons. We hypothesize that during ASs, 5-HT_{2C}R agonists have antiabsence effects by decreasing GABA release form NRT neurons into VB TC neurons leading to a reduced $GABA_A$ phasic and tonic current. Nevertheless, that it is not impossible that 5-HT_{2C}Rs have both anti- and pro-epileptic effects, depending which brain area receptor population is activated. For example in the cortex, 5-HT_{2C}Rs are both highly expressed on inhibitory interneurons (S. Liu, Bubar, Lanfranco, Hillman & Cunningham, 2007) and pyramidal cortical neurons and 5-HT_{2C}R activation can induce increase of thalamic glutamate release (Puig, Celada, Diaz-Mataix & Artigas, 2003).

Another potential way by which 5-HT_{2C}Rs modulate ASs may be through other neurotransmitters such as dopamine, and noradrenaline known to modulate the arousal state and affecting thalamic and cortical pathological oscillations seen in absence epilepsy (Di Giovanni, Di Matteo & Esposito, 2008, 2010; Di Giovanni, 2013).

1.5 Therapeutic Potential of 5-HT2C Drugs in Epilepsy

5-HT modulates normal and pathological brain excitability via the plethora of 5-HTRs. The complexity of this control it might be due to the opposing effects of different receptors and the different 5-HT modulation of the different brain areas involved in the various types of epilepsy. 5-HT_{2C}Rs modulate generalized convulsive tonic-clonic and non-convulsive epilepsy. On the other hand, 5-HT_{2C}Rs do not seem to be involved in focal epilepsy.

Our findings with 5-HT_{2C}R agonists are promising and suggest a therapeutic potential of these drugs for the treatment of human generalized convulsive and non-convulsive epilepsy. Since lorcaserin has received FDA approval for treatment of obesity it will be easy to conduct a well-controlled studies to demonstrate its efficacy in epilepsy.

One of the negative side effects of 5-HT_{2C}R agonists may be their potential anxiogenic effects. As a result, new AEDs based on 5-HT_{2C}R agonism should be lacking of this and other 5-HT_{2C}R aversive off-target effects. Ideally optogenetic treatment of ASs would be devoided of all the typical synthetic 5-HT ligands side effects. Our on-going work using optogenetics to stop ASs will also clarify the role of 5-HT in this type of epilepsy. Increasing our understanding of the role of 5-HT might reveal novel mechanisms of potential translational significance.

1.6 Role of 5-HT in Generalized Epilepsy: Optogenetic Evidence

Optogenetic Studies of the Serotonergic System

The activity of raphe nuclei neurons is tightly correlated with changes in brain state changes (Urbain, Creamer & Debonnel, 2006). While correlating neuronal activity with specific behavioural events is essential for elucidating the neuronal mechanisms underlying a variety of brain functions, establishing causal relationships require

tools to directly interact with neuronal populations to boost or silence their activity and monitor the effects on various physiological and/or behavioural functions. The classical tools to reach these aims were to either electrically stimulate various nuclei or to systemically or locally interact with groups of neurons using pharmacological tools. Electrical stimulation acts by directly triggering action potentials in neurons or axons in the proximity of the stimulating electrode. Because most nuclei consist of populations of neurochemically heterogeneous neurons (see Introduction) and fibers of passage, this technique lacks specificity in terms of selectively influencing the electrical activity of various neurochemically heterogeneous elements of the network albeit and its great temporal specificity. On the other hand, as we showed in the first part of this review pharmacological tools, can be relatively specific, but lack both temporal specificity and when agonists are being used these can reach receptors outside the area of action of axon terminals from which they are released. Recent progress in genetic engineering offers new opportunities to directly control specific neuronal populations. Two light-sensitive proteins, namely Channelrhodopsin-2 (ChR2) and Halorhodopsin (Halo) can now be used to optically activate or silence specific neuronal subtypes (Zhang, Wang, Boyden & Deisseroth, 2006, 2008). The use of these proteins presents compelling advantages: 1) their expression can be targeted to specific cell types and/or subcellular compartments; 2) ChR2 can drive action potential firing with millisecond precision in response to light pulses. Animals expressing ChR2 and/or Halo have already been produced (Gradinaru, Mogri, Thompson, Henderson & Deisseroth, 2009), and optical interfaces for delivering the light to specific brain regions in behaving rodents have been designed (Aravanis et al., 2007). It has been recently shown that when used in combination with electrophysiological recordings, optogenetic techniques can be used to distinguish between action potentials of different classes of neocortical neurons by selectively triggering light evoked action potentials in only one type of cells, a method termed photostimulation-assisted identification of neuronal populations (Lima, Hromadka, Znamenskiy & Zador, 2009). These recently developed optogenetic tools have several advantages over classical techniques: can be targeted to specific neuronal populations, have millisecond temporal precision and can be used to both stimulate and inhibit neuronal activity.

Selectively stimulating raphe nuclei 5-HT neurons using optogenetioc tools has been shown to profoundly affect the spontaneous activity of a large proportion of neurons in the primary olfactory cortex (Lottem, Lörincz & Mainen, 2016). Specifically, the baseline activity of most neurons in the olfactory cortex is reduced in a rapid and transient manner, most neurons being affected by 5-HT photostimulation in less than 100 ms. Optogenetic stimulation of raphe nuclei neurons has been shown to suppress hippocampal ripple activity and inhibition of these neurons increased ripple activity (Wang et al., 2015). Thus, changes in the 5-HT concentration throughout the forebrain can also powerfully influence rhythmic cortical activity.

Optogenetic targeting of various neuromodulatory systems *in vivo* could lead to altered seizure dynamics, the effects being mediated by local and global neuromodulatory actions in the brain causing changes in network dynamics. Given the prominent effect of serotonergic stimulation on cortical activity, the effect on SWDs in animal models would be expected to be dramatic. This could be beneficial for two reasons. First, by changing 5-HTneuromodulatory tone, both globally (using somatic 5-HT neuron photostimulation in the raphe nuclei) and locally (photostimulating ChR2 expressing 5-HT fibers in various cortical and thalamic regions) one could gain new insights in the cellular and network mechanisms involved in the generation of SWDs. This is crucial information for developing more targeted medications. Second, since the effect of 5-HT is rapid ($<$ 100 ms; Lottem et al., 2016), this method could in theory be used for closed-loop seizure detection and ablation. Indeed, although the dominant frequency of SWDs in various animal models is 2-3 times higher than in humans, SWDs could be stopped after only one cycle (the interval between individual spikes of an SWD is $>$ 100 ms) if proper spike detection is used. We are using a closed loop system (Berenyi, Belluscio, Mao & Buzsaki, 2012) in which the first spike of an SWD is used to trigger a series of light flashes delivered from a laser connected to an optic fiber situated in close proximity of ChR2 expressing serotonergic neurons. This way, this simple closed loop system quickly interacts with the generating networks and blocks the seizure before its full blown manifestation. ChR2 can readily be expressed in DRN 5-HT neurons (Dugue et al., 2014; Lottem et al., 2016) and photostimulation, using very low light intensity, reliably evokes action potentials in serotonergic neurons with millisecond precision (Dugue et al., 2014) and our preliminary data (Fig. 1). 5-HT thus has the potential to alter ensemble activity in cortical and/or thalamic networks. Our preliminary data confirm the ability to powerfully affect neocortical neuronal activity as reflected in the suppression of individual neuron firing (Fig. 2).

The activity of unidentified DRN neurons (Lörincz et al., 2007) and identified DRN 5-HT neurons (Zhan et al., 2016) has been shown to be affected during seizures of various types, including SWDs. It has long been thought that, as a neuromodulator, 5-HT can have a sustained influence on its targets without temporal mod-

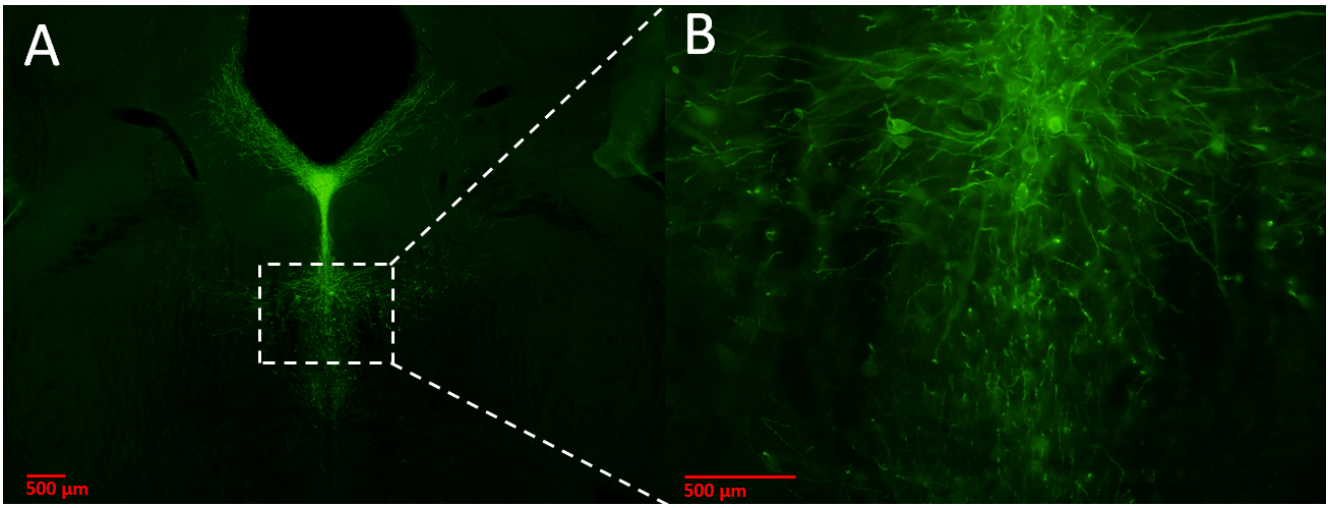

Figure 1: EYFP-ChR2 expression in the DRN of a Tph2-ChR2(H134R)-EYFP mouse (Zhao et al., 2011). (A) Low magnification epifluorescent image of a coronal brainstem section. The boxed area is shown in B. Aq: aqueduct.

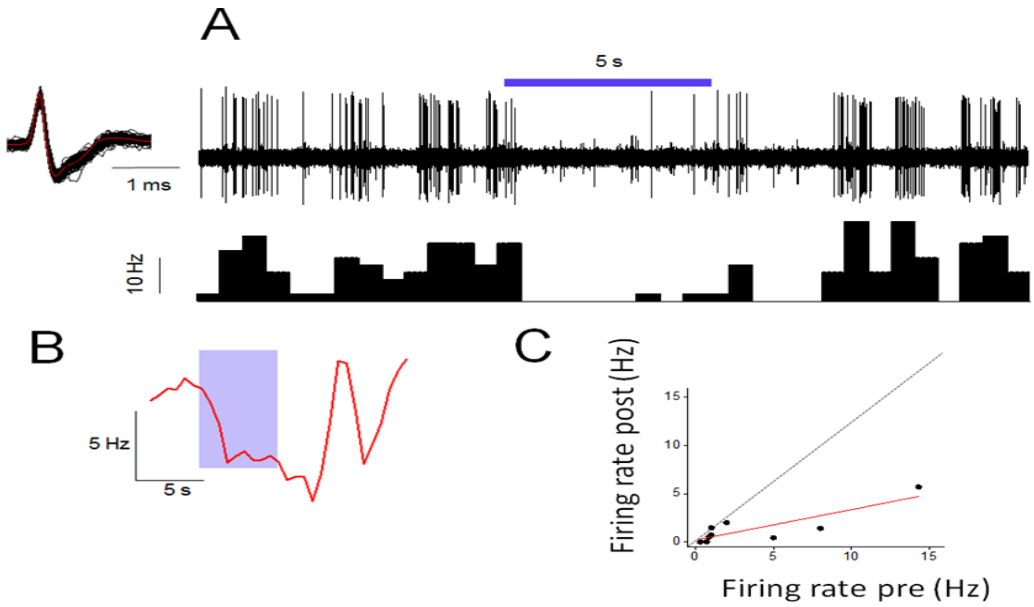

Figure 2: DRN 5-HT photostimulation results in a prominent suppression of neocortical baseline activity. (A) (Top) Example recording of the electrical activity of a neocortical neuron recorded in the motor cortex of an anesthetized mouse. 5-HT photostimulation (indicated by the horizontal blue bar) results in a rapid and prominent suppression of action potential firing. The inset shows the superimposed spikes, red trace is the averaged spike waveform. The blue bar marks the 5-HT photostimulation (train of 10 ms pulses at 10 Hz). (Bottom) Firing rate of the recorded neuron. (B) Peri stimulus time histogram of the neuron illustrated in (A). The photostimulation is illustrated by the blue bar. (C) Scatter plot comparing firing rates under control and photostimulated conditions for 9 recorded neurons. A linear regression fit is superimposed.

ulation. Interestingly, recently it has been shown, that the firing of some DRN neurons is also modulated at a faster timescale and can be phase-locked to specific behavioural events. Specifically, a subset of identified serotonergic neurons show phasic activation to reward predicting cues (Cohen, Amoroso & Uchida, 2015) and encode reward (Z. Liu et al., 2014), suggesting the importance of serotonergic system in guiding behaviour. Given the transient activation of DRN 5-HT neurons linked to specific behavioural effects and the ability of the 5-HT system to rapidly influence cortical electrical activity, the serotonergic system appears as an attractive neuromodulatory candidate to control pathological synchronous cortical electrical activity on a rapid timescale.

2 Conclusion

The findings reviewed here highlight an important role for 5-HT and its receptors, especially the 5-HT_{2C}Rs, in both pathologic neuronal excitability in epilepsy and comorbid affective disorders. The available literature suggests that antagonism at 5-HT_{2C}Rs might have beneficial effects on TLE patients, while their activation shows a clear anti-absence effect. These paradoxical anticonvulsant efficacy of 5-HT_{2C}R antagonists and agonists can be reconciled, taking into consideration that i) the two types of epilepsy have a different network substrate, ii) both agonism and antagonism induce 5-HT_{2C}R desensitization or downregulation (Graybiel, 2004), and/or iii) the existence of different populations of 5-HT_{2C}Rs with different signal transduction mechanisms. Moreover, the anti- versus pro-epileptic effects of the 5-HT_{2C}R activation might depend on the dose of the ligands used, with a pro-convulsive effects being present when the receptors are excessively activated.

Acknowledgements

Our work in this area is supported by the Malta Council of Science and Technology grant R&I-2013-14 (EPILEFREE) to GDG, AAT and VC. Hungarian Scientific Research Fund Grant NF 105083 and Hungarian Brain Research Program Grant KTIA_NAP_13-2-2014-0014 to MLL. SF, GO, RC, GD, FD were supported by fellowships funded by R&I-2013-14.

References

Applegate, C. D. & Tecott, L. H. (1998). Global increases in seizure susceptibility in mice lacking 5-HT_{2C} receptors: a behavioral analysis. *Exp Neurol*, *154*(2), 522–530.

Aravanis, A. M., Wang, L. P., Zhang, F., Meltzer, L. A., Mogri, M. Z., Schneider, M. B. & Deisseroth, K. (2007). An optical neural interface: *in vivo* control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J Neural Eng*, *4*(3), S143–56.

Azmitia, E. C. & Segal, M. (1978). An autoradiographic analysis of the different ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol. 179*(3), 641–667.

Bagdy, G., Kecskemeti, V., Riba, P. & Jakus, R. (2007). Serotonin and epilepsy. *J Neurochem*, *100*(4), 857–873.

Bai, X., Vestal, M., Berman, R., Negishi, M., Spann, M., Vega, C., ... Blumenfeld, H. (2010). Dynamic time course of typical childhood absence seizures: EEG, behavior, and functional magnetic resonance imaging. *J Neurosci*, *30*(17), 5884–5893.

Barnes, N. M. & Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology*, *38*(8), 1083–1152.

Bercovici, E., Cortez, M. A., Wang, X. & Snead, O. C. (2006). Serotonin depletion attenuates AY-9944-mediated atypical absence seizures. *Epilepsia*, *47*(2), 240–246.

Berenyi, A., Belluscio, M., Mao, D. & Buzsaki, G. (2012). Closed-loop control of epilepsy by transcranial electrical stimulation. *Science*, *337*(6095), 735–737.

Berg, A. T., Berkovic, S. F., Brodie, M. J., Buchhalter, J., Cross, J. H., van Emde Boas, W., ... Scheffer, I. E. (2010). Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia*, *51*(4), 676–685.

Bobillier, P., Seguin, S., Petitjean, F., Salvert, D., Touret, M. & Jouvet, M. (1976). The raphe nuclei of the cat brain stem: a topographical atlas of their efferent projections as revealed by autoradiography. *Brain Res*, *113*(3), 449–486.

Bonnycastle, D. D., Giarman, N. J. & Paasonen, M. K. (1957). Anticonvulsant compounds and 5-hydroxytryptamine in rat brain. *Br J Pharmacol Chemother*, *12*(2), 228–231.

Carter, M. E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., ... de Lecea, L. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat Neurosci*, *13*(12), 1526–1533.

Chapin, E. M. & Andrade, R. (2001). A 5-HT(7) receptor-mediated depolarization in the anterodorsal thalamus. II. Involvement of the hyperpolarization-activated current I(h). *J Pharmacol Exp Ther*, *297*(1), 403–409.

Cohen, J. Y., Amoroso, M. W. & Uchida, N. (2015). Serotonergic neurons signal reward and punishment on multiple timescales. *Elife*, *4*.

Cope, D. W., Di Giovanni, G., Fyson, S. J., Orban, G., Errington, A. C., Lörincz, M. L., ... Crunelli, V. (2009). Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. *Nat Med*, *15*(12), 1392–1398.

Coulon, P., Kanyshkova, T., Broicher, T., Munsch, T., Wettschureck, N., Seidenbecher, T., ... Budde, T. (2010). Activity Modes in Thalamocortical Relay Neurons are Modulated by G(q)/G(11) Family G-proteins - Serotonergic and Glutamatergic Signaling. *Front Cell Neurosci*, *4*, 132.

Crunelli, V. & Di Giovanni, G. (2015). Differential Control by Serotonin (5-HT) and 5-HT1A, 2A, 2C Receptors of Phasic and Tonic $GABA_A$ Inhibition in the Visual Thalamus. *CNS Neurosci Ther*, *21*(12), 967–970.

Crunelli, V. & Leresche, N. (2002). Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci*, *3*(5), 371–382.

D'Adamo, M. C., Servettini, I., Guglielmi, L., Di Matteo, V., Di Maio, R., Di Giovanni, G. & Pessia, M. (2013). 5-HT_2 receptors-mediated modulation of voltage-gated K^+ channels and neurophysiopathological correlates. *Exp. Brain Res. 230*(4), 453–462.

D'Amato, R. J., Largent, B. L., Snowman, A. M. & Snyder, S. H. (1987). Selective labeling of serotonin uptake sites in rat brain by [3H]citalopram contrasted to labeling of multiple sites by [3H]imipramine. *J Pharmacol Exp Ther*, *242*(1), 364–371.

Damjanoska, K. J., Muma, N. A., Zhang, Y., D'Souza, D. N., Garcia, F., Carrasco, G. A., ... Van De Kar, L. D. (2003). Neuroendocrine evidence that (S)-2-(chloro-5-fluoro-indol- l-yl)-1-methylethylamine fumarate (Ro 60-0175) is not a selective 5-hydroxytryptamine(2C) receptor agonist. *J Pharmacol Exp Ther*, *304*(3), 1209–1216.

Danober, L., Deransart, C., Depaulis, A., Vergnes, M. & Marescaux, C. (1998). Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog Neurobiol*, *55*(1), 27–57.

Depaulis, A., David, O. & Charpier, S. (2016). The genetic absence epilepsy rat from Strasbourg as a model to decipher the neuronal and network mechanisms of generalized idiopathic epilepsies. *J. Neurosci. Methods*, *260*, 159–174.

Descarries, L. & Mechawar, N. (2000). Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. *Prog Brain Res*, *125*, 27–47.

Descarries, L., Seguela, P. & Wakins, K. C. (1991). Nonjunctional relationships of monoamine axon terminals in the cerebral cortex of adult rat. In K. Fuxe & L. F. Agnati (Eds.), *Transmission in the brain: novel mechanisms for neural transmission* (pp. 53–62). New York: Raven Press.

Dewar, K. M., Reader, T. A., Grondin, L. & Descarries, L. (1991). [3H]paroxetine binding and serotonin content of rat and rabbit cortical areas, hippocampus, neostriatum, ventral mesencephalic tegmentum, and midbrain raphe nuclei region. *Synapse*, *9*(1), 14–26.

Di Giovanni, G. (2013). Serotonin in the pathophysiology and treatment of CNS disorders. *Exp. Brain Res. 230*(4), 371–373.

Di Giovanni, G., Di Matteo, V. & Esposito, E. (2008). *Serotonin–Dopamine Interaction: Experimental Evidence and Therapeutic Relevance*. Progress in Brain Research. Amsterdam: Elsevier.

Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. & Esposito, E. (2006). Central serotonin 2C receptor: From physiology to pathology. *Curr Top Med Chem*, *6*(18), 1909–1925.

Di Giovanni, G., Esposito, E. & Di Matteo, V. (2010). Role of serotonin in central dopamine dysfunction. *CNS Neurosci Ther*, *16*(3), 179–194.

Ding, Y. Q., Marklund, U., Yuan, W., Yin, J., Wegman, L., Ericson, J., ... Chen, Z. F. (2003). Lmx1b is essential for the development of serotonergic neurons. *Nat Neurosci*, *6*(9), 933–938.

Dugue, G. P., Lörincz, M. L., Lottem, E., Audero, E., Matias, S., Correia, P. A., ... Mainen, Z. F. (2014). Optogenetic recruitment of dorsal raphe serotonergic neurons acutely decreases mechanosensory responsivity in behaving mice. *PLoS One*, *9*(8), e105941.

Eban-Rothschild, A., Rothschild, G., Giardino, W. J., Jones, J. R. & de Lecea, L. (2016). VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. *Nat Neurosci*, *19*(10), 1356–1366.

Errington, A. C., Di Giovanni, G. & Crunelli, V. (2014). *Extrasynapitic $GABA_A$ Receptors*. The Receptors. Springer New York.

Errington, A. C., Gibson, K. M., Crunelli, V. & Cope, D. W. (2011). Aberrant $GABA_A$ receptor-mediated inhibition in cortico-thalamic networks of succinic semialdehyde dehydrogenase deficient mice. *PLoS One*, *6*(4), e19021.

Fletcher, A. & Higgins, G. A. (2011). Serotonin and reward-related behaviour: focus on 5-HT_{2C} receptors. In G. Di Giovanni, E. Esposito & V. Di Matteo (Eds.), *5-HT_{2C} Receptor Pathophysiology of CNS Disease* (pp. 293–324). The Receptors. New York: Springer.

Gaspar, P. & Lillesaar, C. (2012). Probing the diversity of serotonin neurons. *Philos Trans R Soc L. B Biol Sci*, *367*(1601), 2382–2394.

Ghanbari, R., El Mansari, M. & Blier, P. (2012). Electrophysiological impact of trazodone on the dopamine and norepinephrine systems in the rat brain. *Eur Neuropsychopharmacol*, *22*(7), 518–526.

Glauser, T. A., Cnaan, A., Shinnar, S., Hirtz, D. G., Dlugos, D., Masur, D., ... Adamson, P. C. (2010). Ethosuximide, valproic acid, and lamotrigine in childhood absence epilepsy. *N Engl J Med*, *362*(9), 790–799.

Gradinaru, V., Mogri, M., Thompson, K. R., Henderson, J. M. & Deisseroth, K. (2009). Optical deconstruction of parkinsonian neural circuitry. *Science*, *324*(5925), 354–359.

Graybiel, A. M. (2004). Network-level neuroplasticity in cortico-basal ganglia pathways. *Parkinsonism Relat. Disord*, *10*(5), 293–296.

Guiard, B. P. & Di Giovanni, G. (2015). Central Serotonin-2A (5-HT_{2A}) Receptor Dysfunction in Depression and Epilepsy: The Missing Link? *Front Pharmacol*, *6:46*.

Hale, M. W. & Lowry, C. A. (2011). Functional topography of midbrain and pontine serotonergic systems: implications for synaptic regulation of serotonergic circuits. *Psychopharmacology*, *213*(2-3), 243–264.

Hamandi, K., Salek-Haddadi, A., Laufs, H., Liston, A., Friston, K., Fish, D. R., ... Lemieux, L. (2006). EEG-fMRI of idiopathic and secondarily generalized epilepsies. *Neuroimage*, *31*(4), 1700–1710.

Heisler, L. K., Chu, H. M. & Tecott, L. H. (1998). Epilepsy and obesity in serotonin 5-HT_{2C} receptor mutant mice. *Ann N Y Acad Sci*, *861*, 74–78.

Higgins, G. A., Silenieks, L. B., Lau, W., de Lannoy, I. A., Lee, D. K., Izhakova, J., ... Fletcher, P. J. (2013). Evaluation of chemically diverse 5-HT_{2C} receptor agonists on behaviours motivated by food and nicotine and on side effect profiles. *Psychopharmacology*, *226*(3), 475–490.

Hioki, H., Nakamura, H., Ma, Y. F., Konno, M., Hayakawa, T., Nakamura, K. C., ... Kaneko, T. (2010). Vesicular glutamate transporter 3-expressing nonserotonergic projection neurons constitute a subregion in the rat midbrain raphe nuclei. *J Comp Neurol*, *518*(5), 668–686.

Holmes, M. D., Brown, M. & Tucker, D. M. (2004). Are "generalized" seizures truly generalized? Evidence of localized mesial frontal and frontopolar discharges in absence. *Epilepsia*, *45*(12), 1568–1579.

Hrdina, P. D., Foy, B., Hepner, A. & Summers, R. J. (1990). Antidepressant binding sites in brain: autoradiographic comparison of [3H]paroxetine and [3H]imipramine localization and relationship to serotonin transporter. *J Pharmacol Exp Ther*, *252*(1), 410–418.

Isaac, M. (2005). Serotonergic 5-HT_{2C} receptors as a potential therapeutic target for the design antiepileptic drugs. *Curr Top Med Chem*, *5*(1), 59–67.

Jacobs, B. L. & Azmitia, E. C. (1992). Structure and function of the brain serotonin system. *Physiol Rev*, *72*(1), 165–229.

Jakus, R. & Bagdy, G. (2011). The Role of 5-HT_{2C} Receptor in Epilepsy. In G. Di Giovanni, E. Esposito & V. Di Matteo (Eds.), *5-HT_{2C} Recept. Pathophysiol. CNS Dis.* (Vol. 22, pp. 429–444). The Receptors. Humana Press.

Jakus, R., Graf, M., Juhasz, G., Gerber, K., Levay, G., Halasz, P. & Bagdy, G. (2003). 5-HT_{2C} receptors inhibit and 5-HT_{1A} receptors activate the generation of spike-wave discharges in a genetic rat model of absence epilepsy. *Exp Neurol*, *184*(2), 964–972.

Kanner, A. M., Schachter, S. C., Barry, J. J., Hersdorffer, D. C., Mula, M., Trimble, M., ... Gilliam, F. (2012). Depression and epilepsy: Epidemiologic and neurobiologic perspectives that may explain their high comorbid occurrence. *Epilepsy Behav. 24*(2), 156–168.

Kiyasova, V., Fernandez, S. P., Laine, J., Stankovski, L., Muzerelle, A., Doly, S. & Gaspar, P. (2011). A genetically defined morphologically and functionally unique subset of 5-HT neurons in the mouse raphe nuclei. *J Neurosci*, *31*(8), 2756–2768.

Lima, S. Q., Hromadka, T., Znamenskiy, P. & Zador, A. M. (2009). PINP: a new method of tagging neuronal populations for identification during *in vivo* electrophysiological recording. *PLoS One*, *4*(7), e6099.

Liu, S., Bubar, M. J., Lanfranco, M. F., Hillman, G. R. & Cunningham, K. A. (2007). Serotonin2C receptor localization in GABA neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. *Neuroscience*, *146*(4), 1677–1688.

Liu, Z., Zhou, J., Li, Y., Hu, F., Lu, Y., Ma, M., ... Luo, M. (2014). Dorsal raphe neurons signal reward through 5-HT and glutamate. *Neuron*, *81*(6), 1360–1374.

Lörincz, M. L. & Adamantidis, A. R. (2017). Monoaminergic control of brain states and sensory processing: Existing knowledge and recent insights obtained with optogenetics. *Prog Neurobiol*, *151*, 237–253.

Lörincz, M. L., Olah, M., Baracskay, P., Szilagyi, N. & Juhasz, G. (2007). Propagation of spike and wave activity to the medial prefrontal cortex and dorsal raphe nucleus of WAG/Rij rats. *Physiol Behav*, *90*(2–3), 318–324.

Lottem, E., Lörincz, M. L. & Mainen, Z. F. (2016). Optogenetic Activation of Dorsal Raphe Serotonin Neurons Rapidly Inhibits Spontaneous But Not Odor-Evoked Activity in Olfactory Cortex. *J Neurosci*, *36*(1), 7–18.

Lowry, C. A., Hale, M. W., Evans, A. K., Heerkens, J., Staub, D. R., Gasser, P. J. & Shekhar, A. (2008). Serotonergic systems, anxiety, and affective disorder: focus on the dorsomedial part of the dorsal raphe nucleus. *Ann N Y Acad Sci*, *1148*, 86–94.

Lu, Y., Simpson, K. L., Weaver, K. J. & Lin, R. C. (2010). Coexpression of serotonin and nitric oxide in the raphe complex: cortical versus subcortical circuit. *Anat Rec*, *293*(11), 1954–1965.

Manning, J. P., Richards, D. A. & Bowery, N. G. (2003). Pharmacology of absence epilepsy. *Trends Pharmacol Sci*, *24*(10), 542–549.

Marescaux, C., Vergnes, M. & Depaulis, A. (1992a). Genetic absence epilepsy in rats from Strasbourg–a review. *J Neural Transm Suppl*, *35*, 37–69.

Marescaux, C., Vergnes, M. & Depaulis, A. (1992b). Neurotransmission in rats' spontaneous generalized nonconvulsive epilepsy. *Epilepsy Res. 8*, 335–343.

McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog Neurobiol*, *39*(4), 337–388.

McCormick, D. A. & Pape, H. C. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *J Physiol*, *431*, 291–318.

McCormick, D. A. & Wang, Z. (1991). Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *J Physiol*, *442*, 235–255.

Meuth, S. G., Aller, M. I., Munsch, T., Schuhmacher, T., Seidenbecher, T., Meuth, P., ... Budde, T. (2006). The contribution of TWIK-related acid-sensitive K^+-containing channels to the function of dorsal lateral geniculate thalamocortical relay neurons. *Mol Pharmacol*, *69*(4), 1468–1476.

Midzyanovskaya, I. S., Kuznetsova, G. D., van Luijtelaar, E. L., van Rijn, C. M., Tuomisto, L. & Macdonald, E. (2006). The brain 5HTergic response to an acute sound stress in rats with generalized (absence and audiogenic) epilepsy. *Brain Res Bull*, *69*(6), 631–638.

Millan, M. J., Marin, P., Bockaert, J. & la Cour, C. M. (2008). Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. *Trends Pharmacol Sci. 29*(9), 454–464.

Monti, J. M. (2010). The role of dorsal raphe nucleus serotonergic and non-serotonergic neurons, and of their receptors, in regulating waking and rapid eye movement (REM) sleep. *Sleep Med Rev*, *14*(5), 319–327.

Munsch, T., Freichel, M., Flockerzi, V. & Pape, H. C. (2003). Contribution of transient receptor potential channels to the control of GABA release from dendrites. *Proc Natl Acad Sci U S A*, *100*(26), 16065–16070.

Navailles, S., Lagiere, M., Le Moine, C. & De Deurwaerdere, P. (2013). Role of 5-HT_{2C} receptors in the enhancement of c-Fos expression induced by a 5-$HT_{2B/2C}$ inverse agonist and 5-HT_2 agonists in the rat basal ganglia. *Exp Brain Res*, *230*(4), 525–535.

Ohno, Y., Sofue, N., Imaoku, T., Morishita, E., Kumafuji, K., Sasa, M. & Serikawa, T. (2010). Serotonergic modulation of absence-like seizures in groggy rats: a novel rat model of absence epilepsy. *J Pharmacol Sci*, *114*(1), 99–105.

Orban, G., Bombardi, C., Marino Gammazza, A., Colangeli, R., Pierucci, M., Pomara, C., ... Di Giovanni, G. (2014). Role(s) of the 5-HT_{2C} receptor in the development of maximal dentate activation in the hippocampus of anesthetized rats. *CNS Neurosci Ther*, *20*(7), 651–661.

Orban, G., Pierucci, M., Benigno, A., Pessia, M., Galati, S., Valentino, M., ... Di Giovanni, G. (2013). High dose of 8-OH-DPAT decreases maximal dentate gyrus activation and facilitates granular cell plasticity *in vivo*. *Exp Brain Res*, *230*(4), 441–451.

Pape, H. C. & McCormick, D. A. (1989). Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. *Nature*, *340*(6236), 715–718.

Pinault, D., Leresche, N., Charpier, S., Deniau, J. M., Marescaux, C., Vergnes, M. & Crunelli, V. (1998). Intracellular recordings in thalamic neurones during spontaneous spike and wave discharges in rats with absence epilepsy. *J Physiol*, *509 (Pt 2)*, 449–456.

Polack, P. O., Guillemain, I., Hu, E., Deransart, C., Depaulis, A. & Charpier, S. (2007). Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci*, *27*(24), 6590–6599.

Popa, D., Lena, C., Fabre, V., Prenat, C., Gingrich, J., Escourrou, P., ... Adrien, J. (2005). Contribution of 5-HT_2 receptor subtypes to sleep-wakefulness and respiratory control, and functional adaptations in knock-out mice lacking 5-HT_{2A} receptors. *J Neurosci*, *25*(49), 11231–11238.

Puig, M. V., Celada, P., Diaz-Mataix, L. & Artigas, F. (2003). *In vivo* modulation of the activity of pyramidal neurons in the rat medial prefrontal cortex by 5-HT_{2A} receptors: relationship to thalamocortical afferents. *Cereb Cortex*, *13*(8), 870–882.

Saavedra, J. M. (1977). Distribution of Serotonin and Synthesizing Enzymes in Discrete Areas of Brain. *Fed. Proc. 36*(8), 2134–2141.

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J. & Scammell, T. E. (2010). Sleep state switching. *Neuron*, *68*(6), 1023–1042.

Siuciak, J. A., Chapin, D. S., McCarthy, S. A., Guanowsky, V., Brown, J., Chiang, P., ... Iredale, P. A. (2007). CP-809,101, a selective 5-HT_{2C} agonist, shows activity in animal models of antipsychotic activity. *Neuropharmacology*, *52*(2), 279–290.

Smidt, M. P. & van Hooft, J. A. (2013). Subset specification of central serotonergic neurons. *Front Cell Neurosci*, *7*, 200.

Soubrie, P., Reisine, T. D. & Glowinski, J. (1984). Functional aspects of serotonin transmission in the basal ganglia: a review and an *in vivo* approach using the push-pull cannula technique. *Neuroscience*, *13*(3), 605–625.

Steinbusch, H. W. (1984). Serotonin-immunoreactive neurons and their projections in the CNS. In T. K. M. J. Björklund A.; Hökfelt (Ed.), *Handb. Chem. Neuroanat. – Class. Transm. Transm. Recept. CNS Part II* (pp. 68–125). Amsterdam.

Steinbusch, H. W., Nieuwenhuys, R., Verhofstad, A. A. & der Kooy, D. (1981). The nucleus raphe dorsalis of the rat and its projection upon the caudatoputamen. A combined cytoarchitectonic, immunohistochemical and retrograde transport study. *J Physiol*, *77*(2-3), 157–174.

Steriade, M. M. & McCarley, R. W. (2005). *Brain Control of Wakefulness and Sleep*. Springer-Verlag US.

Svob Strac, D., Pivac, N., Smolders, I. J., Fogel, W. A., De Deurwaerdere, P. & Di Giovanni, G. (2016). Monoaminergic Mechanisms in Epilepsy May Offer Innovative Therapeutic Opportunity for Monoaminergic Multi-Target Drugs. *Front. Neurosci*, *10*(492).

Tecott, L. H., Sun, L. M., Akana, S. F., Strack, A. M., Lowenstein, D. H., Dallman, M. F. & Julius, D. (1995). Eating disorder and epilepsy in mice lacking 5-HT_{2C} serotonin receptors. *Nature*, *374*(6522), 542–546.

Thevenot, E., Cote, F., Colin, P., He, Y., Leblois, H., Perricaudet, M., ... Vodjdani, G. (2003). Targeting conditional gene modification into the serotonin neurons of the dorsal raphe nucleus by viral delivery of the Cre recombinase. *Mol Cell Neurosci*, *24*(1), 139–147.

Tokuda, S., Kuramoto, T., Tanaka, K., Kaneko, S., Takeuchi, I. K., Sasa, M. & Serikawa, T. (2007). The ataxic groggy rat has a missense mutation in the P/Q-type voltage-gated Ca2+ channel alpha1A subunit gene and exhibits absence seizures. *Brain Res*, *1133*(1), 168–177.

Trudeau, L. E. (2004). Glutamate co-transmission as an emerging concept in monoamine neuron function. *J Psychiatry Neurosci*, *29*(4), 296–310.

Umbriaco, D., Garcia, S., Beaulieu, C. & Descarries, L. (1995). Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1). *Hippocampus*, *5*(6), 605–620.

Upton, N., Stean, T., Middlemiss, D., Blackburn, T. & Kennett, G. (1998). Studies on the role of 5-HT_{2C} and 5-HT_{2B} receptors in regulating generalised seizure threshold in rodents. *Eur J Pharmacol*, *359*(1), 33–40.

Urbain, N., Creamer, K. & Debonnel, G. (2006). Electrophysiological diversity of the dorsal raphe cells across the sleep-wake cycle of the rat. *J Physiol*, *573*, 679–695.

Van Bockstaele, E. J., Biswas, A. & Pickel, V. M. (1993). Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res*, *624*(1–2), 188–198.

van der Kooy, D. & Hattori, T. (1980). Bilaterally situated dorsal raphe cell bodies have only unilateral forebrain projections in rat. *Brain Res*, *192*(2), 550–554.

Venzi, M., David, F., Bellet, J., Bombardi, C., Cavaccini, A., Di Giovanni, G. & Crunelli, V. (2016). Role of Serotonin 2A (5-HT_{2A}) and 2C (5-HT_{2C}) Receptors in Experimental Absence Seizures: An Electrophysiological and Immunohistochemical Study in GAERS and NEC Rats. *Neuropharmacology*, *108*, 292–304.

Wang, D. V., Yau, H. J., Broker, C. J., Tsou, J. H., Bonci, A. & Ikemoto, S. (2015). Mesopontine median raphe regulates hippocampal ripple oscillation and memory consolidation. *Nat Neurosci*, *18*(5), 728–735.

Williams, D. (1953). A study of thalamic and cortical rhythms in petit mal. *Brain*, *76*(1), 50–69.

Xu, M., Chung, S., Zhang, S., Zhong, P., Ma, C., Chang, W. C., ... Dan, Y. (2015). Basal forebrain circuit for sleep-wake control. *Nat Neurosci*, *18*(11), 1641–1647.

Zhan, Q., Buchanan, G. F., Motelow, J. E., Andrews, J., Vitkovskiy, P., Chen, W. C., ... Blumenfeld, H. (2016). Impaired Serotonergic Brainstem Function during and after Seizures. *J Neurosci*, *36*(9), 2711–2722.

Zhang, F., Prigge, M., Beyriere, F., Tsunoda, S. P., Mattis, J., Yizhar, O., ... Deisseroth, K. (2008). Red-shifted optogenetic excitation: a tool for fast neural control derived from Volvox carteri. *Nat Neurosci*, *11*(6), 631–633.

Zhang, F., Wang, L. P., Boyden, E. S. & Deisseroth, K. (2006). Channelrhodopsin-2 and optical control of excitable cells. *Nat Methods*, *3*(10), 785–792.

Zhao, S., Ting, J. T., Atallah, H. E., Qiu, L., Tan, J., Gloss, B., ... Feng, G. (2011). Cell type-specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. *Nat Methods*, *8*(9), 745–752.

Mangion, R. (2017). *Xjenza Online*, 5:15–20.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.02

Review Article

Legislative Entrenchment and Enforcement of Medical and Surgical Practice in Malta, 1801–1901

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Abstract. The late Maltese medical historian Dr Paul Cassar published his magnum opus on Malta's Medical History first in 1964, and is now well over 50 years old but still very valid for elaborations and re-evaluations on the subject-matter. This article is meant to re-visit Dr Cassar's research on medical and surgical practice in Malta (or the Maltese Islands, including Gozo) under the first century of British rule by way of an amplification of the relevant legal sources and literature, and by focusing on the role and function which legislation played as a means of entrenchment and enforcement of the system. It is intended to show that legislation as a social science is more than a document that enjoins principles and concepts, and has been an instrument of application and coercion in relation to vital human interest. This contribution will cover the first 100 years of British rule because in their course the foundations of the present-day medical and surgical system were prepared and laid.[1]

Keywords: legislation, codification, instrument vs. concept, entrenchment, enforcement, medical licence

1 Introduction

"Social science" is a major category of academic disciplines. It is concerned with the scientific and systematic study of the structure and functions of society, and the interplay among persons, individuals or groups (or bodies), within a community, either as a whole or in part. It ramifies into branches such as politics, economics, demography, geography and the rest. It widely comprises the humanistic fields of history and law, and certainly, a combination of both disciplines.[2]

History of law is divided into sub-divisions including history of legislation.[3] History confirms that since at least Aristotle, law including legislation, has two modes of operation: direction and coercion.[4] Both the historian and the lawyer - and the historian of law at that - must deal, characterise and classify legislation, as a type and category of law, according to a number of criteria but particularly pursuant to the level of entrenchment[5] and enforcement.[6] They are bound to deal with the success or failure of legislation (or better legal enactments by an individual or group or body), by tackling it in different contexts not only as a concept but as an instrument of observance and fulfilment.[7]

The late Dr Paul Cassar published Malta's medical history in 1964[8] at the peak of his career as medical historian and his masterpiece always topped his list of scholarly works that run into over 400 entries of his bib-

[1] The author would like to extend his gratitude to Professors Godfrey Baldacchino and Kevin Aquilina for their useful suggestions for the completion of this article.

[2] [1785] Thomas Adams, Letters, 10 September in *Works* (1854) IX, 450, used the term for the first time when he said "The social science will never be much improved, until the people unanimously know and consider themselves as the fountain of power."

[3] Another principal sub-division of legal history that complements legislative history is judicial history or the history of court judgements.

[4] Aristotle *Nicomachean Ethics*. X, 9: 1180a21–22, on the dual operation of law and as means of direction and coercion.

[5] "Entrenchment" in this article means the position at which a clause or provision is fortified or safeguarded against repeal or change in legislation, generally by subjection to sanction in the form of punishment or payment of damages.

[6] "Enforcement" in this article means the process of compelling observance or fulfilment of the law.

[7] See also John Finnis. *Natural Law, Natural Rights* (Oxford University Press, Oxford), X, 260–264, on the two modes of operation of law.

[8] See Paul Cassar. *Medical History of Malta* (Wellcome Historical Medical Library, London, 1963) 586pp. Chapter 47 is 465–496.

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liography.[9] He devotes chapter 47 to 'The Practice of Medicine & Surgery (in Malta)' which he opens with reference to the legal provisions that governed the subject matter from the advent of the Knights Hospitallers (or the Order of Saint John, or "Order", in Malta) in 1530 when they brought with them the *"pragmatiche"* (pragmatic or basic institutes of laws) of Rhodes to Malta.[10] This article utilizes Dr Cassar's retrieval of historical data on health legislation in the course of the first century of the British governance in Malta in order to better stress the extent to which the Maltese legislature or parliament applied entrenchment and enforcement to such a vital area of the legal system of Malta.[11]

2 British Rule

The British became rulers of Malta, *de facto* in 1800, *de jure* in 1814.[12] Governors as one-man sovereigns introduced and promulgated the first system of legislative enactments immediately after the British takeover of the islands in 1800 and some time before they established the first legislature in the form of a Council of Government in 1835.[13] The Knights Hospitallers (1530–1798) had published twice a form of organised collections of *"pragmatiche"* in 1724 (Granmaster De Vilhena's "Code") and 1784 (*Dritto Municipale* or [Grandmaster] De Rohan's "Code").[14] They left an unlimited corpus of edicts or proclamations called *'bandi'* which town-criers announced publicly on the village square but the Order left them in manuscript form until the Knights Hospitaller capitulated to the French troops of Napoleon Bonaparte in 1798.[15]

Remarkably, the Order in general and Grandmasters individually, regulated stringently, and enforced severely, the practice of medicine and surgery, so much so that they did so at a time when the greater part of the laws and legal systems were customary and unwritten and Roman Law - the *jus comune*[16] - was still performing the important function of a supplementary law.[17] The Knights Hospitallers embodied the relevant health provisions in "codifications" considered as statutes invested with a sort of "legal sanctity" that secured them against sudden and sweeping changes.[18] They lived in an era and context within which quacks and charlatans were notorious on the nearby European continent.[19]

The Knights Hospitallers as Heads of State in Malta, with zeal and careful observation obliged doctors to abide by the requirement that they, as practitioners had to report forthwith cases of bodily harm which they committed in the course of their profession, and as absolute one-man rulers-cum-legislators laid down the necessary prerequisites as fundamental criteria in the Code De Vilhena (1724), the supreme legislative framework of Malta at the time.[20]

Sir Alexander Ball, the first Civil Commissioner, at the very outset of British de facto rule (1800–1813) in Malta, enjoined surgeons to make strict observance of the current law, namely the "De Rohan Code" as it was already called,[21] which was set to remain in force for many years after the expulsion of the Order of Saint John and the concomitant establishment of British *de*

[9] See Mary Samut-Tagliaferro and Charles Savona-Ventura. *Dr Paul Cassar (1914–2006) A Biography* (University of Malta Library, 2008) 76pp, for a meticulously researched compilation of Dr Paul Cassar's works.

[10] Alfredo Mifsud. 'I Nostri Consoli e Le Arti e I Mestieri' in *Archivium Melitense, Bollettino della Società Storico-Scientifica Maltese*, III, October 1917, N.1, 40.

[11] See John Selden, *Laws England*, III. i. (1739) who stated "Against this danger he entrenches himself in an Act of Parliament", and John Locke, *An Essay concerning Humane Understanding*, II. xxxi. (1695) 150, who stated that "The Rewards and Punishments... which the Almighty has established as the enforcements of his Law".

[12] Patrick Staines. *Essays on Governing Malta (1800–1813)* (Publishers Enterprises Ltd, Malta 2008) 636pp, that thoroughly covers the period 1800–1813.

[13] Raymond Mangion. Minutes of the Council of Government of Malta 29 December 1835 to 13 August 1849, in *Documentary Sources of Maltese History, Part VI, Constitutional and Legislative Documents* (Malta University Press, Malta, 2009) xvii–xxv *passim*.

[14] Paolo Debono. *Sommario della Storia della Legislazione in Malta* (Tipografia del Malta, Malta, 1897) Capitolo XIV, 194–199, capo xv, 203–210.

[15] Eventually, a selection of *'Bandi'* from 1784 till the advent of formal British rule in 1813 was published in *Collezione di Bandi, Prammatiche ed altri Avvisi Ufficiali pubblicati dal Governo dell'Isola di Malta e Sue Dipendenze, Dal 17 Luglio 1784 al 4 Ottobre 1813* (Stamperia del Governo, Malta, 1840) l86pp.

[16] Giovanni Francesco Abela. *Della Descrittione di Malta Isola nel Mare Siciliano con le sue Antichita ed altre Notitie* (Paolo Bonacorta, Malta, 1647), Libro Quarto, Notitia I, 429. He refers to the *capitolo* or petition of 1458 which the Maltese submitted to the Viceroy of Sicily so that the private law of matrimony in Malta would be governed *secundu jura comuni* and the local custom would be invalid if it were in *contrarium jura comuni*. This is the oldest documented reference to the application of the *jus comune* in Malta.

[17] Hugh Harding, 'Law', in Henry Frendo and Oliver Friggieri (eds), *Malta, Culture and Identity* (Grima Publishing Ltd, Malta, 1994) 211. Roman Law was not the pure Roman Law of Justinian (527–565 A.D.) but Roman Law as modified by the treatises and comments of influential writers as well as by judgments delivered by various continental courts, particularly the Rota Romana.

[18] Albert Venn Dicey. *Lectures on the Relation between Law and Public Opinion in England during the Nineteenth Century* (MacMilland and Co. Ltd, London, 1926) 7, Lecture 1, on the "sanctity" of codifications.

[19] The Library Manuscripts section of the National Library, Valletta, holds several unpaginated manuscripts like Ms. 251–252, 756, 1173 and others that have put on record cases of quacks and charlatans in Malta in the course of the eighteenth century.

[20] *Leggi e Costituzioni Prammaticali, Rinuovate, Riformate ed Ampiate* (Giovanni Andrea Benvenuto, Malta 1724) Titolo 15 - Del Protomedico, 73–76.

[21] *Del Dritto Municipale di Malta, Nuova Compilazione con diverse altre Constituzioni Publicati dal Governo dell'Isola di Malta e Sue Dipendenze Dal 17 Luglio 1784 al 4 Ottubre 1813* (Government Printing Press, Malta 1784), Titolo VI, para ii.

jure rule (1814–1964).[22] In point of fact, the British superseded the Grandmaster's municipal legal tome soon after they laid the foundations of the Police and Criminal Codes in the middle of the nineteenth century.[23] Sir Alexander immediately took steps to ensure the cautious and methodical enforcement of the contemporary written and unwritten laws in the course of the period of transition from the previous rule.[24] He proclaimed that persons engaged in medical (and surgical) practice without a licence were to be severely fined.[25]

In 1813, the British ushered in the printing of the 'Malta Government Gazette' that would contain the texts of legislative enactments.[26] The average person was since then presumed to know the law soon after it was promulgated.[27] London sent Royal Commissioners under the aegis of Civil Commissioner Hildebrand Oakes to enquire into the state of affairs of Malta and they did speak in the sense that they adhered to the compilation of laws such as the Code De Rohan, which they deemed to be more or less a replication of the Code De Vilhena, by which the greater part of the European mainland continued to be governed.[28]

The British rulers certainly evidenced that they were not only well aware but felt constrained to proceed with giving legal profile, by way of codified and primary legislation, to the practice of medicine and surgery.[29] They did not limit themselves to policy, praxis or custom, to ensure that the profession was seriously carried out but in the footsteps of the Knights Hospitallers resorted to stringent enforcement by virtue of legislation to secure legal obedience.[30]

The first Governor, Sir Thomas Maitland, opted for an gubernatorial type of rule and, thus, defied the constitutional "Instructions" to set up an Advisory Council by issuing forthwith instead a Government Notice to clarify that during his "autocratic" reign, a surgeon, physician, apothecary as well as a midwife had to apply for a licence to exercise their profession directly to him, and had to endorse such a submission by a certificate bearing the signature of the Physician-in-Chief.[31] Appreciably, "King Tom" in his capacity of Head of State personally issued, if not crafted, a Government Notice which meant that he exploited, out of his own freewill, the highest legislative action at the time given that he never constituted and composed an Advisory Council, let alone a Legislature.[32]

Sir Thomas did not stop arrogating to himself the ultimate decisions relevant to medical and surgical practice, because he carried on by brushing aside the Physician-in-Chief while granting a Medical Board, which he himself had appointed, the authority to issue warrants to practitioners.[33] He manifested that the best enforcement of health was possible only by subjecting its administration ultimately to himself as the highest political and legislative power, although he did so responsibly through the technical monitoring and recommendation of the most qualified group of experts in the field.[34]

In other words, the rulers and experts, who were connected with the political and technical decision-taking processes as far as medicine and surgery were concerned, followed suit in the first half century of British rule in Malta, whether the Governor constituted a one-man suzerain or sat in the Council of Government along with

[22] *Proclamazione* 18.7.1797 in *Collezione di Bandi, Prammatiche ed altri Avvizi Ufficiali* (Stamperia del Governo, Malta 1840) 41–43.

[23] See also: Albert Ganado. 'Storja tal-Leġislazzjoni ta' Malta' in Toni Cortis (ed.) *Oqsma tal-Kultura Maltija* (Gutenberg Press, Malta, 1991) 254

[24] *Proclamazione* 15.7.1800 in *Collezione di Bandi, Prammatiche ed altri Avvizi Ufficiali* (Stamperia del Governo, Malta 1840) 56 (*Del Dritto Municipale*, Articolo 28, Libro 7, Capitolo 6).

[25] *Proclamazione* 1.11.1799 in *Collezione di Bandi, Prammatiche ed altri Avvizi Ufficiali* (Stamperia del Governo, Malta 1840) 53.

[26] The *"Giornale di Malta"* was issued between 7th January, 1812, and 20th October, 1813, then the *"Gazzetta del Governo di Malta"* (GGM) from 27th October, 1813 to 31st July, 1816, and finally the *"Malta Goverment Gazette"* (MGG) as from 7th August, 1816.

[27] *Parliamentary Debates, Legislative Assembly, Session, 1924–27* (G.P.O, Malta, 1928), Volumes 12 and 13, Tuesday, 9th March, 1926, 5273–5274. Dr Giuseppe Micallef LL.D., as a member of the Maltese Legislature, made an interesting reference during a debate in the Legislative Assembly when a one-article bill was introduced to impose a short but general moratorium on the effects of future enactments. He explained that *"ignorantia juris neminem excusat" "(no excuse for ignorance of the law)"* was a true legal maxim, but he reminded that Maltese laws of yore used to be called *"bandi"*, because the town or village-crier would announce them three times on the main public squares in the presence of the town or country-folk as soon as they were decreed.

[28] The Royal Commissioners' findings are in Hildebrand Oakes, William A'Court and John Burrows, *Report of His Majesty's Commissioners For enquiring into the affairs of Malta, Malta 30 August 1812*, 259pp.

[29] N(ational) A(rchives) R(abat) M(alta), Despatches (Duplicates) 1/2/14, 226r–227r. Sir Frederick Hankey, Chief Secretary to Governor, explained in a Memorandum annexed to a Report dated 26th January 1836 on the state of the laws in Malta that it was expected that the promulgation of the "new Codes of Law" would bring about beneficial results in perspective, certainty and lasting character of the law on the lines of the then European Codes.

[30] See also Hugh Harding. *Maltese Legal History under British Rule (1901–1836)* (Progress Press Ltd, Malta, 1968) 61 *passim.*

[31] GGM 36, Mercoledì 29.6.1814, *Notificazione* 18.6.1814, 143

[32] John Joseph Cremona. *The Maltese Constitutions and Constitutional History Since 1813* (PEG Ltd, Malta) 2. He states that the Advisory Council turned out to be a still-born.

[33] MGG 387, Wednesday 28.3.1821, Minute, 3.1821, 2567.

[34] John Joseph Cremona. *Malta and Britain, The Early Constitutions* (Publishers Enterprises Group Ltd, Malta, 1996) 35. Indeed, Maitland remained the sole legislature in Malta but it was at times unclear which of the official notifications issued by him were of a legislative nature.

official and elected councillors. Remarkably, those at the behest of law and policy all assumed a tight and adamant method towards strict and efficacious legislative enforcement as with the practice of medicine and surgery, as their period coincided with a rational and philosophical approach to disease and a cumulus of landmark achievements in the fields of cure and therapy.[35]

Local scientists and doctors in line with advances overseas, registered significant progress in various areas of medicine and surgery as well as in the use of clinical instruments and plastic appliances,[36] operating theatres,[37] and laboratory work such as microscopic and bacteriological analyses.[38] Over and above, the British rulers embarked on the building of the General Central Hospital at Floriana, the Lunatic Asylum (later the Mental Diseases Hospital, now the Mount Carmel Hospital) at Ħ'Attard, and the Saint Vincent De Paule Hospital at Ħal Luqa-Ħal Qormi.[39]

3 Entrenchment and Enforcement by Codification

In the middle of the nineteenth century, the authorities were still bringing the law to bear very effectively in terms of the De Rohan Code until the end of its operation especially with reference to foreigners who tinkered with medicine and surgery without a licence, did not report cases of negligence in the course of the exercise of their profession, and came in for censure. The Council of Government evidently assured that a fine would be imposed on any physician or surgeon who disclosed any secret obtained in confidence by him – yes, no woman was a doctor at the time.[40] It gave full recognition to the notion of the inviolate nature of professional secrecy among medical and surgical practitioners by attributing enforcement to it by way of penal sanction.[41]

In 1854, the Governor promulgated the Code of Police Laws (or Police Code), along with the (substantive, penal and procedural) Criminal Code (or the "Penal Code"), as the first in a series of massive codifications and quasi-codifications.[42] He brought into effect two major enactments of the Council of Government of the first elected minority which meant that he sealed the whole legislature's decision to substitute the sections of the De Rohan Code, or last digest of laws under the Order, by engrafting the germane dispositions into the Police Code (Chapter XIV).[43]

The Council of Government, thus, drew on primary legislation, or written law fashioned by the most qualified technical advisors and approved by the highest legislature or legislative process, but enshrined the mechanisms of enforcement within the highest consolidated systems of laws of general and binding character, or more specifically, endorsed them as collections of laws arranged by way of chapterisation, and substantively compartmentalised them according to distinct themes subject to penalties or payment of damages in case of infringement or non-observance.[44]

No doubt, the British gave a humane character to the application of punishment with regard to medical and surgical persons who caused the death of a patient. They were influenced by the humanitarianism that prevailed throughout the continent and Europe in general throughout the nineteenth century to the extent of limiting the death penalty only to the most wilfully perpetrated crimes under the Criminal Code (1854).[45] They left the penalty of suspension from the exercise of the profession solely to apply to situations where medical and surgical interventions procured abortion.[46] However, they extended the liability of a practitioner to damages for negligence and imprudence in the treatment of his patients to the Civil Code.[47]

At the same time, the Governor through the Legislature or Council of Government continued to tighten up the process leading to medical and surgical practice in Malta by amending the Code of Police Laws.[48] In 1894, the Government adopted legislative measures with retroactive effect as from 1886 so that physicians and

[35] Dr Tommaso Chetcuti, *Discorso Inaugurale Pronunciato nella Tornata Pubblica Del 16 Ottobre All'Apertura Dell'Anno Accademico 1846–47 Nella Società Medica D'Incoraggiamento* (Tipografia di Filippo Izzo, Malta, 1846) 3–25.

[36] *Storia della Società Medica d'Incoraggiamento* (Tipografia di F.Izzo, Malta 1845) xiv–xli.

[37] Dr Ludovico Bernard. *Sull'Utilità e Necessità della Chirurgia, Discorso Pronunziato Nella Tornata Pubblica De' 28 April 1865* (Tipografia Anglo-Maltese, Malta 1866) 18.

[38] See, for instance, Salvatore L.Pisani, *Report on the Cholera Epidemic in the year 1887* (Malta 1888) 6–18.

[39] Richard Micallef. *Origin and Progress of the Government Charitable Institutions in Malta and Gozo* (Malta, Malta 1901) 18–24.

[40] Albert E. Abela. *Grace and Glory. Malta: People, Places and Events* (Progress Press, Valletta, 1997) 94–95. Blanche Huber was the first woman to graduate doctor of medicine from the University of Malta in 1925.

[41] *Leggi Criminali per L'Isola di Malta e Sue Dipendenze* (Tipografia di Paolo Cumbo, Malta 1854), Capitolo X, Articolo 245.

[42] Originally, the Criminal Code and the Code of Police Laws were meant to be one Codex but they were separated as two independent codifications before their promulgation.

[43] *Leggi e Regolamenti Di Polizia per L'Isola di Malta e Sue Dipendenze* (Tipografia di Paolo Cumbo, Malta 1854) 13–14.

[44] NARM, C(hief) S(ecretary) (to) G(overnor) (Files), 3561/1853.

[45] Andrew Jameson. *Report on the Proposed Code of Criminal Laws for the Island of Malta and its Dependencies* (Government Press, Malta, 1844) 1–21 (Introduction) where he explicates in detail to what extent the proposed (Substantive, Penal and Procedural) Criminal Laws were given a 'humane' treatment.

[46] *Leggi Criminali per L'Isola di Malta e Sue Dipendenze* (Tipografia di Paolo Cumbo, Malta, 1854), Capitolo XII, Articolo 257.

[47] *Leggi Criminali per L'Isola di Malta e Sue Dipendenze* (Tipografia di Paolo Cumbo, Malta, 1854), Capitolo XII, Articolo 239.

[48] *Leggi di Polizia per L'Isola di Malta e Sue Dipendenze* (Government Printing Office, Malta, 1872) Capitolo XIV, 41.

surgeons would not be licensed to practise their profession unless they had trained in medicine and surgery for at least a year after passing the final examinations for obtaining the necessary degree (M.D.).[49]

4 The "Sanitary" Ordinances

At the turn of the twentieth century, the political-cum-legislative powers-that-be proceeded with regulating scrupulously the system by giving a legislative character to its machineries of application. Rather, the Council of Government enacted a series of so-called "Sanitary Ordinances", four in all, to give a statutory character of the primary level to the system in general, and mark a period during which the foundations of future medical and surgical legislation were laid.[50] The highest legislative process composed of the Head of State and a mixture of elected and official representatives of the people, discussed and approved legislative enactments that placed the medical and surgical profession as well as the relevant Government departments on a more solid organisation.[51]

In 1901, the Council of Government enacted Ordinances VII[52] and XVII[53] to lay down that any person who wished to obtain a warrant to practise as medical doctor or surgeon had to satisfy the Medical Board that he was a British subject, had attained the age of 21 years, was of a good character and possessed the degree of Doctor of Medicine and Surgery of the Royal University of Malta.[54] Other applicants who held a qualification from another academic institution had to be interrogated by the Medical Board. Those who were provided with a warrant from the Malta Government were eligible for registration under the Medical Register of the United Kingdom.[55] In 1901, the Council of Government approved an enactment to extend effectively the UK Medical Act of 1886[56] and, thus, establish reciprocity between Britain and Malta concerning the most vital profession in the world.[57]

The Governor was empowered on the advice of the Medical Board, by virtue of the Ordinances of 1901, to issue a temporary licence to a foreign physician or surgeon. The Council of Government debated such a provision that was meant to afford the necessary facilities to the local public to consult specialists from overseas. The Government sought not only politically but certainly legally to bind itself with respect to the enforcement of such a measure subject to technical consultation on the strength of primary legislation. The Government reiterated and confirmed its predisposition and commitment to resort to the highest statutory and legal instrument other than mere policy for the purpose of honouring its obligation towards entrenchment and coercion of the most qualitative medical and surgical practice.

In 1901, the Government revised and modified the laws governing the medical and surgical professions to ensure that they suited modern requirements. It rendered definite and explicit the principle of the co-relation of medicine and surgery but of their independence from pharmacy.[58] However, it did not stop there. It amended the Criminal Code such that the law would contemplate other crimes in relation to medical persons who were attended by the disqualification to practise, and the relevant penalties involved capital punishment or hard labour.[59]

The then Crown Advocate Sir Giuseppe Carbone insisted that the provision concerned had to be inserted notwithstanding the fact that no case had ever occurred which vindicated the inclusion of the clause in the Criminal Code, or otherwise the "Penal Code". The Crown Advocate argued that it was the duty of the legislator to foresee that he did not only lay down the law but codified it to render it enforceable as best he could even before any such instances actually took place.[60] The Government through the Legislature incorporated the mechanisms of enforcement by way of the most stringent punitive sanctions.

5 Conclusion

In 1901, the Government and the Legislature enacted and promulgated the "Sanitary Ordinances" to lay the foundations of a future well-structured legislation and system governing medical and surgical practice in Malta. However, the British rulers in line with their predecessors, the Order of Saint John, had already tried their utmost to subject the profession of the *"Magnificus et Excellens Doctor"*, who had always enjoyed a position

[49] *Leggi di Polizia per L'Isola di Malta e Sue Dipendenze* (Government Printing Office, Malta, 1894) Capitolo XIV, 40.

[50] See also Raymond Mangion, *Constitutions and Legislation in Malta* (Russell Square Publishing, London 2016).

[51] See University of Malta, Faculty of Laws, Adrian Cutajar, *The Four Sanitary Ordinances 1900–1908, A History 1900–2000*, LL.D. thesis 2003, 146pp.

[52] D(ebates) (of the) C(ouncil) (of) G(overnment), Session 1899–1900, Sitting 23, Wednesday 14.3.1900, Volume XXIV, 1011.

[53] DCG, Session 1899–1900, Sitting 36, Wednesday 19.6.1901, Volume XXV, 1190–1191.

[54] MGG 4363, Thursday 30.5.1901, Supplement, i–xi; MGG 4376, Tuesday 2.7.1901, i–xii.

[55] See also MGG 10326, Wednesday 3.6.1953, Supplement 3.6.1953, 709. ACT VIII of 1953.

[56] 49 & 50 Victoria, Chapter 48.

[57] MGG 4405, Monday 7.10.1901, 850–851.

[58] See also NARM, CSG, 726/1900, on the restructuring of the medical profession and the grant of power to the Medical Board to decide on the issue of warrants to practise according to set criteria.

[59] *Leggi Criminali per L'Isola di Malta e Sue Dipendenze* (Tipografia di Paolo Cumbo, Malta, 1854), Capitolo XII, Articolo 239.

[60] DCG, Session 1899–1900, Sitting 14, Wednesday 10.1.1900, Volume XXIV, 527–566.

of trust and respect in the country as elsewhere, to the highest levels of entrenchment and enforcement by virtue of the Police and Criminal Codes that the British had promulgated as part of the massive legislative codifications of the nineteenth and twentieth century.

The British rulers, whether of their sole disposition and personal management in an autocratic role or jointly with a legislative body, all-nominated or partly elected, in the course of the first 100 years from their takeover of Malta's sovereignty, showed the extent to which they intimately comprehended the ropes of legislative entrenchment and enforcement as far as medical and surgical practice was concerned. They did so by ensuring that they conferred the necessary licence to the properly qualified persons.

The British were legislatively so strict in safeguarding medical and surgical practice from all kinds of unlawfulness that they drew the attention of Malta's judicature. Legal and medical historians have often cited the case *La Polizia v. Giovanni Salunto* per judge Luigi Camilleri (Preziosi) of 8th April 1922, as a leading judicial pronouncement to the effect that a person does not need to make use habitually of the unlawful exercise of medical or surgical practice and no purpose of gain is necessary for him because the medical and surgical profession and the laws that govern it have the "noble and elevated aim" to protect from harm every single individual as well as public health in general.[61]

This article has looked, from the perspective of law, at a chapter of Dr Paul Cassar's book on medical history of Malta where he deals with the development of legislation governing the issue of medical and surgical licence in the first century of British rule in Malta, 1801–1901. It was written with the aim to render more explicit and emphatic Dr Cassar's reference to the status and quality of legislative enactments as legal instruments of entrenchment (or level of protection against change or repeal) and enforcement (or extant of application by way of force) under this first part of British rule in Malta.

[61] *Collezione di Decisioni delle Corti Superiori dell'Isola di Malta* (Stamperia del Governo, Malta, 193–), Vol. xxv, Parte Quarta, Appello Criminale, 914–917. See also the Police v. Lorenzo Mifsud determined by judge William Harding (2.12.1939) in William Harding, *Recent Criminal Cases Annotated* (Malta, Lux Press, 1943) 176–178. *"...non solo per il fatto che la azione curativa direttamente esercitata sul paziente può apportagli nocumento, ma ancora perchè l'empirico guadagnando la fiducia dei malati alle sue strane operazione fa loro trascurare qualsisasi elementare principio di cura razionale..."*.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.03

Research Article

Matching Biological Motion Across Viewpoints

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Abstract. There has been much debate as to how objects can be recognized across viewpoint changes. Here we ask whether viewpoint changes affect performance when participants make judgements about human actions depicted as point-light stimuli. Previous research has suggested that bodies may be "special" objects and may thus be immune to such viewpoint costs. We used a concurrent matching task in which three dynamic point-light figures performed familiar actions taken from a standard biological motion database. On each trial the action performed by the central "target" figure was also performed by one of the two flanking figures. The task was to make a speeded left/right response to indicate which flanker was copying the target. Separate, random depth orientations were assigned to the two flanking figures and the target could either have the same orientation or appear with an offset of 45° or 90° relative to the matching flanker. The starting animation frame was randomly chosen for each of the three figures. We found that viewpoint differences between the target and matching flanker affected both speed and accuracy. This indicates that the recognition of human bodies depicted as biological motion stimuli is viewpoint-dependent, as with many other types of object. We also suggest that concurrent matching is a flexible tool for exploring biological motion as decisions can be made on a variety of actions without the need for explicit action-naming or training.

Keywords: Biological Motion, Action Understanding, Object Recognition, Viewpoint Dependence, Concurrent Matching, Object Constancy

1 Introduction

Helping us perceive and understand the actions of other people is a primary function of the human visual system. Vision allows us to adaptively interact with others in our own social environment and to comprehend the meaning, goals and intentions behind behaviours we see from afar (Johnson & Shiffrar, 2012; Knoblich, Thornton, Grosjean & Shiffrar, 2006). The importance of action understanding is reflected in the wide range of brain areas and brain networks that become activated whenever we watch other people behave (Downing & Peelen, 2011; Giese & Poggio, 2003; Grossman & Blake, 2002; Peuskens, Vanrie, Verfaillie & Orban, 2005; Rizzolatti, Fogassi & Gallese, 2001; Saygin, 2007; Thompson & Parasuraman, 2012).

One particular research area has emphasised how *movement* of our bodies, rather than their form (e.g., size or shape), is important for action understanding. This field of "biological motion" research dates back to the classic work of Gunnar Johansson, who first demonstrated the way in which dynamic point-light figures could be used in an experimental setting (Johansson, 1973, 1976; Marey, 1895, see Fig. 1). Point-light stimuli – where movement is conveyed by the relative motions of a small number of bright markers located on the head and the principal joints (i.e., shoulders, elbows, wrists, hips, knees, ankles) – remains the most popular technique for isolating the dynamic aspects of action and is the method we also use in the current work (Blake & Shiffrar, 2007; for a review of the point-light technique, see Thornton, 2006). What is most salient about these stimuli is the fact that when they are viewed as static images, they appear to the naïve observer only as a collection of random dots. However, when set in motion the underlying human behaviour is immediately revealed.

Johansson's original stimuli were created by filming actors in low-lighting conditions while they were wearing light sources attached to their joints. Later techniques included computer simulations (Cutting, 1978),

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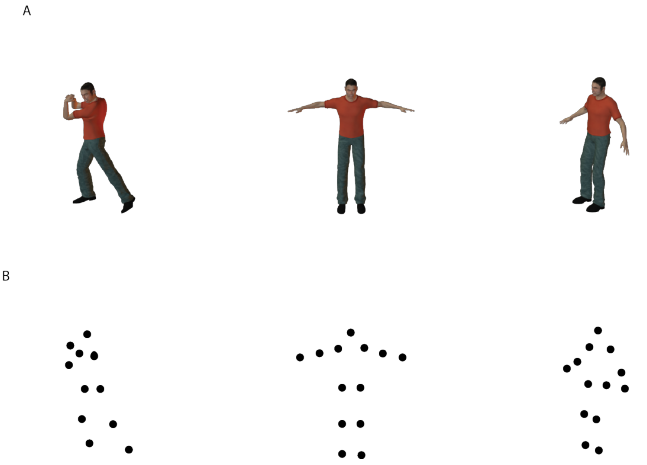

Figure 1: Concurrent Matching Task. A) Three example actions. B) Point-light depictions of the same actions, as shown in the current experiment. On each trial, the task was to decide if the left or right flanking figure performed the same action as the central target, irrespective of difference in depth orientation and action cycle.

identifying joints within each frame of a video sequence (Ahlström, Blake & Ahlström, 1997) and now most commonly purpose-built motion capture systems (Dekeyser, Verfaillie & Vanrie, 2002; Giese & Poggio, 2003; Ilg, Bakir, Mezger & Giese, 2004; Manera, Schouten, Becchio, Bara & Verfaillie, 2010; Troje, 2002; Vanrie & Verfaillie, 2004). This latter technique – made popular by movies such as The Lord of the Rings and Beowulf – has a number of advantages, but in particular records the position of each point as a 3D coordinate. This means that actions can be replayed from any viewpoint, a feature we exploit in the current work, as described shortly.

Regardless of how the stimuli are created, point-light figures share the characteristic of rapidly conveying a range of information about the underlying action. Not only can naïve participants quickly report that the figure represents a human actor, they can usually identify the action (Dittrich, 1993; Hemeren, 2008), and also extract a range of other characteristics, such as the gender (Kozlowski & Cutting, 1977; Pollick, Kay, Heim & Stringer, 2005) and emotional state (Atkinson, Dittrich, Gemmell & Young, 2004; Dittrich, Troscianko, Lea & Morgan, 1996; Pollick, Paterson, Bruderlin & Sanford, 2001) of the actor. The ability to perceive biological motion with point-light figures appears to be robust against a number of manipulations, such as masking with additional "noise" dots (Bertenthal & Pinto, 1994; Cutting, Moore & Morrison, 1988), spatial and temporal degradation (Beintema & Lappe, 2002; Thornton, Pinto & Shiffrar, 1998; Thurman & Grossman, 2008) and large changes in apparent distance (Thornton, Wootton & Pedmanson, 2014). The rapid and robust processing of point-light stimuli has led to suggestions that both passive, bottom-up mechanisms (Bosbach, Prinz & Kerzel, 2004; Johansson, 1973, 1976; Mather, Radford & West, 1992; Thornton & Vuong, 2004) and active, top-down mechanisms (Bertenthal & Pinto, 1994; Chandrasekaran, Turner, Bülthoff & Thornton, 2010; Thompson & Parasuraman, 2012; Thornton, Rensink & Shiffrar, 2002) are brought to bear by the visual system in order to solve the problem of action understanding.

In the current paper, there were two main objectives. First, we wanted to further develop a novel matching task for biological motion that we had previously introduced as a new method to study the perception of action at extreme distances (Thornton et al., 2014). On each trial of this task, three point-light figures are presented, one central target and two flanking figures. The two flanking figures always perform different actions, and the target figure copies one of these actions (see Fig. 1). The participant's task is simply to report whether the left or right flanker matches the target. The task is flexible because the nature of actions displayed (e.g., novel or familiar) and the characteristics of the figures (e.g., relative size, step-cycle, intact or scrambled) can all be manipulated independently without affecting the basic response demands. Participants always simply have to match the left or right flanker.

Here, we show how the task can be easily adapted to answer other biological motion research questions. Specifically, our second objective was to explore the effect that *viewpoint* has on the recognition speed and accuracy of a range of human actions. In the current study, we focus on viewpoint change involving a rotation in depth around the vertical axis. To take walking as an example, rotations in depth would vary whether the figure appears to be moving to the left, right or towards an observer. Within the context of our matching task, in the current study we systematically varied the viewpoint difference between the target and flanking figures.

There have been many previous studies of biological motion that have examined picture plane rotation – turning the figures upside down (e.g., Bertenthal & Pinto, 1994; Hemeren, 2008; Pavlova & Sokolov, 2000, 2003; Sumi, 1984; Troje, 2003). Indeed, perceptual difficulty in processing such "inverted" point-light stimuli – as well as similar manipulations using other forms of body representations (Reed, Stone, Bozova & Tanaka, 2003; Slaughter, Stone & Reed, 2004) – is often cited as one of the main lines of evidence that the human body is "special", similar to claims for holistic processing of faces (Diamond & Carey, 1986; Rhodes, Brake, Taylor & Tan, 1989; Richler, Mack, Gauthier & Palmeri, 2009; Rossion, 2008; Van Belle, De Graef, Verfaillie, Busigny & Rossion, 2010; Yin, 1969; Young, Hellawell & Hay, 1987). Although some caution has been urged in us-

ing picture plane rotation as a "gold standard" for global processing of biological motion (Pinto & Shiffrar, 2009; Troje & Westhoff, 2006), it remains by far the most common form of viewpoint manipulation. As we note shortly, far fewer studies have examined viewpoint changes in depth, and here we try to link this manipulation more directly to the object recognition literature. Specifically we explore the nature of *object constancy* in the context of human point-light actions other than walking.

Object constancy refers to the ability to recognise an object despite spatial transformations (i.e., changes in orientation, position in the space, size) that give rise to large variations in the image that is projected onto the retina (Graf, 2006; Lawson, 1999). Such variations naturally occur in our everyday experience as we move around the world or objects move relative to us. It is clear that we are able to solve this problem of object constancy as object identity is not usually lost during everyday dynamic interactions. However, there has been considerable debate within the object recognition literature as to how such constancy is achieved.

One class of theories has suggested that recognition is achieved by matching current visual experience to 3D, object-centred representations that are inherently *viewpoint independent* (Biederman, 1987; Marr & Nishihara, 1978). In contrast, another class of theories suggests that our object knowledge consists of stored templates related to previous experience with specific 2D views (Bülthoff & Edelman, 1992; Tarr & Bülthoff, 1995). In order to recognise an object from a specific viewpoint, some sort of internal transformation process is required in order to align the current viewpoint with previously stored *viewpoint dependent* representations. Empirically, the crucial difference between these two theoretical approaches is that *viewpoint dependent* theories predict there will be a perceptual/behavioural cost when tasks involve viewpoint changes, while *viewpoint independent* theories do not.

There has been relatively little previous research directly addressing the question of object constancy in relation to biological motion processing. Several studies have examined viewpoint dependency in specific relation to walking. Karl Verfaillie for example, used a sequential priming paradigm and found reliable RT advantages in both object (human or non-human decision; Verfaillie, 1993) and action (left or right facing decision; Verfaillie, 2000) related tasks. Stimuli were always profile views of walking figures and speed of response was found to be faster when the primed and target view were facing in the same direction. Mark Bradshaw and colleagues used a detection task with normal and spatially scrambled walkers embedded in noise masks (Bradshaw, Leach, Hibbard, van der Willigen & Rushton, 1999). They found detection thresholds were reliably lower when the walker was facing the observer than when oriented at 30°, 60° or 90° away from the line of sight. Troje, Westhoff and Lavrov (2005) used a person identification task with walking patterns and found that performance consistently dropped when different study and test views were used. Interestingly, the same group found some evidence for viewpoint independent processing of walking patterns when they used a more basic person identification task but included the condition where one of the target figures belonged to the observer (Jokisch, Daum & Troje, 2006). They found that the recognition of one's own walking patterns was viewpoint independent, whereas the recognition of all other walking patterns was more accurate from a frontal and half-profile view than a profile view, consistent with findings of Bradshaw et al. (1999).

Moving beyond point-light walkers, Daems and Verfaillie (1999) used photographic depictions of possible and impossible actions in a priming task. They only found facilitation when prime and target stimuli were physically plausible and had the same in-depth orientation, consistent with the idea of viewpoint dependent action recognition. Two more recent studies, however, have produced somewhat conflicting results with respect to question of viewpoint dependency. de la Rosa, Mieskes, Bülthoff and Curio (2013) examined the ability to recognise dyadic interactions (e.g., handshake, hug) using stick-figure stimuli. They found both reaction time and accuracy costs such that each type of interaction had a preferential viewpoint. In contrast Platonov and Orban (2016) used video sequences of observed manipulative actions (e.g., rolling or rotating an object) and found no viewpoint costs. They specifically suggest that action observation may differ from object recognition in being "for the most part viewpoint-independent" (p. 10).

From the above brief review, it is clear that there is still fairly limited – and somewhat conflicting – evidence concerning the question of viewpoint dependency and action recognition in general (as opposed to walking specifically). In the current paper we restrict ourselves to full-body, point-light stimuli and ask the question of whether there are viewpoint costs – the signature of viewpoint dependent representations – when matching biological patterns other than walking.

2 Method

2.1 Participants

Sixteen observers from the University of Malta took part in the current experiment. All participants had normal or corrected to normal vision and none reported any history of motor impairments. Prior to data collection, all participants gave written informed consent, although,

the precise research question was only explained during debriefing. They were paid €5 for taking part in the single experimental session which lasted approximately 15 minutes. All aspects of current study were reviewed and approved by the Research Ethics Committee of the Faculty of Media and Knowledge Sciences, University of Malta.

2.2 Equipment

Stimuli were displayed and data collected on a Macintosh Mini Computer connected to Fujitsu B24T-7 LCD display. The display had a visible area of 54×30 cm, a resolution of 1280×1024 pixels and a refresh rate of 75 Hz. Custom written code was developed in Matlab, using the Psychophysics Toolbox extensions (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997). The viewing distance was approximately 90 cm.

2.3 Stimuli

In the current study we used the stimulus display shown in Fig. 1B. On each trial there were always three dynamic point-light figures. The two flanking figures always performed two different actions. The central target copied the action of one of the two flankers. Each figure was composed of 13 white dots drawn on a uniform black background. Note that for illustration purposes, the contrast of figure dots and background have been reversed in Fig. 1B. The 13 dots represent head, shoulders, elbows, wrists, hips, knees and ankles. All dots were always visible, even when they would have ordinarily been occluded by other parts of the body. Each dot subtended approximately 0.22° visual angle. The figures were orthographically projected and aligned so that their centres were at the vertical midline of the screen. Each figure was the same size and subtended approximately 5° visual angle in height. The target figure was positioned at the very centre of the display area and the flankers were offset approximately 8° to the left and right of the target.

Each figure performed a familiar, periodic human action. The 11 actions performed by the figures were chosen from those within the database created by Vanrie and Verfaillie (2004). Specifically, the actions used were: chop, jump, mow, paint, pump, saw, shoot, spade, sweep, tap and wave. Note that walking was specifically not chosen for the current study as previous work had already explored this action in detail. We used all other actions from the database that were periodic and had a basic upright posture. The actions were randomly selected from this set on a trial-by-trial basis. The starting frame of each figure was always determined randomly. The "yaw" rotation around the y-axis (360° orientation range) of the two flanking figures was independently randomised. The orientation of the target was constrained to be offset 0°, 45° or 90° with respect to the orientation of the matching flanker. Trial order was determined randomly for each participant. Animations were displayed at 30 frames/s, and all of the three actions continued to play until a response was made.

2.4 Task

The task was the same as that used by Thornton et al. (2014). On each trial the participant had to decide which of the two flanking figures was performing the same action as the central target figure. Responses were indicated by pressing designated keys on a standard USB keyboard. They used the right hand to press the "l" key if the right flanker matched the target and the "s" key with the left hand if the left flanker matched the central target.

2.5 Design

Participants worked through two experimental blocks consisting of 90 trials in each block. The first block was preceded by approximately 20 familiarization trials. Each block was composed of 15 repetitions × 2 matching flanker positions (flanker matching on the right or on the left of the target) × 3 orientations offsets (0°, 45° or 90°). The 11 actions were not parametrically varied within the design. Rather, on each trial, two actions were selected at random. Trial order was determined separately for each block and participant.

2.6 Procedure

The experiment took place in a silent and dimly lit room. Participants were familiarised with the task and method of responding. They were instructed to respond as quickly and accurately as possible. Trials were separated by an inter-trial interval of 0.5 seconds. Errors were indicated by a visible "Error" message on the screen and a short additional pause of 0.5 seconds. The opportunity to rest was given to participants after the first experimental block. The entire experiment lasted about 15 minutes.

2.7 Data Analysis

Response time and error rates were analysed separately using the same One-Way repeated measures ANOVA. The independent variable, Viewpoint, had three levels (0°, 45° and 90°) reflecting the angular difference between target and flanker. We used planned linear contrasts to explore whether performance degraded in a systematic manner with increasing viewpoint differences. When data violated the assumption of sphericity, as assessed with Mauchly's test, we applied the Greenhouse-Geisser correction to the degrees of freedom.

3 Results

The left axis of Fig. 2 shows the pattern of median reaction times for correct responses. There are two points to note. First, overall responses times are relatively slow in

this task. The time needed to compare at least two actions that evolve over time means that participants are not able to make the rapid reactions typical of simple detection or direction discrimination tasks. Second, there is a very clear pattern of increasing reaction time as a function of the variation in viewpoint. Specifically, the time needed to recognize two identical actions increases in proportion to the angular rotation difference between target and flanker. Participants were fastest when target and flanker had the same viewpoint (0°) and increased in the 45° and 90° conditions.

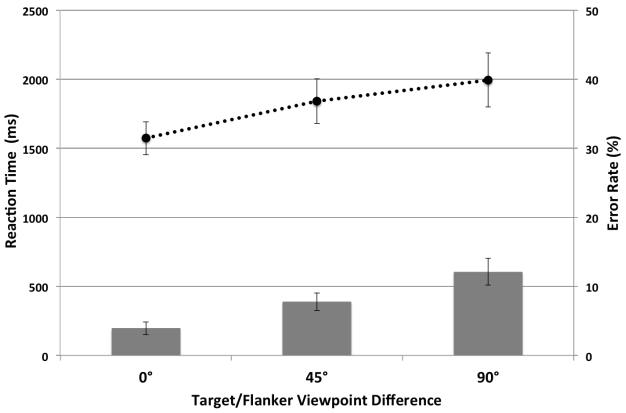

Figure 2: The left axis (line) shows median reaction time data as a function of the viewpoint difference between target and flanker in a concurrent matching task. The right axis (bar) shows percentage error rates as a function of viewpoint difference. Error bars indicate one standard error of the between-participant means.

Consistent with these patterns there was a clear main effect of Viewpoint, $F_{(2,30)} = 10.5$, MSE = 69345, $p < 0.001$, $\eta^2{}_p = 0.41$. Within subjects contrasts indicated that a linear model was the best fitting prediction of the overall pattern seen in the reaction time data, $F_{(1,15)} = 14.6$, MSE = 97425, $p < 0.01$, $\eta^2{}_p = 0.49$.

The right axis of Fig. 2 shows the pattern of error data. Again, there are two points to note. First, the overall level of performance was very good, with error rates remaining below 15% in all conditions. This indicates that, as with other familiar objects, participants are able to generalise quite well across viewpoints changes. Second, despite this general level of performance, there were clear costs associated with viewpoint change that mirror those seen with response times. Participants were most accurate when making judgements about actions shown from the same orientation (0°), while performance dropped in the other two conditions (45° and 90°).

There was again a main effect of Viewpoint, $F_{(1.4,20.8)} = 15.18$, MSE = 0.003, $p < 0.001$, $\eta^2{}_p = 0.5$. As with the RT data, within subjects contrasts indicated that a linear increase in error rates was the best fitting model for the overall pattern of data, $F_{(1,15)} = 23.1$, MSE = 0.002, $p < 0.001$, $\eta^2{}_p = 0.61$.

4 Discussion

In this paper, we examined the perception of point-light actions across viewpoint change using a concurrent matching task. Consistent with previous studies that had used only point-light walkers (Bradshaw et al., 1999; Jokisch et al., 2006; Troje et al., 2005; Verfaillie, 1993, 2000), we found that for a range of actions both reaction time and accuracy performance grew consistently worse as the angular difference between target and matching flanker increased. While the magnitude of these viewpoint costs were relatively small – consistent with the general notion of object constancy for human actions – their presence supports the notion that the representations underlying action understanding – as with both static (e.g., Bülthoff & Edelman, 1992; Lawson, 1999; Tarr & Bülthoff, 1995) and dynamic (e.g., Chuang, Vuong & Bülthoff, 2012; Friedman, Vuong & Spetch, 2010; Vuong, Friedman & Plante, 2009) object recognition – are viewpoint dependent.

The present study also demonstrates how the simple concurrent matching task we had previously developed (Thornton et al., 2014) could be used as a tool for exploring other biological motion questions and, more generally, help shed light on action understanding. As mentioned in the Introduction, we believe this task has advantages over other methods used to examine biological motion – such as direction discrimination and action naming – in that the action content can be changed quite dramatically without the need for familiarization and/or training, as the basic response demands always stay the same.

In general terms, the current task would be classified as an ABX design (Kingdom & Prins, 2016; Macmillan & Creelman, 1991), where A & B are samples, and X is the to-be-matched target. This is a standard psychophysical task, originally introduced for comparing auditory samples (Munson & Gardner, 1950), but one that can be applied to any sensory dimension. Presenting two sample stimuli concurrently, with random key assignments on a trial-by-trial basis reduces the likelihood that systematic response bias will affect the outcome, for example, a tendency to always respond "same" in a Same/Different design. Typically, ABX designs have sequential presentation of each item, but concurrent presentation also has the advantage of reducing memory load and the tendency to prefer the first or second presented sample.

Within the context of biological motion research, we have already used the task to explore distance perception (Thornton et al., 2014). In addition to exploring viewpoint costs, as we do in the current pa-

per, the task could also be used whenever an explicit discrimination is required. For example, it could be used to assess how well people are able to distinguish male and female actors, the speed with which actions are being performed, whether actions are synchronised/desynchronized or even which of the two flanking actions is being performed by the same actor as the target. While many of these questions have been addressed with other methods, we stress again the advantages of the basic ABX design mentioned above, and also note that because the same left/right decision is used regardless of the underlying decision, performance across different types of discrimination could also be compared. Of course, the task may not be appropriate for exploring some issues, for example, in studies that test the ability to process biological motion incidentally (Bosbach et al., 2004; Thornton & Vuong, 2004; Veto, Einhäuser & Troje, 2017, 2013).

Three further points are worth noting about the observed viewpoint costs in the context of current matching. First, as all stimuli were always visible, it suggests that the viewpoint dependent processing we observe with this task relate to immediate perceptual or very short-term/working memory representations. It has long been known that perceptual transformations – such as mental rotation (Shepard & Metzler, 1971) – do occur when participants have to make judgements about concurrently visible stimuli. It is our suggestion that such transformations underlie the current performance deficits. As mentioned in the Introduction, the need to make such transformations is the hallmark of viewpoint dependent processing.

Second, we did not constrain the absolute orientation of the figures. That is, the orientation of the two flanking figures was independently selected at random on each trial from the full 360° options. The target figure orientation was then constrained to be either 0°, 45° or 90° relative to the matching flanker orientation. While this does not allow us to examine possible preferential viewpoints for processing point-light stimuli (Bradshaw et al., 1999; Jokisch et al., 2006), it does suggest that transformation costs generalise and are unlikely to arise just because of problems perceiving point-light figures from specific angles.

Third, our design, was also random with respect to the action that was selected on a given trial. One of our main goals was to extend previous findings beyond simple walking patterns and our design included 11 actions from the Vanrie and Verfaillie (2004) database. In future studies, it would be interesting to systematically examine the costs associated with particular actions, as this might shed further light on the nature of the perceptual transformations involved. Here, we can simply note that an item analysis – collapsing across participants –

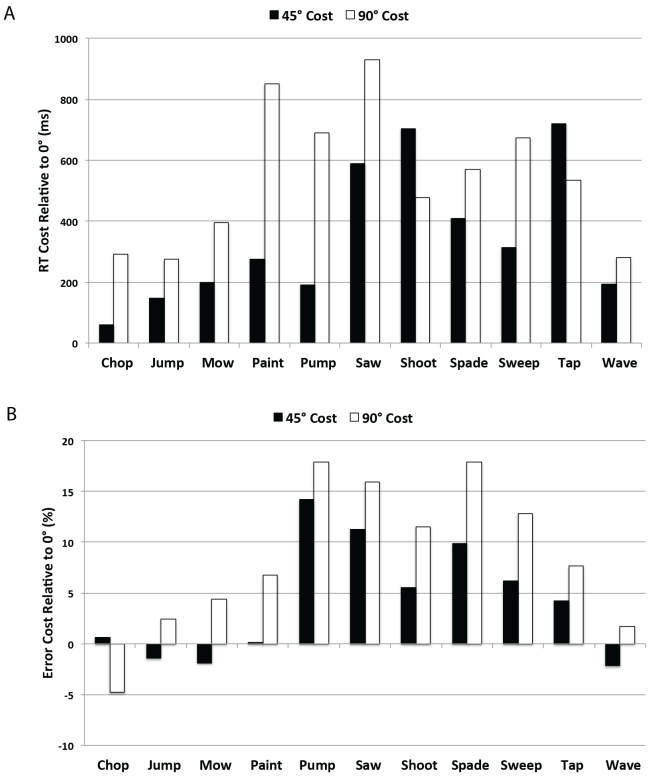

Figure 3: A) Reaction time costs in milliseconds; B) Percentage error rate costs for both the 45° and 90° trials relative to 0° difference. In both panels data have been collapsed across participants and are shown separately for each of the 11 actions.

showed reaction time viewpoint costs for all 11 actions. However, there were considerable differences in the magnitude of such costs as a function of action. Similar variation was seen in an accuracy item analysis, although error rates were higher for the majority (7 out of 11) of actions when the target and flanker had different viewpoints. These cost patterns are illustrated in Fig. 3.

To conclude, it seems highly likely that the perception of full-bodied point light actions is achieved via mechanisms that are viewpoint dependent. Previous studies using walking patterns with a variety of tasks have generally come to the same conclusion. Here, we have generalised this finding across a broader range of actions using concurrent matching. This does not necessarily mean that all forms of action understanding rely on viewpoint dependent representations. Indeed, above we have noted exceptions to this rule in previous studies using both point-light (Jokisch et al., 2006) and video (Platonov & Orban, 2016) stimuli. Although the viewpoint dependent and viewpoint invariant views of object recognition are often thought of as being mutually exclusive, a number of authors have pointed out that the visual system is more likely to adopt a flexible approach and use whatever form of representation suits the cur-

rent stimuli and task (Foster & Gilson, 2002; Vanrie, Willems & Wagemans, 2001). Nevertheless, our current reading of the literature, together with the results of this experiment lead us to speculate that viewpoint dependent action understanding is the rule, rather than the exception.

Acknowledgements

The authors would like to thank Quoc Vuong, Paul Hemeren, Peter Veto & Karl Verfaillie for useful comments on earlier versions of this manuscript. Nicola Ballarini's work at the University of Malta was supported by the Erasmus+ programme of the European Union. Part of this work was presented at the 6th Mediterranean Neuroscience Conference, Malta, June 2017.

References

Ahlström, V., Blake, R. & Ahlström, U. (1997). Perception of Biological Motion. *Perception*, *26*(12), 1539–1548.

Atkinson, A. P., Dittrich, W. H., Gemmell, A. J. & Young, A. W. (2004). Emotion Perception from Dynamic and Static Body Expressions in Point-Light and Full-Light Displays. *Perception*, *33*(6), 717–746.

Beintema, J. A. & Lappe, M. (2002). Perception of biological motion without local image motion. *Proc. Natl. Acad. Sci. 99*(8), 5661–5663.

Bertenthal, B. I. & Pinto, J. (1994). Global Processing of Biological Motions. *Psychol. Sci. 5*(4), 221–225.

Biederman, I. (1987). Recognition-by-components: a theory of human image understanding. *Psychol. Rev. 94*(2), 115–147.

Blake, R. & Shiffrar, M. (2007). Perception of human motion. *Annu. Rev. Psychol. 58*, 47–73.

Bosbach, S., Prinz, W. & Kerzel, D. (2004). A Simon Effect With Stationary Moving Stimuli. *J. Exp. Psychol. Hum. Percept. Perform. 30*(1), 39–55.

Bradshaw, M. F., Leach, R., Hibbard, P. B., van der Willigen, R. & Rushton, S. K. (1999). The walker's direction affects the perception of biological motion. In M. A. Grealy & J. A. Thomson (Eds.), *Studies in perception and action V/Tenth International Conference Perception Action Aug. 8–13, 1999, Edinburgh, Scotland* (pp. 3–6). Mahwah, N.J: L. Erlbaum Associates.

Brainard, D. H. (1997). The Psychophysics Toolbox. *Spat. Vis. 10*, 433–436.

Bülthoff, H. H. & Edelman, S. (1992). Psychophysical support for a two-dimensional view interpolation theory of object recognition. *Proc. Natl. Acad. Sci. 89*(1), 60–64.

Chandrasekaran, C., Turner, L., Bülthoff, H. H. & Thornton, I. M. (2010). Attentional networks and biological motion. *Psihologija*, *43*(1), 5–20.

Chuang, L. L., Vuong, Q. C. & Bülthoff, H. H. (2012). Learned Non-Rigid Object Motion is a View-Invariant Cue to Recognizing Novel Objects. *Front. Comput. Neurosci. 6*.

Cutting, J. E. (1978). A program to generate synthetic walkers as dynamic point-light displays. *Behav. Res. Methods Instrum. 10*(1), 91–94.

Cutting, J. E., Moore, C. & Morrison, R. (1988). Masking the motions of human gait. *Percept. Psychophys. 44*(4), 339–347.

Daems, A. & Verfaillie, K. (1999). Viewpoint-dependent Priming Effects in the Perception of Human Actions and Body Postures. *Vis. cogn. 6*(6), 665–693.

de la Rosa, S., Mieskes, S., Bülthoff, H. & Curio, C. (2013). View dependencies in the visual recognition of social interactions. *Front. Psychol. 4*, 752.

Dekeyser, M., Verfaillie, K. & Vanrie, J. (2002). Creating stimuli for the study of biological-motion perception. *Behav. Res. Methods, Instruments, Comput. 34*(3), 375–382.

Diamond, R. & Carey, S. (1986). Why faces are and are not special: An effect of expertise. *J. Exp. Psychol. Gen. 115*(2), 107–117.

Dittrich, W. H. (1993). Action Categories and the Perception of Biological Motion. *Perception*, *22*(1), 15–22.

Dittrich, W. H., Troscianko, T., Lea, S. E. G. & Morgan, D. (1996). Perception of Emotion from Dynamic Point-Light Displays Represented in Dance. *Perception*, *25*(6), 727–738.

Downing, P. E. & Peelen, M. V. (2011). The role of occipitotemporal body-selective regions in person perception. *Cogn. Neurosci. 2*(3-4), 186–203.

Foster, D. H. & Gilson, S. J. (2002). Recognizing novel three-dimensional objects by summing signals from parts and views. *Proc. R. Soc. B Biol. Sci. 269*(1503), 1939–1947.

Friedman, A., Vuong, Q. C. & Spetch, M. (2010). Facilitation by view combination and coherent motion in dynamic object recognition. *Vision Res. 50*(2), 202–210.

Giese, M. A. & Poggio, T. (2003). Cognitive neuroscience: Neural mechanisms for the recognition of biological movements. *Nat. Rev. Neurosci. 4*(3), 179–192.

Graf, M. (2006). Coordinate transformations in object recognition. *Psychol. Bull. 132*(6), 920–945.

Grossman, E. D. & Blake, R. (2002). Brain Areas Active during Visual Perception of Biological Motion. *Neuron*, *35*(6), 1167–1175.

Hemeren, P. E. (2008). *Mind in action: action representation and the perception of biological motion*. Lund University cognitive studies. Lund: Univ.

Ilg, W., Bakir, G. H., Mezger, J. & Giese, M. A. (2004). On the representation, learning and transfer of spatio-temporal movement characteristics. *Int. J. Humanoid Robot. 1*(04), 613–636.

Johansson, G. (1973). Visual perception of biological motion and a model for its analysis. *Percept. Psychophys. 14*(2), 201–211.

Johansson, G. (1976). Spatio-temporal differentiation and integration in visual motion perception. *Psychol. Res. 38*(4), 379–393.

Johnson, K. & Shiffrar, M. (2012). *People watching: Social, perceptual, and neurophysiological studies of body perception*. Oxford University Press.

Jokisch, D., Daum, I. & Troje, N. F. (2006). Self Recognition versus Recognition of others by Biological Motion: Viewpoint-Dependent Effects. *Perception*, *35*(7), 911–920.

Kingdom, F. A. A. & Prins, N. (2016). *Psychophysics: a practical introduction* (Second edition). Amsterdam: Elsevier/Academic Press.

Kleiner, M., Brainard, D. H., Pelli, D. G., Ingling, A., Murray, R., Broussard, C. et al. (2007). What's new in Psychtoolbox-3. *Perception*, *36*(14), 1.

Knoblich, G., Thornton, I. M., Grosjean, M. & Shiffrar, M. (Eds.). (2006). *Human body perception from the inside out*. Advances in visual cognition. Oxford ; New York: Oxford University Press.

Kozlowski, L. T. & Cutting, J. E. (1977). Recognizing the sex of a walker from a dynamic point-light display. *Percept. Psychophys. 21*(6), 575–580.

Lawson, R. (1999). Achieving visual object constancy across plane rotation and depth rotation. *Acta Psychol. (Amst). 102*(2–3), 221–245.

Macmillan, N. A. & Creelman, C. D. (1991). *Detection theory: a user's guide*. New York: Cambridge University Press.

Manera, V., Schouten, B., Becchio, C., Bara, B. G. & Verfaillie, K. (2010). Inferring intentions from biological motion: A stimulus set of point-light communicative interactions. *Behav. Res. Methods*, *42*(1), 168–178.

Marey, É.-J. (1895). *Movement: The Results and Possibilities of Photography (tr. E. Pritchard)*. London: William Heinemann. [French original: 1894. Le Mouvement. Paris: Masson].

Marr, D. & Nishihara, H. K. (1978). Representation and Recognition of the Spatial Organization of Three-Dimensional Shapes. *Proc. R. Soc. B Biol. Sci. 200*(1140), 269–294.

Mather, G., Radford, K. & West, S. (1992). Low-Level Visual Processing of Biological Motion. *Proc. R. Soc. B Biol. Sci. 249*(1325), 149–155.

Munson, W. A. & Gardner, M. B. (1950). Standardizing Auditory Tests. *J. Acoust. Soc. Am. 22*(5), 675.

Pavlova, M. & Sokolov, A. (2000). Orientation specificity in biological motion perception. *Percept. Psychophys. 62*(5), 889–899.

Pavlova, M. & Sokolov, A. (2003). Prior Knowledge about Display Inversion in Biological Motion Perception. *Perception*, *32*(8), 937–946.

Pelli, D. G. (1997). The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spat. Vis. 10*(4), 437–442.

Peuskens, H., Vanrie, J., Verfaillie, K. & Orban, G. A. (2005). Specificity of regions processing biological motion. *Eur. J. Neurosci. 21*(10), 2864–2875.

Pinto, J. & Shiffrar, M. (2009). The visual perception of human and animal motion in point-light displays. *Soc. Neurosci. 4*(4), 332–346.

Platonov, A. & Orban, G. A. (2016). Action observation: the less-explored part of higher-order vision. *Sci. Rep. 6*(1).

Pollick, F. E., Kay, J. W., Heim, K. & Stringer, R. (2005). Gender Recognition From Point-Light Walkers. *J. Exp. Psychol. Hum. Percept. Perform. 31*(6), 1247–1265.

Pollick, F. E., Paterson, H. M., Bruderlin, A. & Sanford, A. J. (2001). Perceiving affect from arm movement. *Cognition*, *82*(2), B51–B61.

Reed, C. L., Stone, V. E., Bozova, S. & Tanaka, J. (2003). The Body-Inversion Effect. *Psychol. Sci. 14*(4), 302–308.

Rhodes, G., Brake, S., Taylor, K. & Tan, S. (1989). Expertise and configural coding in face recognition. *Br. J. Psychol. 80*(3), 313–331.

Richler, J. J., Mack, M. L., Gauthier, I. & Palmeri, T. J. (2009). Holistic processing of faces happens at a glance. *Vision Res. 49*(23), 2856–2861.

Rizzolatti, G., Fogassi, L. & Gallese, V. (2001). Neurophysiological mechanisms underlying the understanding and imitation of action. *Nat Rev Neurosci*, *2*(9), 661–670.

Rossion, B. (2008). Picture-plane inversion leads to qualitative changes of face perception. *Acta Psychol. (Amst). 128*(2), 274–289.

Saygin, A. P. (2007). Superior temporal and premotor brain areas necessary for biological motion perception. *Brain*, *130*(9), 2452.

Shepard, R. N. & Metzler, J. (1971). Mental Rotation of Three-Dimensional Objects. *Science*, *171*(3972), 701–703.

Slaughter, V., Stone, V. E. & Reed, C. (2004). Perception of Faces and Bodies: Similar or Different? *Curr. Dir. Psychol. Sci. 13*(6), 219–223.

Sumi, S. (1984). Upside-down Presentation of the Johansson Moving Light-Spot Pattern. *Perception*, *13*(3), 283–286.

Tarr, M. J. & Bülthoff, H. H. (1995). Is human object recognition better described by geon structural descriptions or by multiple views? Comment on Biederman and Gerhardstein (1993). *J. Exp. Psychol. Hum. Percept. Perform. 21*(6), 1494–1505.

Thompson, J. & Parasuraman, R. (2012). Attention, biological motion, and action recognition. *Neuroimage*, *59*(1), 4–13.

Thornton, I. M. (2006). Biological Motion: Point-Light Walkers and Beyond. In G. Knoblich, I. M. Thornton, M. Grosjean & M. Shiffrar (Eds.), *Human body perception from the inside out* (pp. 271–303). Advances in visual cognition. Oxford ; New York: Oxford University Press.

Thornton, I. M., Pinto, J. & Shiffrar, M. (1998). The Visual Perception of Human Locomotion. *Cogn. Neuropsychol. 1998*(15), 535–552.

Thornton, I. M., Rensink, R. A. & Shiffrar, M. (2002). Active versus Passive Processing of Biological Motion. *Perception*, *31*(7), 837–853.

Thornton, I. M. & Vuong, Q. C. (2004). Incidental Processing of Biological Motion. *Curr. Biol. 14*(12), 1084–1089.

Thornton, I. M., Wootton, Z. & Pedmanson, P. (2014). Matching biological motion at extreme distances. *J. Vis. 14*(3), 13.

Thurman, S. M. & Grossman, E. D. (2008). Temporal "Bubbles" reveal key features for point-light biological motion perception. *Journal of Vision*, *8*(3), 28.

Troje, N. F. (2002). Decomposing biological motion: A framework for analysis and synthesis of human gait patterns. *J. Vis. 2*(5), 2.

Troje, N. F. (2003). Reference Frames for Orientation Anisotropies in Face Recognition and Biological-Motion Perception. *Perception*, *32*(2), 201–210.

Troje, N. F. & Westhoff, C. (2006). The Inversion Effect in Biological Motion Perception: Evidence for a "Life Detector"? *Curr. Biol. 16*(8), 821–824.

Troje, N. F., Westhoff, C. & Lavrov, M. (2005). Person identification from biological motion: Effects of structural and kinematic cues. *Percept. Psychophys. 67*(4), 667–675.

Van Belle, G., De Graef, P., Verfaillie, K., Busigny, T. & Rossion, B. (2010). Whole not hole: Expert face recognition requires holistic perception. *Neuropsychologia*, *48*(9), 2620–2629.

Vanrie, J. & Verfaillie, K. (2004). Perception of biological motion: A stimulus set of human point-light actions. *Behav. Res. Methods, Instruments, Comput. 36*(4), 625–629.

Vanrie, J., Willems, B. & Wagemans, J. (2001). Multiple Routes to Object Matching from Different Viewpoints: Mental Rotation versus Invariant Features. *Perception*, *30*(9), 1047–1056.

Verfaillie, K. (1993). Orientation-dependent priming effects in the perception of biological motion. *J. Exp. Psychol. Hum. Percept. Perform. 19*(5), 992–1013.

Verfaillie, K. (2000). Perceiving Human Locomotion: Priming Effects in Direction Discrimination. *Brain Cogn. 44*(2), 192–213.

Veto, P., Einhäuser, W. & Troje, N. F. (2017). Biological motion distorts size perception. *Sci. Rep. 7*, 42576.

Veto, P., Thill, S. & Hemeren, P. (2013). Incidental and non-incidental processing of biological motion Orientation, attention and life detection. In *Cooperative Minds: Social Interaction and Group Dynamics: Proceedings of the 35th Annual Meeting of the Cognitive Science Society Berlin, Germany, July 31–August 3, 2013*. Cognitive Science Society, Inc.

Vuong, Q. C., Friedman, A. & Plante, C. (2009). Modulation of Viewpoint Effects in Object Recognition by Shape and Motion Cues. *Perception*, *38*(11), 1628–1648.

Yin, R. K. (1969). Looking at upide-down faces. *J. Exp. Psychol. 81*(1), 141–145.

Young, A. W., Hellawell, D. & Hay, D. C. (1987). Configurational Information in Face Perception. *Perception*, *16*(6), 747–759.

Aquilina, B. and Cauchi, R. J. (2017). *Xjenza Online*, 5:30–35.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.04

Research Article

Tourette Syndrome: Do Reduced Histamine Levels Induce an Increase in Spontaneous Repetitive Behaviour?

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Abstract. Gilles de la Tourette syndrome (TS) is a disabling neuropsychiatric disorder characterised by persistent motor and vocal tics. Comorbidity of TS with other neuropsychiatric conditions such as obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD) and autism is frequent. TS has a significant genetic contribution and, in this regard, several susceptibility loci have been identified including the *histidine decarboxylase* (*HDC*) gene, which encodes an enzyme that is essential for histamine synthesis. Animal models of human disease are key to identify genetic and, importantly, pharmacological modifiers of phenotypes that mimic those present in the human condition. *HDC* is highly conserved throughout different species including the fruit fly *Drosophila melanogaster*. Aiming at uncovering TS-like phenotypes, in the present study we investigated repetitive grooming behaviour in flies that have reduced histamine levels as a result of a mutation in the *hdc*-encoding gene. We find that histamine deficiency in *Drosophila* is not associated with an increase in spontaneous repetitive grooming behaviour but rather a decrease. We speculate that the grooming behaviour in *Drosophila hdc* knockouts is not a translationally relevant TS phenotype. Future work should investigate whether stereotypy can be induced in the same mutants after pharmacological challenge or stress induction.

Keywords: Tourette Syndrome; *Drosophila*; grooming behaviour; histamine; histidine decarbyoxylase; model organism

Abbreviations

ADHD, attention-deficit hyperactivity disorder; *HDC*, *histidine decarboxylase*; OCD, obsessive-compulsive disorder; *SLITRK1*, *SLIT and TRK-like family member 1*; TS, Gilles de la Tourette syndrome

1 Introduction

Gilles de la Tourette syndrome (TS) is a disabling neuropsychiatric disorder where persistent motor and vocal tics are hallmark features. Comorbidity of TS with other neuropsychiatric conditions such as obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD) and autism is frequent, implying a common aetiology. There is as yet no cure for this disorder although pharmacological treatment and behavioural therapies can reduce symptoms. Twin studies have identified a significant genetic contribution to TS, where concordance rates over 50% were observed in monozygotic twins compared to rates below 10% for dizygotic twins (Hyde, Aaronson, Randolph, Rickler & Weinberger, 1992; Price, Kidd, Cohen, Pauls & Leckman, 1985). In addition, family studies show a greater rate of TS or chronic tics in first-degree relatives compared to rates in relatives of controls (Hebebrand et al., 1997; Kano, Ohta, Nagai, Pauls & Leckman, 2001; Pauls, Raymond, Stevenson & Leckman, 1991; Saccomani, Fabiana, Manuela & Giambattista, 2005). In this context, several association studies as well as linkage screens have identified and probed several TS susceptibility loci (Deng, Gao & Jankovic, 2012; O'Rourke, Scharf, Yu & Pauls, 2009; State, 2010, 2011).

Considerable excitement in the field was triggered by the discovery of two genes with a substantial role in TS including *L-histidine decarboxylase* (*HDC*), which encodes the rate limiting enzyme in histamine biosynthesis (Ercan-Sencicek et al., 2010), and the *SLIT and TRK-like family member 1* (*SLITRK1*), which encodes a transmembrane protein with strong homology to the axon guidance molecule, SLIT and the neurotrophin receptor, TRK (Abelson et al., 2005). Mouse knockouts

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of these two genes are available. Animal models are vital for modelling the human condition, thereby allowing the possibility of dissecting the function of disease-linked proteins, the uncovering of relevant disease pathways as well as the development and testing of novel therapeutic strategies. *Slitrk1*-knockout mice have a reduced body weight, slightly decreased viability, an elevated anxiety-like and depression-like behaviour. Although anxiety and depression are at times clinical features of TS, *Slitrk1*-null mice have no compulsive and/or tic-related behaviours. *Hdc*-knockout mice are viable but have reduced spontaneous locomotor activity in the dark as well as decreased exploratory activity in an illuminated open-field. Interestingly, under different testing conditions, *Hdc*-null mice display phenotypes that resemble features of TS including anxiety, potentiated tic-like stereotypic movements and increased grooming behaviour (Castellan Baldan et al., 2014; Cauchi & Tarnok, 2012; Xu, Li, Ohtsu & Pittenger, 2015).

In contrast to *SLITRK1*, *HDC* is highly conserved throughout different species including the fruit fly *Drosophila melanogaster* (Saenz-de-Miera & Ayala, 2004). *Drosophila* has a rich history with regards to its role in deciphering the genetic basis of behaviour and, in this regard, this model organism has been successful in modelling several neurological conditions including neuropsychiatric conditions such as schizophrenia, bipolar disorder and autism (Cauchi & van den Heuvel, 2006; Grice, Sleigh, Liu & Sattelle, 2011; Grice, Praveen, Matera & Liu, 2013; O'Kane, 2011; Sokolowski, 2001). *HDC* is essential for the production of histamine, which has diverse functions. In humans, histamine can act as a signalling molecule with important functions in gastrointestinal, immune, cardiovascular, respiratory and reproductive functions. In the nervous system, histamine acts as a transmitter with histaminergic neurons being involved in homeostatic brain functions and neuroendocrine control. Contribution to sensory and motor functions, cognition, attention, and learning as well as memory is also well-documented (extensively reviewed in Haas, Sergeeva & Selbach, 2008). In *Drosophila*, mutations in the *hdc* gene disrupt adult but not larval photoreceptor synaptic transmission and, hence, mutant flies are visually impaired in adulthood (Burg, Sarthy, Koliantz & Pak, 1993; Melzig et al., 1996, 1998). Furthermore, in view of various mechanosensory deficiencies displayed by *hdc* mutants, it was concluded that histamine is a major functional neurotransmitter for mechanosensory receptors. The *hdc* gene and other genes encoding for proteins involved in histamine signalling were recently identified in a screen for genes involved in sensing ambient temperature and in responding to its change (Hong et al., 2006).

Based on recent evidence implicating the *HDC* gene and histaminergic neural pathways in the aetiology of TS, we hypothesised that histamine deficiency induces TS-like behaviours including repetitive grooming. To this end, and aiming at developing a *Drosophila* model of TS, in the present study we investigated the spontaneous grooming behaviour of flies with a homozygous deficiency of the *hdc* gene.

2 Materials & Methods

2.1 Fly Stocks

Flies were cultured at 25 °C on standard molasses/maizemeal and agar medium in plastic vials under a 12 h/12 h light/dark cycle. The wild-type strain was *y w*. The previously-characterised hdc^{JK910}, hdc^{P218}, hdc^{P211} and hdc^{P217} are ethyl methanesulfonate (EMS) mutants of the *hdc* gene and were generous gifts from William Pak (Purdue University, West Lafayette, Indiana, USA) (Burg et al., 1993; Melzig et al., 1996, 1998).

2.2 Grooming Assay

Grooming assays of mutants and controls were conducted on the same day and during the daytime to minimise potential effects of circadian rhythm and climatic variations. Single male flies were transferred into a well-illuminated 1 cm^3 observation chamber, allowed to acclimatise in the new environment for 5 minutes, and were then video-recorded by a Sony DCR-SX53E video camera for 5 minutes. The captured video was analysed by one observer (B.A.). Video annotation were performed by the iMovie software (Apple) to determine the (1) percentage of time the fly spent grooming, (2) the number of grooming bouts, and (3) the duration of individual grooming bouts. Groom bouts was considered complete when the fly stopped grooming by either remaining motionless or walking for more than 2 seconds. Flies were analysed at different time points during adulthood and $n \geq 15$ for each time point.

2.3 Statistical Analyses

The unpaired *t*-test was used to compare wild-type and mutant fly populations. All data are shown as mean ± S.E.M. Statistically significant comparisons are depicted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$, and $^{****}p < 0.0001$.

3 Results

hdc mutant flies are adult viable, but were reported to display defects in visual and mechanosensory behaviours (Burg et al., 1993; Melzig et al., 1996, 1998). Aiming at uncovering phenotypes that mimic those present in TS, an attempt at assessing the grooming behaviour of these flies and its variation with age was undertaken. It was hypothesised that based on recent work pointing to *HDC*

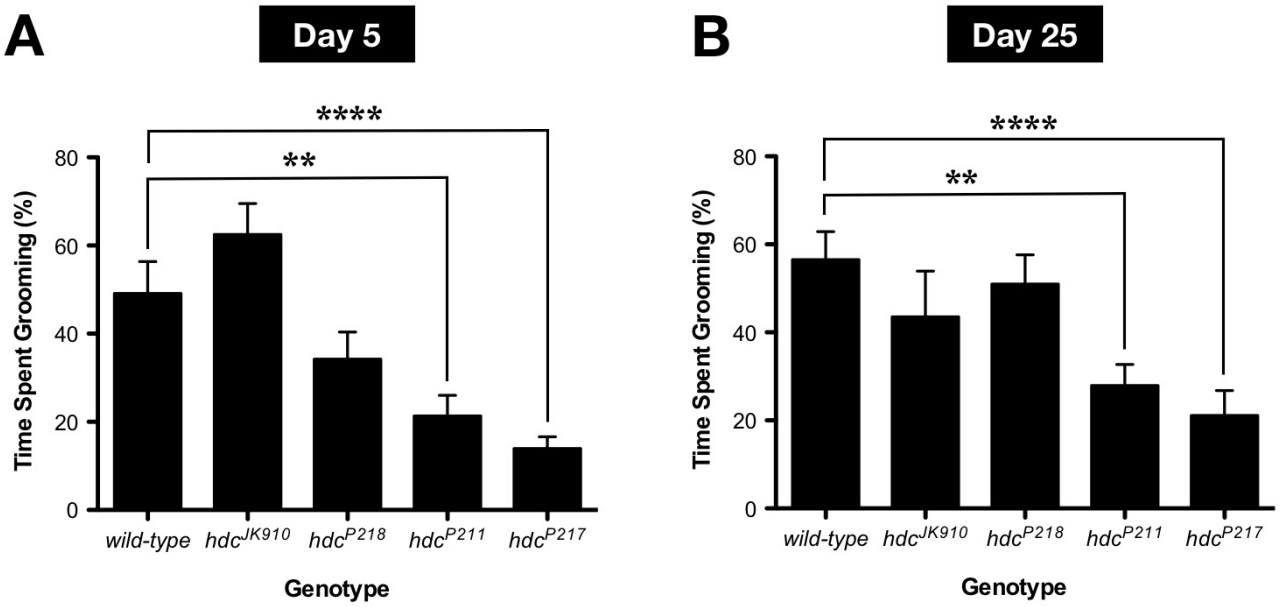

Figure 1: hdc^{P211} and hdc^{P217} mutant flies exhibit reduced grooming throughout adulthood. (A) At 5 days post-eclosion, hdc^{P211} and hdc^{P217} flies groom significantly less that control wild-type flies. This phenotype is not exhibited by hdc^{JK910} and hdc^{P218} flies. (B) A similar trend can be noted at 25 days post-eclosion. Data are represented as the mean ± S.E.M. percentage of time single male flies spend grooming during a 5 min observation period. $^{**}p < 0.01$, $^{****}p < 0.0001$, and $n \geq 15$ flies for each genotype at each time point.

as a TS susceptibility locus (Ercan-Sencicek et al., 2010; Karagiannidis et al., 2013), repetitive behaviours, and, hence, excessive grooming, are likely to be prominent in flies with homozygous loss-of-function *hdc* alleles.

To study baseline or spontaneous grooming behaviour, the activity of individual male flies was recorded in a small well-illuminated observation chamber. At 5 days old, hdc^{JK910} and hdc^{P218} flies groomed, on average, for 63% and 34% of the 5 min observation period, respectively. This result was not significantly different from that of controls, which were observed to spend about half (49%) of the time grooming. In contrast, age-matched hdc^{P211} and hdc^{P217} flies spent 21% and 14% of the observation window grooming, which was significantly lower than that of wild-type control flies ($p < 0.001$; Fig. 1A). This allelic-specific trend in grooming behaviour persisted with age, hence similar comparisons can be made at 25 days (Fig. 1B) and 35 days (data not shown) post-eclosion.

In addition to the total amount of time flies spent grooming, the duration and number of individual grooming bouts were assessed. At 5 and 25 days, the average duration of individual grooming bouts of both hdc^{JK910} and hdc^{P218} flies was found not to be statistically different from controls. Conversely, at these two time points, hdc^{P211} and hdc^{P217} flies had on average significantly shorter grooming bouts in comparison to wild-type counterparts (Fig. 2). Similar results were obtained at 35 days (data not shown). On investigating the number of grooming bouts occurring during the observation interval, hdc^{JK910} flies had a significantly lower number of grooming bouts at all time points measured. hdc^{P218} and hdc^{P217} flies only showed notable difference at day 25 whereas hdc^{P211} flies displayed no difference at all from control wild-type flies (Fig. 3).

In summation, our findings show that only the hdc^{P211} and hdc^{P217} alleles curb the amount of time dedicated to grooming as well as the duration of each grooming bout. However, the hdc^{JK910} and hdc^{P218} alleles exhibit a reduction in the number of grooming bouts. In this context, deficiency of histamine is associated with reduced rather than excessive spontaneous grooming activity in *Drosophila*.

4 Discussion

Animal models of human disease are key to identify genetic and, importantly, pharmacological modifiers of phenotypes that mimic those present in the human condition. In view of only a partial overlap with TS clinical features, *Hdc* (and *Slitrk1*) mouse knockouts might not be good models of this disorder (Cauchi & Tarnok, 2012). In the present study, we investigated whether *Drosophila* can serve as a TS animal model. To this end, we observed the grooming behaviour of adult flies at both an early and late stage during adulthood. In contrast to an earlier study, in which grooming was

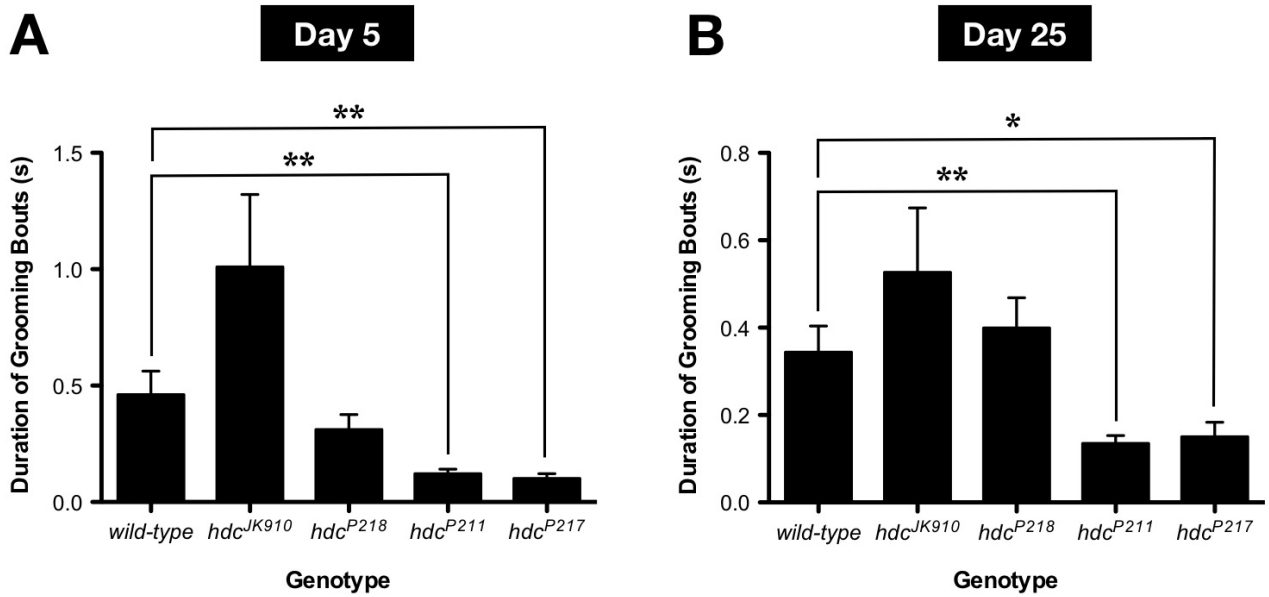

Figure 2: hdc^{P211} and hdc^{P217} mutant flies display a reduction in the duration of individual grooming bouts. At day 5 (A) and day 25 (B) of adulthood, the duration of individual grooming bouts is significantly shorter than controls. No change is however observed for hdc^{JK910} and hdc^{P218} mutant flies. Data are represented as the mean ± S.E.M. percentage of time single male flies spend grooming during a 5 min observation period. $^*p < 0.05$, $^{**}p < 0.01$, and $n \geq 15$ flies for each genotype at each time point.

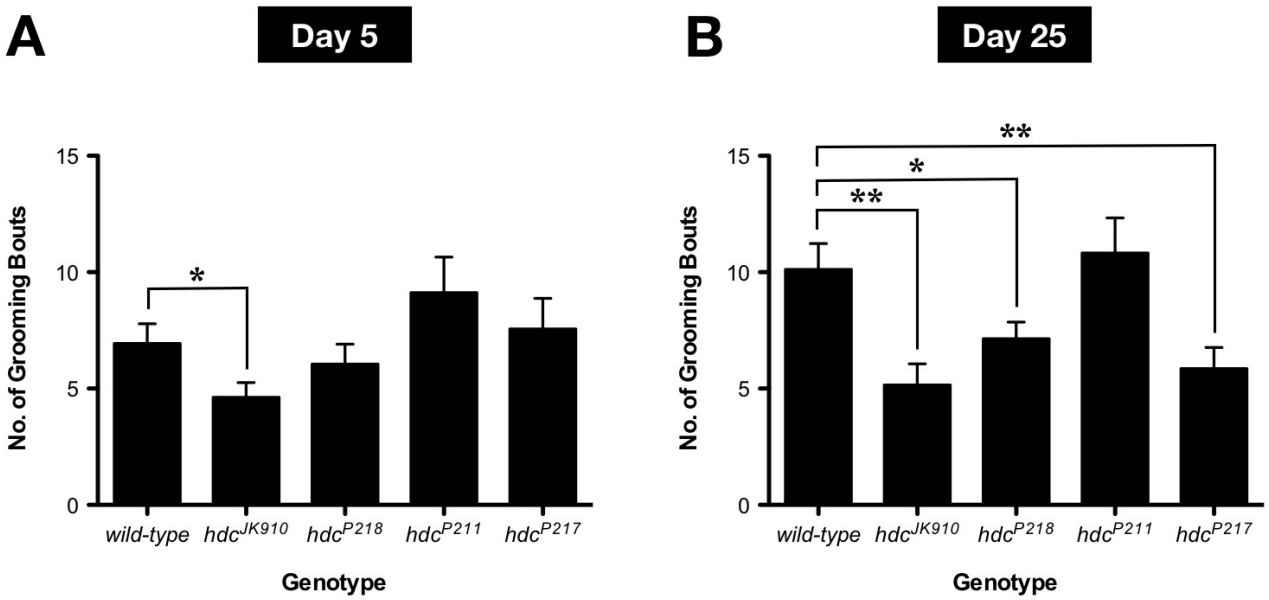

Figure 3: *Histamine deficiency reduces the number of grooming bouts.* (A) Day 5 hdc^{JK910} mutant flies exhibit a significant decrease in the number of grooming bouts. At this time point, the other *hdc* alleles analysed displayed no change compared to control. (B) At day 25, all *hdc* mutant flies except hdc^{P211} have a significant decrease in the number of grooming bouts. Data are represented as the mean ± S.E.M. percentage of time single male flies spend grooming during a 5 min observation period. $^*p < 0.05$, $^{**}p < 0.01$, and $n \geq 15$ flies for each genotype at each time point.

induced by coating flies with dust (Melzig et al., 1996), our study investigated baseline or spontaneous grooming. We hypothesised that spontaneous rather than prompted grooming, if repetitive, would closely mimic the persistent motor ticks that are a hallmark feature of TS. We find that histamine deficiency in *hdc* mutant flies does not induce an increase in spontaneous repetitive grooming behaviours but rather a decrease. Taking also into consideration the findings of Melzig et al. (1996), *hdc* mutants therefore have a reduction in both spontaneous (our study) as well as dust-induced grooming activity (Melzig et al., 1996).

Our results might imply that in humans, histaminergic neurons might have functions that are not present in *Drosophila*. Nonetheless, it is highly likely that the grooming behaviour in *Drosophila hdc* knockouts is not a translationally relevant TS phenotype. Future work should investigate whether repetitive grooming behaviour can be induced in the same mutants after pharmacological challenge or stress induction. This was found to be the case in mouse models. Hence, increased grooming and stereotypy were obvious in mouse *Hdc* knockouts only after amphetamine administration (Castellan Baldan et al., 2014) or following the stress induced by the presentation of a conditioned fear stimulus (Xu et al., 2015). These findings in *Hdc* knockout mice enhanced their validity as a pathophysiologically informative model of TS in addition to strengthening the value of the mouse model for drug discovery. Further investigations in fly *hdc* mutants with the aim of inducing stereotypy either by stress or drugs are therefore warranted. If successful, such studies can potentially validate the suitability of *Drosophila* as a novel animal model of TS.

References

Abelson, J. F., Kwan, K. Y., O'Roak, B. J., Baek, D. Y., Stillman, A. A., Morgan, T. M., ... State, M. W. (2005). Sequence variants in SLITRK1 are associated with Tourette's syndrome. *Science (80-.). 310*(5746), 317–320.

Burg, M. G., Sarthy, P. V., Koliantz, G. & Pak, W. L. (1993). Genetic and molecular identification of a Drosophila histidine decarboxylase gene required in photoreceptor transmitter synthesis. *EMBO J. 12*(3), 911–919.

Castellan Baldan, L., Williams, K. A., Gallezot, J. D., Pogorelov, V., Rapanelli, M., Crowley, M., ... Pittenger, C. (2014). Histidine decarboxylase deficiency causes tourette syndrome: parallel findings in humans and mice. *Neuron*, *81*(1), 77–90.

Cauchi, R. J. & Tarnok, Z. (2012). Genetic animal models of Tourette syndrome: The long and winding road from lab to clinic. *Transl. Neurosci. 3*(2), 153–159.

Cauchi, R. J. & van den Heuvel, M. (2006). The fly as a model for neurodegenerative diseases: is it worth the jump? *Neurodegener. Dis. 3*(6), 338–356.

Deng, H., Gao, K. & Jankovic, J. (2012). The genetics of Tourette syndrome. *Nat. Rev. Neurol. 8*(4), 203–213.

Ercan-Sencicek, A. G., Stillman, A. A., Ghosh, A. K., Bilguvar, K., O'Roak, B. J., Mason, C. E., ... State, M. W. (2010). L-histidine decarboxylase and Tourette's syndrome. *N. Engl. J. Med. 362*(20), 1901–1908.

Grice, S. J., Praveen, K., Matera, A. G. & Liu, J. L. (2013). Spinal Muscular Atrophy: Insights from the Fruit Fly. In R. J. Cauchi (Ed.), *Drosoph. melanogaster model. mot. neuron dis.* (Chap. 7, pp. 171–184). N.Y.: Nova Biomedical.

Grice, S. J., Sleigh, J. N., Liu, J. L. & Sattelle, D. B. (2011). Invertebrate models of spinal muscular atrophy: insights into mechanisms and potential therapeutics. *Bioessays*, *33*(12), 956–965.

Haas, H. L., Sergeeva, O. A. & Selbach, O. (2008). Histamine in the nervous system. *Physiol. Rev. 88*(3), 1183–1241.

Hebebrand, J., Klug, B., Fimmers, R., Seuchter, S. A., Wettke-Schafer, R., Deget, F., ... Remschmidt, H. (1997). Rates for tic disorders and obsessive compulsive symptomatology in families of children and adolescents with Gilles de la Tourette syndrome. *J. Psychiatr. Res. 31*(5), 519–530.

Hong, S. T., Bang, S., Paik, D., Kang, J., Hwang, S., Jeon, K., ... Kim, J. (2006). Histamine and its receptors modulate temperature-preference behaviors in Drosophila. *J. Neurosci. 26*(27), 7245–7256.

Hyde, T. M., Aaronson, B. A., Randolph, C., Rickler, K. C. & Weinberger, D. R. (1992). Relationship of birth weight to the phenotypic expression of Gilles de la Tourette's syndrome in monozygotic twins. *Neurology*, *42*(3 Pt 1), 652–658.

Kano, Y., Ohta, M., Nagai, Y., Pauls, D. L. & Leckman, J. F. (2001). A family study of Tourette syndrome in Japan. *Am. J. Med. Genet. 105*(5), 414–421.

Karagiannidis, I., Dehning, S., Sandor, P., Tarnok, Z., Rizzo, R., Wolanczyk, T., ... Paschou, P. (2013). Support of the histaminergic hypothesis in Tourette syndrome: association of the histamine decarboxylase gene in a large sample of families. *J. Med. Genet. 50*(11), 760–764.

Melzig, J., Buchner, S., Wiebel, F., Wolf, R., Burg, M., Pak, W. L. & Buchner, E. (1996). Genetic depletion of histamine from the nervous system of Drosophila eliminates specific visual and mechanosensory behavior. *J. Comp. Physiol. A. 179*(6), 763–773.

Melzig, J., Burg, M., Gruhn, M., Pak, W. L. & Buchner, E. (1998). Selective histamine uptake rescues photo- and mechanoreceptor function of histidine decarboxylase-deficient Drosophila mutant. *J. Neurosci. 18*(18), 7160–7166.

O'Kane, C. J. (2011). Drosophila as a model organism for the study of neuropsychiatric disorders. *Curr. Top. Behav. Neurosci. 7*, 37–60.

O'Rourke, J. A., Scharf, J. M., Yu, D. & Pauls, D. L. (2009). The genetics of Tourette syndrome: a review. *J. Psychosom. Res. 67*(6), 533–545.

Pauls, D. L., Raymond, C. L., Stevenson, J. M. & Leckman, J. F. (1991). A family study of Gilles de la Tourette syndrome. *Am. J. Hum. Genet. 48*(1), 154–163.

Price, R. A., Kidd, K. K., Cohen, D. J., Pauls, D. L. & Leckman, J. F. (1985). A twin study of Tourette syndrome. *Arch. Gen. Psychiatry*, *42*(8), 815–820.

Saccomani, L., Fabiana, V., Manuela, B. & Giambattista, R. (2005). Tourette syndrome and chronic tics in a sample of children and adolescents. *Brain Dev. 27*(5), 349–352.

Saenz-de-Miera, L. E. & Ayala, F. J. (2004). Complex evolution of orthologous and paralogous decarboxylase genes. *J Evol Biol*, *17*(1), 55–66.

Sokolowski, M. B. (2001). Drosophila: genetics meets behaviour. *Nat. Rev. Genet. 2*(11), 879–890.

State, M. W. (2010). The genetics of child psychiatric disorders: focus on autism and Tourette syndrome. *Neuron*, *68*(2), 254–269.

State, M. W. (2011). The genetics of Tourette disorder. *Curr. Opin. Genet. Dev. 21*(3), 302–309.

Xu, M., Li, L., Ohtsu, H. & Pittenger, C. (2015). Histidine decarboxylase knockout mice, a genetic model of Tourette syndrome, show repetitive grooming after induced fear. *Neurosci Lett*, *595*, 50–53.

Mifsud, S. (2017). *Xjenza Online*, 5:36–56.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.05

Research Article

A Preliminary Survey and Taxonomy of Wild Roses (*Rosa* Spp.) Occurring on the Maltese Islands

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Abstract. Rosa spp. are neglected and understudied for the Maltese flora, where only three species have been reported. *R. sempervirens* L. is the only native rose established in at least 12 locations in Malta. *R. gallica* L. s. l. (including hybrids) and *R. canina* L. s. l. are only mentioned in historical literature with doubtful occurrences. However, there are several other roses occurring naturally in Malta, which are probably not studied due to the difficulties in identification. A study carried out between May and July 2016 has resulted in the examination of 27 populations of wild or naturally occurring roses, of which twelve species, hybrids or cultivars have been recorded, eight of which are new for the Maltese flora. In addition, new locations or rediscoveries of *R. canina* and *R. gallica* s. l. have been found and, hence, are still extant in the Maltese Islands.

Keywords: *Rosa* spp.; Rosaceae; Flora of Malta; Mediterranean region

1 Introduction

The study of native and naturalized roses (hereafter referred to as wild roses) on the Maltese islands has been neglected partly because of their intricate taxonomy, resulting in difficulties to reach concrete identification. This is chiefly because of the widespread hybridisation including the creation of numerous cultivars in this genus. Moreover, they are given little importance to the flora of Malta, partly because they are often dismissed as horticulture escapes or casual aliens.

The last updated work on *Rosa* spp. goes back to the flora of Borg (1927) and subsequent accounts of floras such as by Haslam, Sell and Wolseley (1977), Lanfranco (1989), Weber and Kendzior (2006), Casha (2013) consist of repetitions of the same records. Albeit, roses in Malta have been mentioned, as early as the 16th century. One of the earliest official descriptions of Malta was by the ancient French writer, Jean Quintin (1500–1561), who reports the abundant occurrence of very fragrant roses he called "*Rose di Malta*" (translation: Maltese roses) as cited by Gianfrangisk Abela in 1647 (Ciantar, 1772, update reprint of Abela's work). According to Borg (1927), these Maltese roses were a fragrant variety of *Rosa gallica* L.

Only *Rosa sempervirens* L. is native to the Maltese islands. It is an evergreen rambling shrub found in few rocky valleys in mainland Malta (Haslam et al., 1977). It is considered a rare and threatened species for the Maltese Islands (Lanfranco, 1989) and is consequently protected (LN311, 2006, Dec 7, :schedule III). It was first recorded by Grech Delicata (1853) from Buskett, and subsequently reported in other rocky valleys by various authors (Sommier & Caruana Gatto, 1915; Borg, 1927; Lanfranco, 1989; Tabone, 2008). Another rose reported in historical records is *R. dumetorum* Thuill. reported by Gulia (1872) from Ta' Ċenċ in Gozo, which was cited with the same name by Sommier and Caruana Gatto (1915); under the taxon *R. canina* L. var. *dumetorum* by Borg (1927); and as *R. corymbifera* Borkh. by Haslam et al. (1977). The population at Ta' Ċenċ was declared as "disappeared" already in 1911 by Sommier and Caruana Gatto (1915) and corroborated by Haslam et al. (1977). Substantiated records of *R. canina* s.l. from Malta have not been reported and recent work sometimes simply cites the old record. However, approximately forty years ago, Michael Briffa had encountered and photographed *R. canina* from Wied Inċita, Attard (pers. comm. Michael Briffa, 2007) and, upon examining this photograph, the present author confirmed its identity in a wide sense. However, no recent sighting of this rose was ever made from this valley which has suffered massive quarrying activity over the last twenty

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years. *R. canina* might as well represent a native rose as is the case for Sicily (Giardina, Raimondo & Spadaro, 2007), but with the current scarce data available, its status cannot be confirmed.

Finally, the species *R. damascena* Mill. was reported by Borg (1927) from many gardens and sometimes became naturalised in the Maltese islands, although no specific localities were given. Borg (*op. cit.*) thought that this fragrant rose is a hybrid between *R. gallica* and *R. canina*, but now it is known to be complex hybrid involving, at least, the parents *R. gallica* and *R. moschata* (Huxley, Griffiths, Levy & Royal Horticultural Society, 1992; Grant, 2000; HMF, 2016). Presumably, it was quite a popular cultivar, owing to its several Maltese names (Warda tal-Madonna, Warda tal-Ħall and Warda ta' Malta) and possibly, it was the same "*Rose di Malta*" mentioned in the 16$^{\text{th}}$ century (Ciantar, *op. cit.*). Despite its popularity, this 'Maltese rose' has not been mentioned or reported in any recent floristic account (e.g. Haslam et al., 1977; Weber & Kendzior, 2006; Weber, 2008; Casha, 2013; Mifsud, 2002–2014; Lanfranco & Bonett, 2015).

In fact, recent floristic accounts or papers only give *R. sempervirens* to be currently present for the Maltese islands, occasionally citing some of the historical records (e.g. *R. gallica*) without any recent confirmation or personal findings. However, the present author, apart from *Rosa sempervirens*, also published the presence of *R. gallica* and *R. rubiginosa* from the Maltese islands (Mifsud, 2010a, 2010b). Bakay, Racek, Rovná and Kerényi-Nagy (2015) also recorded *R. rubiginosa* from a disturbed area in Xagħra, Gozo. Apart from these examples, the author was also aware of several other roses occurring naturally in the Maltese islands, but these were never studied in any depth and identified, mainly due to the difficulty of identification that this genus is renowned for.

During Spring 2016, a fresh botanical and taxonomical study on all wild roses known to the author, and other specimens shown or indicated, thanks to the help of few contributors (see acknowledgements), was carried out. This was principally motivated because of the lack of taxonomical information and knowledge about the genus *Rosa* for the flora of the Maltese islands. Even if the present study may not convey a perfect identification of all hybrid-complexes of roses reported here, it shall fulfil the aim to highlight in detail which roses occur in Maltese ecosystems, hopefully eliciting rhodologists to share their expertise and propose better identification of the Maltese roses from those conveyed in this account. Finally, this study provides tentative names of roses which are suitable for landscaping and embellishment projects in the Maltese Islands, because such vegetatively naturalized roses are likely to be successful if cultivated in similar natural conditions around the Maltese islands.

2 Methodology and Materials

The methodology employed in this study consisted of three steps: (i) selection of roses to be considered to be native, naturalised or occurring naturally, which hereafter they are referred to as wild; (ii) a detailed morphological description of the species or cultivar, including habitat and taxonomical remarks supported by photographs and (iii) identification of the collected specimens, with the aid of various publications and online sources (Chapman, 2012; Clapham, Tutin & Warburg, 1962; Grant, 2000; HMF, 2016; HWR, 2009; Klastersky, 1968; Pignatti, 1982; Phillips & Rix, 1993; Pottier-Alapetite, 1979; Redell, 1998; Silvestre & Montserrat, 1998; Stace, 2010; VRA, 2016).

The main criteria by which roses were selected for this study are the following:

i. Individuals or populations in rural areas where they are autochthonous (e.g. in valleys or valley sides) or growing naturally in abandoned cultivated areas, and hence growing on their own over a long period of time.
ii. Individuals or populations in semi-urban areas, for example close to agricultural areas or abandoned dwellings, where they are not given any form of assistance or care by man.
iii. Roses were not included in this study when located in parks, public or private gardens and embellishments, or in rural areas where there were obvious signs of cultivation.

It was not always easy to assess whether some individuals were adventive or not, but individuals were not studied if they had signs of pruning, irrigation or any care from man, and if located in cultivated or tilled fields in which agriculture takes place actively, especially if roses formed attractive and fragrant flowers. For example roses found at the terrace of Miżieb pumping station, agricultural areas near Santa Luċija chapel in Mtarfa (Malta) and tilled fields at Bidnija and Żebbuġ, Gozo receive some care from man and therefore excluded.

For each rose species, hybrid or cultivar, a detailed botanical description, based on the material used in this study, is given in this account, with emphasis on critical distinctive characters such the leaflet margin, stipules, sepals, and styles. Herbarium specimens were prepared and kept in the author's private collection and close-up photographs were taken for future reference and re-examination. In addition, the local distribution, habitat, historical notes, taxonomic remarks and status on the Maltese islands are also supplied in detail. Locations marked by an [!] in the distribution list indicate

that the species was observed by the present author over the last 15 years.

The status was based on the terminology and definitions provided by Nesom (2000), where many populations or individuals were found to have spread vegetatively by underground shoots or suckers, sometimes scrambling for several meters over surrounding vegetation after many years of abandoned cultivation or introduction. Since many roses only reproduce vegetatively, either because they are sterile hybrids or because the habitat is not suitable for seed germination, they do not naturalize in the classic sense of dispersing away to form new populations, albeit they are still naturally occurring in the wild. According to Nesom (2000), the term *"persisting non-native plants"* is coined for such situations, defined as perennial plants originally cultivated for ornament or interest and remaining in place of origin without human assistance after the site has returned to a more natural state, and not reproducing or at least not spreading beyond the original planting. If populations are seen to be very old and have grown for several meters and intermixed with surrounding local flora (usually *Rubus ulmifolius* L.), they are here referred to established, persisting non-native roses.

3 Results

A total of 27 populations or individuals of *Rosa* spp. have been examined in this survey, of which 24 were considered native or naturally occurring (Table 1). They were classified into twelve different taxonomic entities (species, bi-parental hybrids or complex hybrids) as listed in Table 2 further below.

1. *Rosa sempervirens* L. [Figure 1A]

   Distribution: MALTA: Buskett Woodland(!), Wied Inċita(!) (f. *floribunda* according to Borg, 1927), Wied Għar Dalam (Duthie, 1872); Wied il-Għasel at iż-Żenqa (f. *microphylla* according to Borg, 1927); Wied Anġlu(!), Wied Ħażrun, Ta' Baldu, Santa Katarina (Lanfranco, 1989); scree around Inquisitor's Palace, Laferla Cross (overlooking Wied Fulija), Wied Għomor(!) (Tabone, 2008); Wied l-Speranza (! 19-Nov-2009, the largest population known to the author), Wied il-Kbir, Qormi (! 10-May-2016, pers. comm. Anthony Chircop & Owen Mifsud). GOZO: "*Migiarro*" (Gulia, 1872), unsubstantiated and ambiguous record possibly referring either to Mġarr ix-Xini (Ta' Sannat) or one of the rocky valleys near the coast of Mġarr (Għajnsielem) such as Wied Biljun or iż-Żewwieqa. Also observed in cultivation at a park close to Skorba Temples, Mġarr, Malta.

   Habitat: Exposed rocky valley sides or valley beds, except at Wied il-Buskett where it is sheltered within a woodland but close to the valley bed. This shaded specimen was never observed to flower, it was possibly planted a long time ago in an unsuitable shaded area.

   Description (specimen examined #3, ref. Table 1): Evergreen shrub with long trailing branches, often found rambling over neighbouring vegetation. **Stems** and branches glabrous, greyish-green, flexuous, sparsely prickled, but rather numerous on the lower branches. **Prickles** narrow, slightly curved with an abruptly widening base, reddish-brown becoming greyish brown, 4–6 mm long with a 4–9 mm base. Acicles absent. **Leaves** imparipinnate, 4–8(10) cm long with 5 or 7 leaflets, glabrous, dark green and slightly glossy. Leaflets usually sessile except the terminal, (15)20–50 × (10)12–24 mm, narrowly ovate with an obtuse base and a rather acute to subacuminate tip, margin crenate-uniserrate almost around entire leaf. **Stipules** glabrous, (4)7–15 × 2–5 mm, linear-oblong, margins often revolute smooth or sometimes sparsely punctate with tiny red glands, terminal auricles divergent, deltate-acuminate, 3–5 mm long. **Inflorescence** simple or corymb-like, producing terminal, white, single flowers, 4–6 cm across, faintly fragrant. Pedicels, hypanthium and sepals covered with purple-red stipitata glands about 0.5 mm long. **Petals** 5, white, 12–25 mm wide, emarginate. **Sepals** lanceolate with an entire margin and an acuminate tip, about 12 × 5 mm, finely velutinose at the adaxial surface, patent at flowering. **Stamens** numerous forming an open ring around the style, 3–8 mm long; filaments white, anthers golden yellow. **Styles** fused in a pubescent column emerging from a pinkish hypanthium floor and ending with a capitate apex of pale-green, glabrous (or slightly pubescent) stigma. **Hips** dark red, subglobular, smooth or with sparse stipitate glands at the base, 8–10 mm across and 10–12 mm long. Flowering May–June.

   Taxonomy: A variable species were all described infraspecific taxa (3 subspecies, 27 varieties, and 1 form) are currently being treated either as synonyms of the nominal taxon or unresolved (ThePlantList, 2014). Borg (1927) reports the forms f. *floribunda* Guss., and f. *microphylla* D.C. (= f. *minor* Guss.) from Malta, of which none are regarded to have any taxonomic value.

   Status: Native, scarce-rare, can spread extensively in undisturbed valleys, apparently absent from

Table 1: Specimens or populations of roses examined in this study giving the date first observed, locality, altitude above sea level within the nearest 50 m, habitat and taxon entity. Legend: *Specimens considered as cultivated or aided by the intervention of man (excluded), ?*doubtful status, abandoned recently or found very near cultivated areas (included).

Pop.	Date found	Isl.	Locality	Toponym	Altitude (m)	Habitat or location	Taxon
1	31/05/2006	Malta	Dingli	Buskett	200	Woodland	1
2	27/05/2006	Malta	Attard	Wied Inċita	100	Rocky valley bank	1
3	19/11/2009	Malta	Mosta	Wied Speranza	150	Rocky valley bank	1
4	17/05/2016	Malta	Għargħur	Wied Anġlu	150	Rocky valley bank	1
5	01/06/2015	Malta	San Ġwann	Wied Għomor	100	Abandoned fields close to valley bank	1
6	10/05/2016	Malta	Qormi	Wied il-Kbir	100	Valley bed	1
*	03/05/2016	Malta	Mġarr	Park near Skorba temples	150	Small park	1
7	05/05/2006	Malta	Rabat	Wied il-Qlejgħa	150	Valley side lined by fields	6
8(?*)	26/01/2008	Malta	Qrendi	Ħagar Qim	100	Rubble wall along footpath	6
9	07/05/2009	Malta	Marsa	Old power station	50	Disturbed area possibly in an abandoned field	6
10	03/05/2016	Malta	Għargħur	Wied il-Faħam	150	Degraded garigue	6
11	22/05/2016	Malta	Siġġiewi	Fawwara chapel	200	Footpath within an agricultural area	6
12	28/05/2016	Malta	Mosta	Tarġa Gap	150	Steppe/disturbed garigue	6
*	21/05/2016	Malta	Rabat	Mtarfa (Santa Luċia)	200	Footpath near cultivated fields	6
13	22/05/2016	Malta	Dingli	Il-Qaws	250	Terrace of an explosive factory naturalising in footpath and steppe below	7
14	01/06/2006	Malta	Għargħur	Wied id-Dis	150	Terrace of an abandoned dwelling in valley bed	12
15	01/06/2006	Malta	Għargħur	Wied id-Dis	150	Terrace of an abandoned dwelling in valley bed	8
16	26/12/2005	Gozo	Ta' Sannat	Mġarr ix-Xini	50	Sheltered valley side	9
17	10/05/2016	Gozo	Nadur	Wied ir-Riħan	100	Valley bed	3
18	19/11/2009	Malta	Mosta	Wied Speranza	150	Valley side	4
19	07/05/2016	Gozo	Nadur	Wied ir-Riħan	100	Valley side	5
20	07/05/2016	Gozo	Nadur	Għajn Qasab	100	Rubble wall within an agricultural area	5
21(?*)	28/05/2016	Malta	Mġarr	Dwejra area	150	Agricultural fields	5
*	28/05/2016	Malta	Bidnija/Mġarr area	Ħal-Dragu	150	Agricultural fields	5
22	16/06/2010	Malta	Siġġiewi	Fawwara	200	Disturbed roadside	10
23	26/06/2016	Malta	Siġġiewi	Fawwara chapel	200	Rubble wall of terraced field	11
24	19/05/2010	Gozo	Kerċem	Wied il-Lunzjata	100	Against rubble wall of terraced field	2

Figure 1: **A**. *Rosa sempervirens* L. left to right: inflorescence and buds; pedicel, hypanthium and sepals; and hip (Wied l-isperenza, Mosta, 25-May-2016). **B**. Rosa 'Albéric Barbier' left: inflorescence (near Wied il-Faħam, Għargħur, 3-May-2016); centre: buds and calyx (Ħagar Qim, Qrendi, 3-May-2016); right: stipule (Santa Luċia, Mtarfa, 21-May-2016). **C**. *Rosa* 'Léontine Gervais' left to right: inflorescences; pedicel, hypanthium and calyx; stipule (il-Qaws, Dingli, 22-May-2016). Photos Stephen Mifsud.

Comino and Gozo, vulnerable with a restricted distribution in the Maltese islands (Lanfranco, 1989), legally protected (LN311, 2006, Dec 7, :schedule III). The sites reported from Wied il-Kbir and Wied Speranza are new records and both harbour amongst the largest populations on the Maltese Islands.

2. *Rosa rubiginosa* L. (syn. *R. eglanteria* L.) [Figure 4C]

Distribution: Gozo: Wied il-Lunzjata (! 19-May-2010); Xagħra (Bakay et al., 2015), but no longer extant (see details below).

Habitat: Sheltered beside a rubble wall close to a maquis in the vicinity of a perennial valley.

Description (specimen examined #24, ref. Table 1): Suberect shrub, moderately branched up to 1.8 m long. **Stems** glabrous, straight or slightly arching, matte green becoming maroon-brown in areas exposed to direct sunlight with several prickles. **Prickles** narrow, straight to slightly curved then abruptly hooked at the tip, variable in size, 3–12 mm long with a 2–5 mm wide base, yellowish-pink, then maroon-red with a yellowish-green tip finally becoming light brown in old stems. Acicles present at random distribution on the stems, perhaps more frequent on young unweathered stems. **Leaves** imparipinnate, 5–11 cm long, with 5–7(–9) leaflets, light to medium green, glabrous (or puberulent) above, hairy below especially on the veins. **Leaflets** 1.5–2.5 × 1.0–1.8 cm, broadly elliptic to suborbicular, with a rounded base and obtuse to rounded tip; margin uniserrate or vaguely biserrate and with minute sessile or shortly stalked glands, also abundant on the abaxial surface and giving a characteristic of a fruity apple-like fragrant scent. **Stipules** 10–14 × 3–5 mm, oblong with two small dentate (-lanceolate) auricles each 2–3 mm long, margin entire and undented, lined with shortly stipitate glands and some pubescence from the lower surface of the stipule. **Rachis** pubescent, glandular, without prickles. **Inflorescence** solitary or up to three per branch. Buds pink to pale purple developing into pink flowers. **Corolla** single, moderately fragrant, 3.0–4.5 cm in diameter. **Sepals** 1.0–1.6 cm long, patent in flower, narrow linear-lanceolate tapering into an acuminate tip, weakly pinnatifid or with 2–3 pairs of small lobes; abaxial surface with numerous shortly-stipitate glands and sparsely minutely tomentose increasing in density towards the margin and lobes, adaxial surface entirely velutinose-pubescent and paler (hoary-grey) in colour. **Petals** 5, 10–12 × 10–12 mm, shorter from the sepals, purple-pink becoming paler at the base. **Hypanthium** fusiform, 6 mm long, generally glabrous; pedicels up to 10 mm long, densely glandular. **Stamens** numerous, up to 4–7 mm long, filaments and anthers golden yellow. **Styles** pilose, free but compact together in a short head, about 3—4 mm long and exserted through a small orifice about 1 mm wide in a flattened or shallowly concave disc; stigma pale green. **Hips** fusiform, bright red, glabrous, sometimes with some persistent sepals, although usually they fall off when fully ripe, 14–20 mm long, about 10 mm in diameter. Flowering between May and beginning of July.

Taxonomy: The hairy leaves with translucent glands emitting a scent of fresh apples, the slender prickles and the erect habit are important distinguishing characters for this species (Clapham et al., 1962; Klastersky, 1968; Pignatti, 1982; Silvestre & Montserrat, 1998; Graham & Primavesi, 2005). The closely related species *Rosa micrantha* Borrer ex SM. differs by having pedicels longer than 1 cm, glabrous styles, and very reflexed sepals which fall early after anthesis (Silvestre & Montserrat, 1998; Graham & Primavesi, 2005).

History: This species was first recorded by the present author from Wied il-Lunzjata, Gozo in May 2010 (Mifsud, 2010b). Another record was later reported from Xagħra (Bakay et al., 2015) providing a brief description and only a picture of two dry hips. Doubt about the authenticity of *R. rubiginosa* was risen from the illustrated subglobose shape of the hips, the long pedicels (approximately 2 cm from the scale provided) and that the inflorescences were produced in clusters of 2–7. In contrast, *R. rubiginosa* has elongated fusiform fruit on short pedicels (about 1 cm long) and inflorescences single or up to three flowers (Clapham et al., 1962; Graham & Primavesi, 2005). Owing to this uncertainty, two surveys at the location corresponding to the given GPS co-ordinates of this individual was carried out in September 2015 and May 2016, but no rose was found in that area.

Upon making contact with the corresponding author (L. Bakay), it was confirmed that the area where he found the rose was the same area searched by the present author and hence it was

deduced that this individual is not further extant. Bakay found and examined the rose in August 2013 (Bakay L. pers. comm., May 2016) when the flowers were already over. This entails that further examination of this specimen in flower to confirm *R. rubiginosa* or the closely related *Rosa micrantha* or perhaps a hybrid of *R. rubiginosa* is not possible. Owing to the experience on this genus of the authors (Bakay et al., 2015), this rose is best referred to as *Rosa* cf. *rubiginosa*. The location was very disturbed and no other rose species was found during two surveys about 150 m away from the location that Bakay et al. (2015) spotted this individual.

Status: New, unrecorded non-native species for the Maltese flora. Persisting, but not spreading vegetatively due to unsuitable habitat around. Very rare (one locality in Gozo).

3. *Rosa canina* L. [Figure 3A]

Distribution: GOZO: Wied ir-Riħan, Nadur, Gozo (! 10-May-2016).

Habitat: Damp sheltered area close to valley bed.

Description: Climbing to sub-erect shrub with few-branched stems shrub up to 2–3 m long. **Stems** glabrous, vivid green with few prickles at the lower parts of the stem, decreasing gradually towards the flowering parts. **Prickles** rather narrow, very curved with a hooked tip, 5–7 mm long with a broad base about 5 mm wide, pinkish-yellow then becoming grey. Acicles absent. **Leaves** imparipinnate, 8–12 cm long, (5)–7 leaflets, adaxial surface dark green, glabrous, slightly polished, abaxial surface similar but slightly paler. **Leaflets** 2.8–4.0 × 1.4–2.2 cm, narrowly lanceolate with a rounded or obtuse base and an acute to shortly acuminate tip; margin uniserrate all round; teeth alternating between two sizes, each with a tiny reddish gland at the tip (not always detected in mature leaves). **Stipules** 14–18 × 4–5 mm, oblong with two lanceolate auricles each 3–4 mm long, entirely green, margin partly revolute, sparsely glandular increasing in frequency towards the auricles, otherwise rather smooth. Rachis glabrous, green, occasionally with few pricklets. **Inflorescence** in 3–11 corymbose clusters. **Buds** rosy-pink developing into pale baby pink flowers. **Corolla** single, mildly fragrant, 4–6 cm in diameter. **Sepals** 2–3 cm long, reflexed in flower and fruit, narrow-lanceolate with a long acuminate tip which broadens again in some sepals, conspicuously lobed by 4–10 oblong or linear-lanceolate lobes (up to 6 mm long), the larger ones with few secondary lobes, abaxial surface glabrous, adaxial surface entirely velutinose-pubescent and paler (hoary-grey) in colour, sepal-lobes dotted with red glands (absent along the main margin). **Petals** 5, 13–17 × 10–13 mm, pale pink paler or white towards the base. **Hypanthium** ovoid with a small constriction below the calyx, 9–11 mm long, glabrous; pedicels glabrous 1–2 cm long. **Stamens** numerous, up to 10 mm long, filaments pale green, anthers golden yellow. **Styles** tightly packed but free, pubescent, short (c. 4 mm) and inserted in a flattened-conical disc through a small orifice about 1 mm wide, stigma amber-yellow, slightly swollen-coralloid, covering the hypanthium disc. **Hips** reddish brown, shortly ellipsoid to pyriform, glabrous, glossy, 15–24 mm long.

Taxonomy: The single pale-pink flower, multilobed sepals, red, curved prickles, free styles covering the hypanthium disc and the hairless leaflets with gland-tipped teeth are excellent matching characters for *R. canina* (Clapham et al., 1962; Klastersky, 1968; Pottier-Alapetite, 1979; Pignatti, 1982; Silvestre & Montserrat, 1998; Graham & Primavesi, 2005; Stace, 2010).

Status: Rediscovered species? The species is frequent and native in Sicily (Giardina et al., 2007) and perhaps the same may apply for Malta. This assumption would be strengthened if the old record of *R. canina* from Ta' Ċenċ (Gulia, 1872), also situated in Gozo, was autochthonous, but this population is no longer extant (Sommier & Caruana Gatto, 1915) and its status cannot be verified. Moreover, an unpublished record by Michael Briffa collected on May 1977 from the valley of Wied Inċita (pers. comm., 2007) appears to be autochthonous, but due to the lack of any recent sightings and because of the extensive quarrying and disturbance which took place at this site in the last few decades, it is also considered extinct by the present author. It cannot be completely excluded that this individual shrub at Wied Riħan, has escaped and persisted from abandoned cultivation, but it is unlikely because farmers used to cultivate more attractive and fragrant roses. With only one substantiated locality, the status of *R. canina* in Malta remains uncertain. Very rare (one locality in Gozo).

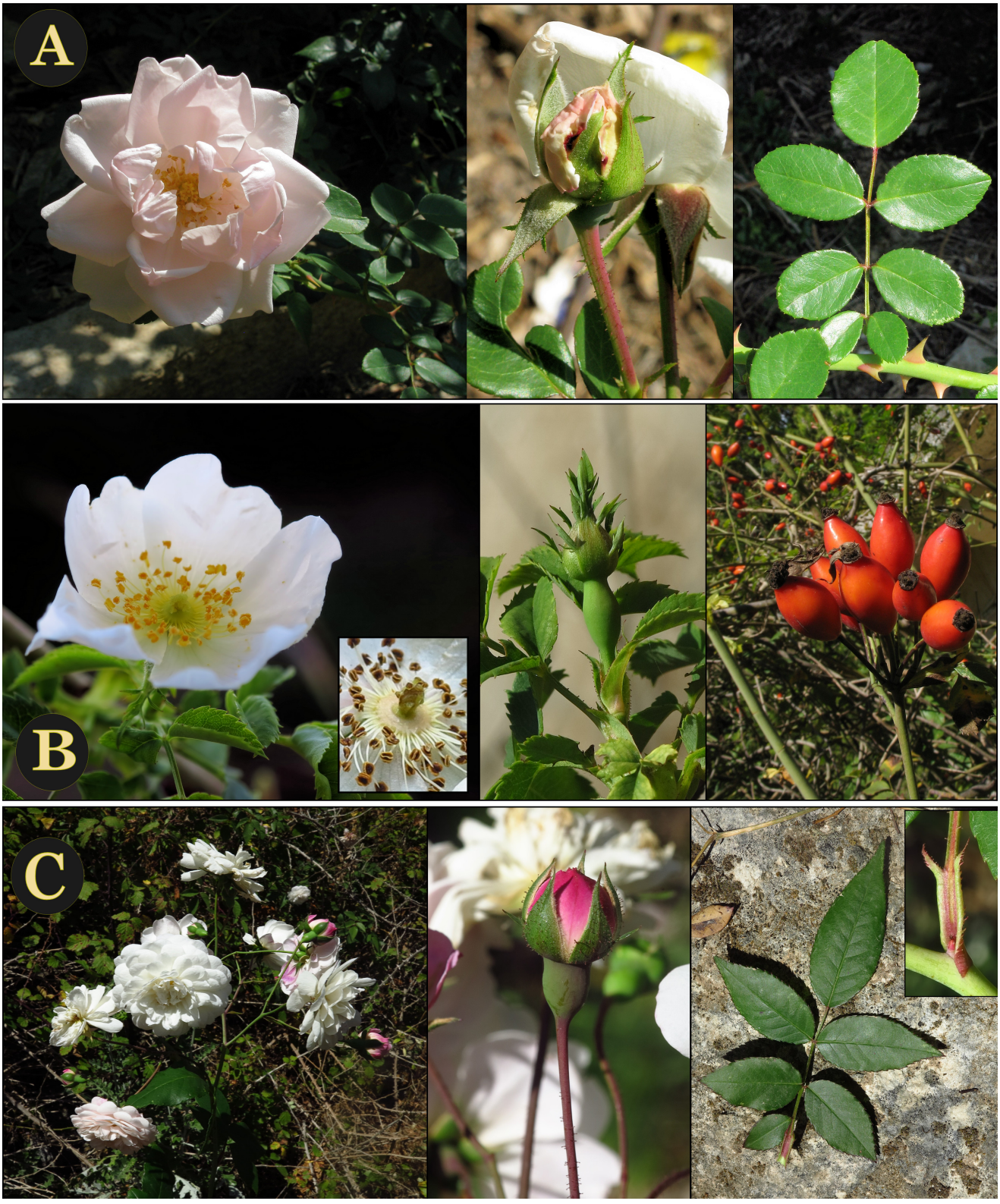

Figure 2: **A**. *Rosa* cf *luciae* s.l. unidentified left to right: corolla; buds showing sepals; leaf and prickles (Wied id-Dis, Għargħur, 3-May-2016). **B**. *Rosa stylosa* Desv.× *R. arvensis* Huds (= *R.* ×*pseudorusticana* Crepin ex Rogers) left to right: corolla (single); hypanthium and sepals; hips (Wied id-Dis, Għargħur, 3-May-2016). **C**. *Rosa* 'Adélaide D'Orleans' left to right: corymbose inflorescence; bud showing sepals (initially magenta then fading to white); leaf (Wied Mġarr ix-Xini, Ta' Sannat, Gozo, 10-May-2016). Photos Stephen Mifsud.

4. *Rosa gallica* L. s.l. [Figure 3B]

Distribution: Malta: Wied Speranza (! 19-Nov-2009).

Habitat: Rocky valley sides.

Description: Deciduous with many suckers and shoots spreading and forming an expanded cushion-shaped shrub not more than 1 m high. **Stems** 0.5–1.0 m, glabrous, pale to glaucous green with many prickles on all parts of the stem. **Prickles** thin, almost straight and linear, reddish-mauve, variable in size from pricklets of about 4 mm in length to larger prickles not more than 12 mm long, intermixed with many gland-tipped acicles (c. 2 mm long) and stiff stipitate glands (0.5 mm long). **Leaves** imparipinnate, 7–10 cm long, 3–5(–7) leaflets, glabrous, medium dark green above (fading with age), greyish or paler matte green below due to the presence of fine pubescence, which with age becomes restricted only on the main veins. **Leaflets** 2.8–5.5 × 1.8–2.5 cm, broadly elliptic with a rounded to subcordate base and an acute to blunt tip; margin crenate-uniserrate all round with tiny translucent glands at the lower half of the margin, emitting a resinous scent. **Stipules** 14–18 × 3–5 mm, oblong with two small rounded-lanceolate auricles each 2 mm long, entirely green, finely tomentose below (extending to the margins), margin lined by shortly-stipitate colourless or pale red glands. Rachis shortly tomentose below, unarmed, hoary green. **Inflorescence** single terminal flowers, rarely twos or threes subtended by a short green pedicel. **Corolla** single, moderately fragrant, 4–6 cm in diameter. **Sepals** 8–12 mm long, patent in flower, persistent but deciduous when hips mature, deltate gradually tapering to a shortly acuminate tip, margin with 1 or 2 pairs of distinct, linear-oblong lobes (up to 4 mm long); abaxial surface tomentose and densely glandular, adaxial surface entirely velutinose-pubescent and paler (hoary-grey) in colour. **Petals** 5 to 8, 3–4 mm wide, vivid purple fading to pink towards the base, overlapping, sometimes cup-shaped. **Hypanthium** ovoid with a small constriction below the calyx, 7–8 mm long, glabrous becoming hispid-glandular below; pedicels 1–2 cm long, with several red-stalked hispid glands of various sizes. Stamens numerous, up to 10 mm long, filaments and anthers golden yellow. **Styles** tightly packed but free, villous, short (c. 4 mm) and inserted in a flattened-conical disc through a small orifice about 1 mm wide, stigma yellow, slightly swollen-coralloid, partly covering the hypanthium disc. **Hips** bright red, ellipsoid to subglobular, hispid-glandular, 10–12 mm long.

Taxonomy: Identification through keys of Klastersky (1968), Silvestre and Montserrat (1998), Graham and Primavesi (2005), Stace (2010) led to *R. gallica*. The single terminal flowers (sometimes cup-shaped); broadly ovate leaflets, the numerous small prickles and acicles along the stems and the glandular pedicels with a resinous scent are typical characters of *R. gallica*.

There are some varieties with single or semi-double corolla, amongst these, the most popular are the cultivar *Rosa gallica* 'Complicata' (syn. *Rosa gallica* 'Ariana d'Algier'), but it is very fragrant and almost thornless; *Rosa gallica* 'Alain Blanchard' also with 5–10 petals but mottled and darker; and *R. gallica* 'Officinalis' which is a semi-double rose with more (10–16) petals (HMF, 2016). It is difficult to determine if the population found at Wied Speranza is a pure *R. gallica*, or one of its many varieties and cultivars. It has many distinct characters of *R. gallica* and the population is located in a very natural place, away from anthropogenic activity, but then, it has some flowers with 7 petals, although according (HMF, 2016), *R. gallica* can have up to 8 petals. Under these circumstances the wide sense of *R. gallica* is the best treatment for this rose.

History: *Rosa gallica* is one of the oldest roses, where its history is believed to date back to the year 1200 BC (Herbs2000, 2002–2016). Numerous hybrids and cultivars have been formed and now placed in what is known as the gallica cultivar group, where over 2000 gallica cultivars were commercially available at the beginning of the 19th century (Haynes, 2012b). The oldest and most common cultivar is *Rosa gallica* 'Officinalis', known as the Provence rose or Apothecary rose, the cultivation of which for cosmetic, medicinal and culinary use was documented since the 13th century (HistoricRoses, 2009). Much older is the Damask rose, a hybrid between *R. gallica* rose with *R. moschata* (= *R.* ×*damascena*), which is documented since 900 BC (Herbs2000, 2002–2016), and reported from the Maltese islands at least since the 16th century (see below).

Status: Persisting and naturalizing locally but non-invasive. Examined material was found in a rocky area along a valley side and appears to

be a very old population which has expanded vegetatively by underground suckers to a low shrub about 6–7 m across. It can even be an archeophytic survivor. Rare.

5. *Rosa* ×*damascena* Mill. (*R. gallica* × *R. moschata* (×*R. fedtschenkoana*)) [Figure 3C]

Distribution: MALTA: fields below Dwejra, close to main road to Mġarr village (! 28-May-2016, pers. comm. Brian Restall); GOZO: Wied ir-Riħan (! 07-May-2016, pers. comm Richard Grech); Għajn Qasab (! 07-May-2016, pers. comm Richard Grech). Also observed in a cultivated area named Ħal-Drago at Bidnija (! 28-May-2016, pers. comm. Brian Restall), and was regarded as an adventive specimen, although uncertain if it receives aid from man.

Habitat: Damp areas beside valley beds, tributary streamlets or damp cultivated fields with clayey soil.

Description (specimen examined #19, ref. Table 1): Erect, moderately long-branched shrub with straight branches up to 2–3 m long. **Stems** glabrous, pale green, old stems with longitudinal, pale brown, shallow scars (fissures), densely prickled, sometimes found in irregular patches. **Prickles** very narrow, almost straight to slightly curved, typically with various sizes from 1 mm to 7 mm long (base 0.5 mm to 3 mm), initially pinkish-salmon then becoming beige-gray. Pricklets and acicles present, randomly distributed with prickles more frequent along main stems and primary branches, acicles increasing on leaf rachis and petioles. **Leaves** imparipinnate, 8–14 cm long, (3)5–7 leaflets, adaxial surface bright green, glabrous, rugose and coarse, abaxial surface hoary and slightly greyish, covered with dense, fine hair less than 0.5 mm long, sometimes restricted on the veins. **Leaflets** 3.0–6.0 × 2.2–4.0 cm, broadly ellipsoid with rounded or sub-truncate base and an obtuse tip, crenate-uniserrate, margin hairy from pubescence of abaxial surface. **Stipules** 12–20 × 4–5 mm, oblong (slightly concave below auricles) with two lanceolate-acuminate auricles, each 3—5 mm long, pale green, abaxial surface pubescent, margin pubescent, entire with red sessile glands towards the auricles, partly involute. Rachis densely pubescent with few red stipitate glands and small pricklets at the leaf nodes below. Flowers in (1)3–6 corymbose clusters. **Buds** magenta-pink developing into rosy-purple flowers. **Corolla** semi-double or double, highly fragrant, 5–8 cm in diameter, single or in few-numbered corymbs. **Sepals** 2–3 cm long, reflexed or patent-reflexed in flower and fruit, narrow-lanceolate with a long acuminate tip, conspicuously lobed by (0)1–3(5) narrow long lobes, (sometimes these with a secondary small teeth), abaxial surface pubescent becoming more dense towards the margin and with numerous red stipitate glands, adaxial surface entirely velutinose-pubescent and paler (hoary-grey) in colour. **Hypanthium** elongated-ellipsoid with a small constriction below the calyx, 10–14 mm long, hairless but with numerous red stipitate glands especially at the base; pedicels distinctly long (about 3–4 cm) with many stipitate glands. **Stamens** numerous, 3–8 mm long, with white filaments and amber-yellow anthers. **Styles** free, 3–5 mm long, densely pubescent overtopping and covering the hypanthium disc below, stigma pale green, slightly swollen-coralloid, orifice wide, circa 2 mm. After anthesis, the immature fruit, has a distinctive fragrant scent similar to pine resin. **Hips** ovoid-elliptic, glabrous, vivid red 15–20 mm long, 8–11 mm across. Flowering between (April) May-June.

Taxonomy: The influence of *R. gallica* in the material examined is evident, from the numerous, straight prickles of various sizes, the lobed to pinnatifid sepals with red stiff glands, and glandular pedicels, sepals and stipule margin. However, it differs by having double-flowers, a deep stronger fragrance and a more erect non-suckering habit. A well-known, strongly musk-scented rose is *R.* ×*damascena*, a hybrid between *R. gallica*, *R. moschata* and possibly *R. fedtschenkoana* (Haynes, 2012a), which most of its diagnostic characters matched with the specimens examined. Another well-known gallica hybrid is *R.* ×*centifolia* L., but this rose has flowers with much more numerous petals (more than 100), stronger pinnatifid sepals and usually a characteristic moss-like growth on the pedicels and sepals (Graham & Primavesi, 2005; HMF, 2016). Flowers of examined specimens had 60–80 petals and lacked the moss-like growth on the pedicels, hence none are attributed to *R.* ×*centifolia*.

History: *R.* ×*damascena* is one of the oldest and most successful hybrid roses, which was introduced from Syria and then cultivated in most of southern Europe. It is thought that the crusader Robert de Brie introduced it sometime between 1254 and 1276, although other routes of introduction have

also been hypothesised (Haynes, 2012a). Having attractive and fragrant flowers, roses were amongst the first plants reported from Malta, and the cultivation of what was known as "*Rose di Malta*" (= Maltese roses) goes back to the 16th century by the ancient French writer, Jean Quintin (1500–1561) cited later by Gianfrangisk Abela in 1647 (Ciantar, 1772, update reprint of Gianfrangisk Abela). According to Borg (1927), the Maltese roses are a fragrant hybrid of *R. gallica* and were common in cultivation at that time. Their cultivation seems to have been replaced gradually by the importation of different and fancier roses and additionally, decreasing rapidly by the destruction or development of old gardens.

Status: Persistent, non-native hybrid with few populations questionably adventive (e.g. at Ħal-Drago, Bidnija). Being already established and popular in Malta in the 16th century, and considered as a very old rose, it can be assumed that the Damask rose is an archaeophyte. Rare in the Maltese Islands, but more individuals are possibly present in private fields and farms and ancient gardens from old cultivations.

6. *Rosa* 'Albéric Barbier' (*Rosa luciae* × *Rosa* 'Shirley Hibberd') [Figure 1B]
Barbier Frères & Compagnie, 1900 (France)

Distribution: MALTA: Wied il-Qlejgħa (! 5-May-2006); rubble wall between Ħaġar Qim and Mnajdra Temples, Qrendi (! 26-Jan-2008); Marsa, along road to old Power Station (! 7-May-2009); Għargħur, close to Wied il-Faħam (! 3-May-2016); Fawwara chapel (! 22-May-2016, pers. comm. Edwin Lanfranco), Tarġa Gap, Mosta (! 28-May-2016, pers. comm. Brian Restall). Also observed in private cultivation and overhanging a wall at Santa Luċia street, Mtarfa (! 21-May-2016) as well as in fields close to Santa Luċija chapel, Mtarfa (! 21-May-2016, pers. comm. Carmen Chetcuti), which are regarded as cultivated or receiving the attention of man.
Habitat: Rambling on vegetated valley sides or old walls, over rubble walls or spreading on disturbed garigue. They are likely to be introduced from propagules that may have been dumped or planted long time ago and naturalising vegetatively. In the UK, this rose is reported to become naturalised in open ground and in scrub, usually near the sea (Stace, 2010).

Description (specimen examined #10, ref. Table 1): Evergreen, profusely branched, rambling or prostrate shrub with its branches trailing up to 5 m long. **Stems** glabrous, bright green turning maroon at exposed parts or terminal branches, variably prickled, scarcely so along the young stems where only a pair of prickles are usually present just below the leaf node. **Prickles** narrow, slightly curved, reddish-maroon, 5–8 mm long with a 5 mm base. Acicles absent. **Leaves** imparipinnate, 8–14 cm long with (5)7 leaflets, glabrous, bright medium green and very glossy on both sides, petioles often reddish. **Leaflets** 4.0–6.5 × 2.0–2.8 cm, ovate with an obtuse-cuneate base and a rather acute tip, crenate-uniserrate at the upper two-thirds of the leaf margin. **Stipules** 15–20 mm long, linear-oblong with two lanceolate-acuminate auricles each 4–6 mm long, margin with herbaceous linear teeth, up to 2 mm long with reddish-maroon tips, rarely subglandular. Rachis maroon, with several pricklets below, 0.3–0.9 cm long. **Inflorescences** in few-numbered corymbose clusters, rarely single. Buds pale yellow or champagne, sometimes with brownish edges if exposed to wind. **Corolla** double, slightly fragrant, variable in size, 3–6 cm in diameter, white-cream with a pale yellow centre, which gradually fades to a complete white flower when fully mature; in rare occasions, outermost petals stained pink. **Sepals**, reflexed, narrowly lanceolate-deltate, subacuminate, coriaceous, abaxial surface glabrous becoming densely felty-pubescent towards the margin, adaxial surface entirely pubescent and paler (hoary-grey) in colour, margin entire often with 1–2 small linear lobes or appendages, up to 3 mm long. **Hypanthium** globose, 4–7 mm long, glabrous; pedicels reddish-maroon (less so if in a shaded location), glabrous or occasionally with few short acicles. **Stamens** numerous, short, pale mustard yellow, encircling the hypanthium margin. **Styles** free or subfused at the base, 8–13 mm long, densely pubescent, stigma glabrous, pale green, coralloid. **Hips** deciduous, falling early. Flowering between (April–) May–June.

Taxonomy: The prostrate rambling habit, the evergreen, glabrous glossy leaves, red-maroon petioles and pedicels (less so in shaded habitats), and the subfused styles are key to *Rosa luciae* Franchet & Rochebr (= *Rosa wichuraiana* Crép.) (Graham & Primavesi, 2005), but the double flowers and their styles, which are not completely fused, suggest one of its more popular hybrids. The 'Albéric Barbier' rose is the best matching cultivar (Grant, 2000; Stace, 2010; Chapman,

2012; HMF, 2016), but the *Rosa* 'Aviateur Blériot' (*R. luciae* × 'William Allen Richardson') (Fauquel, 1909) is a suitable alternative although this has coppery orange buds which blossom to larger flowers stained in a more vivid yellow (Grant, 2000; RHS, 2016; HMF, 2016). The population at Fawwara was slightly different in being more vigorous, thicker stems and larger leaves, prickles and flowers. These differences are most probably owned to the fact that specimen was found growing close to a water reservoir and hence in a more fertile ground, although it can be attributed to the closely related hybrid *Rosa* 'Gardenia' (*R. luciae* × 'Perle des Jardins') (Horvath, 1899), which is described to be more vigorous and with undulated leave margins as in the specimen, but the flowers were not strongly fragrant that the 'Gardenia' is renowned for (Grant, 2000; HMF, 2016, 2016; VRA, 2016).

History: The 'Albéric Barbier' rose is a common hybrid from *R. luciae*, which was bred in 1900 by the Barbier's nursery and generated by a cross between *Rosa luciae* and 'Shirley Hibberd'. 'Shirley Hibberd' is an 1874 Tea rose, bred by Antoine Levet in Lyon and named after James Shirley Hibberd (1825–1890), who was one of the most popular and successful gardening writers of the Victorian era (Chapman, 2012).

Status: New, unrecorded, non-native taxon for the Maltese flora. Persisting and well established rose, spreading and naturalizing locally in some populations but not invasive. Scarce (at least six populations in the wild).

7. *Rosa* 'Léontine Gervais' (*Rosa luciae* × 'Souvenir de Catherine Guillot') [Figure 1C]
Barbier Frères & Compagnie, 1903 (France)

Distribution: MALTA: il-Qaws, Dingli (! 22-May-2016, pers. comm. Edwin Lanfranco).

Habitat: Terrace and wall of an abandoned building with shoots naturalized in footpath and disturbed steppic ground several meters away. Building possibly still visited by people but the rose is completely abandoned with naturalized shoots emerging and spreading in the surrounding natural habitats.

Description: Same as **2** above but with smaller leaflets (3.0–5.0 × 2.0–2.8 cm) and stipules (13–17 mm). Stipule teeth smaller (<1 mm) with a glandular tip which is conspicuous in young leaves and then weathers away. Buds rose-purple. Corolla semi-double, 5–8 cm in diameter, salmon pink flushing gently to yellowish tones at the centre, becoming pale pink when mature, loose-petalled and open at the centre to expose their colourful stamens and styles; quite fragrant with a fruity scent. Sepals without lobes or if present much reduced to tiny linear appendages. Stamens longer (up to 13 mm) pinkish yellow, bearing amber-orange anthers. Styles free and densely pubescent, but conspicuously deep red at the upper half tipped with an amber-orange stigma. Hips deciduous (not observed). Flowering between May–July.

Taxonomy: The resemblance with the Wichuraiana ramblers (*R. luciae* hybrids) such as *Rosa luciae* × 'Shirley Hibberd' described above is evident in many morphological characters, most important being in its habit, glossy glabrous leaves, stipules, sepals, free hairy styles, reddish petioles and fruit falling prematurely. Only the colour of the corolla, stamens and styles and the stronger fragrance of the flower were essentially different. Hybrids of *R. luciae*, which display this flower colour are *Rosa* 'Leontine Gervais' (*R. luciae* × 'Souvenir de Catherine Guillot') and *Rosa* 'René André' (*R. luciae* × 'L'Ideal'). According to Grant (2000), HWR (2009), Chapman (2012), the rambling Wichuraiana rose studied from Dingli corresponds best with 'Leontine Gervais'.

History: This rambler was created by Barbier and Compaigne of Orleans in 1903 (Grant, 2000). Its name is thought to be dedicated to someone who worked in the Barbier nursery (Chapman, 2012).

Status: New, unrecorded, non-native taxon for the Maltese flora. Persisting and well established rose spreading vegetatively to the surrounding natural habitats, but not invasive. Very rare (one locality).

8. *Rosa* × *pseudorusticana* Crepin ex Rogers (*Rosa stylosa* × *R. arvensis*) [Figure 2B]

Distribution: MALTA: Wied id-Dis (! 1-Jun-2006).

Habitat: Abandoned cultivation in a terrace of an unused dwelling close to valley bed.

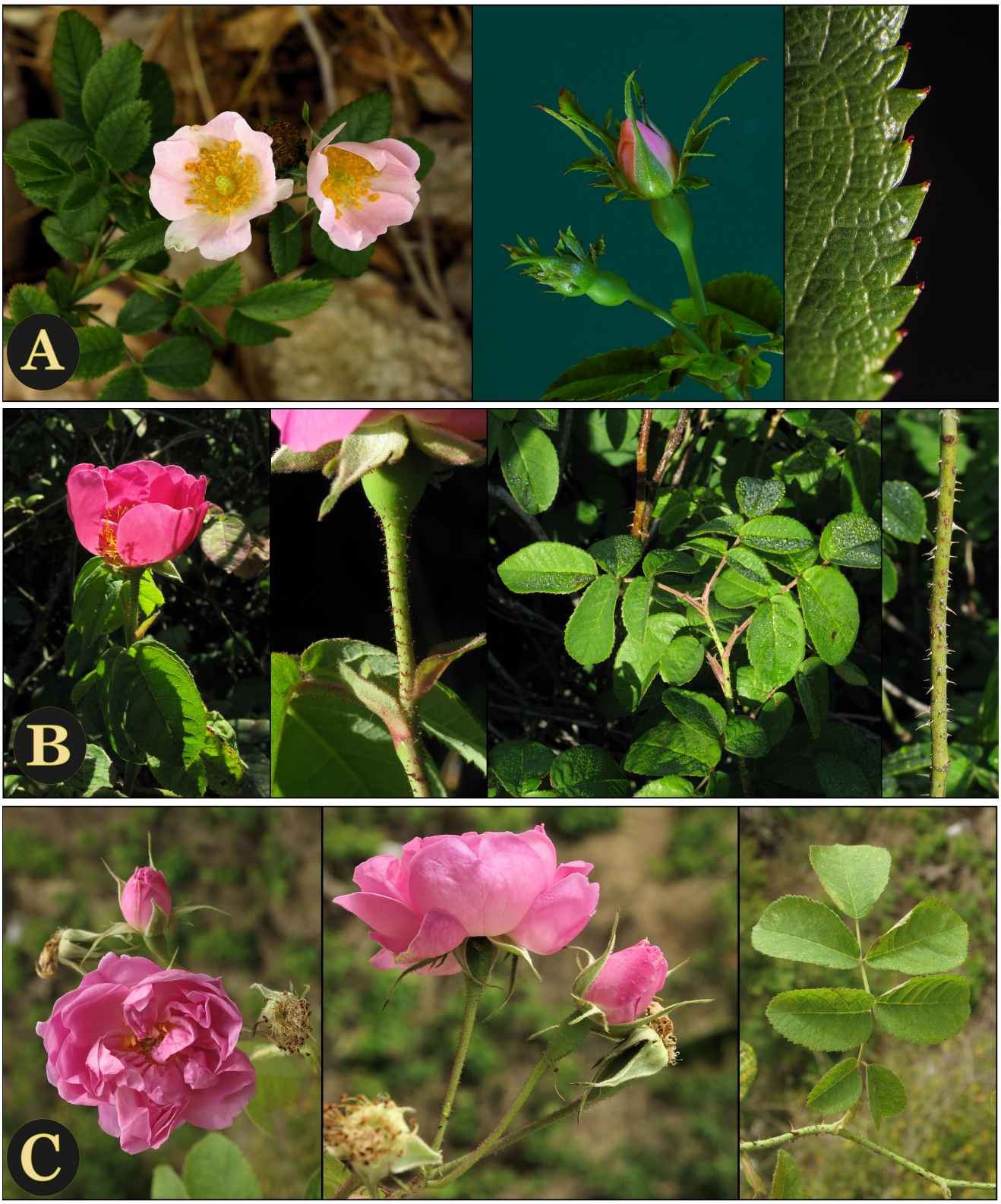

Figure 3: **A**. *Rosa canina* L. left to right: inflorescences; calyx showing lobed sepals; leaf margin showing terminal astipitate red gland at the tip of teeth (Wied ir-Riħan, Nadur, Gozo, 10-May-2016). **B**. *Rosa gallica* L. left to right: inflorescence; pedicel and hypanthium with red stipitate glands; broad, ovate leaves; stem showing its numerous prickles, pricklets and acicles (Wied l-isperanza, Mosta, 19-Nov-2009). **C**. *Rosa* ×*damascena* Mil. left to right: inflorescences; pedicel, hypanthium and lateral view of corolla; broad, ovate leaves (Wied ir-Riħan, Nadur, Gozo, 10-May-2016). Photos Stephen Mifsud.

Description: Deciduous sub-erect shrub with straight to arching stems, about 1–2 m long, few-branched. **Stems** and branches glabrous, greyish-green, rigid to moderately flexuous. **Prickles** very few, narrow, slender, slightly curved sometimes almost straight, pale brown, 4–5 mm long with a 3–4 mm base. Acicles. **Leaves** imparipinnate, 4–5(6) cm long with 5 leaflets, glabrous, glossy dark green above, matte greyish-green below. **Leaflets** (12)15–20 × (10)12–15 mm, broadly ovate with a rounded or broadly cuneate base and an acute tip, margin uniserrate, teeth large at the apical third then gradually diminishing towards the base. **Stipules** glabrous, 10–13 × 5–7 mm, oblong to broadly fusiform, margins lined with spaced stalked red glands, auricles divergent, narrow-deltate, 3–4 mm long. **Corolla** single, pure white, 4–5 cm across, mildly fragrant. Pedicels and hypanthium glabrous. **Sepals** narrow lanceolate, lobed, especially around the acuminate tip, strongly reflexed at flowering, 15–17 × 4–5 mm (not including the lateral lobes), abaxial side glabrous, adaxial surface finely velutinose. **Stamens** numerous of unequal length ranging between 3–10 mm long, filaments pale green, anthers golden yellow. **Styles** connivent-fused in a very short, 3–4 mm long column with bulging pale green glabrous stigma. **Hips** dark red, fusiform, sepals caducous, smooth and glossy, 10–13 mm across and 18–25 mm long. Flowering between May and June.

Taxonomy: This single-flowered rose has characters of both putative parents, most importantly the narrow prickles, white flowers, rounded leaflets and lobed sepals of *R. arvensis* Huds. and the elongated, larger fusiform hips and fused styles of *R. stylosa* Desv. The styles of some flowers were subfused at the base or tightly clumped, which became loose when gently pulled apart, rather heterogenous between different flowers. This is considered as an intermediate character between the free styles of *R. arvensis* and fully-fused in *R. stylosa*. Moreover, the eglandular, large and coarsely uniserrated margin of the leaflets are also considered as a good character of this hybrid (Stace, 2010; Graham & Primavesi, 2005).

Status: New, unrecorded, non-native taxon for the Maltese flora. Perhaps best assigned as a persisting non-native rose since no influx of human activities have been detected, but not invasive. Very rare (one locality).

9. *Rosa* 'Adélaide D'Orleans' (*Rosa sempervirens* × 'Old Blush') [Figure 2C]
Jacques, 1826 (France)

Distribution: GOZO: Mġarr ix-Xini (! 26-Dec-2005).

Habitat: Lower parts of a damp, sheltered valley side with *Rubus ulmifolius* L.

Description: Evergreen, profusely branched, rambling to prostrate shrub with its branches trailing up to 6 m long. **Stems** glabrous, bright green turning maroon at exposed parts and pedicels, scarcely to moderately prickled unevenly. **Prickles** narrow most of their length then expanding abruptly to a wide base, slightly curved, dull brown with a paler copper-brown terminal half, 5–8 mm long with a 5–9 mm base. Acicles absent. **Leaves** imparipinnate, 9–12 cm long with 5 (7) leaflets, glabrous, adaxial surface glossy, abaxial surface matte and slightly grayish. **Leaflets** 3.5–6.0 × 1.7–2.5 cm, the terminal conspicuously larger, lanceolate with an obtuse or rounded base and an acute-subacuminate tip, crenate-uniserrate around the entire margin. **Stipules** 12–16 mm long, linear-oblong with two lanceolate-acuminate auricles each 4–5 mm long, green or maroon, margin with herbaceous linear teeth up to 2 mm long with reddish tips (or entirely red), rarely with stipitate glands at the base or auricles. Rachis reddish-maroon, with few pricklets below at the leaflet nodes, c. 4 mm long. **Flowers** in many-numbered corymbose clusters, usually more than 10 and up to 25. **Buds** rosy-pink developing into white flowers with pinkish petals at the base. **Corolla** double, very slightly fragrant, 4–8 cm in diameter, larger in few-numbered corymbs. Petals numerous, white or with a very faint pink hue. **Sepals** patent in flower, becoming partly reflexed in fruit, lanceolate with a subacuminate tip, unlobed or one pair of very narrow lobes, abaxial surface with many, unevenly distributed stalked glands, adaxial surface entirely velutinose-pubescent and paler (hoary-grey) in colour, margin, velutinose-pubescent. **Hypanthium** oblong with a rounded base, 7–8 mm long, glabrous, pedicels with sparse stalked glands, maroon, distinctly long (about 3–4 cm). **Stamens** numerous, short, pale green filaments with yellow anthers. **Styles** mostly free but clumped very close together, few styles fused at the base, 5 mm long, sparsely pubescent, stigma glabrous, pale green, coralloid. Hips absent.

Taxonomy and History: This rose was identified as 'Adélaide D'Orleans', one of the many hybrids produced from *Rosa sempervirens* known as Sempervirens hybrids or Sempervirens ramblers. These hybrids have evergreen dark leaves, whitish flowers, yellow stamens and stipules very similar to *R. sempervirens*. 'Adélaide D'Orleans' has a characteristic small rosy-pink buds, which fade to a white corolla when mature (Phillips & Rix, 1993; Grant, 2000). It was bred in 1826 by Antoine A. Jacques, head gardener to the Duc d'Orléans at Château de Neuilly (1824 to 1832) by crossing the Southern Europe species Rosa sempervirens with a Chinese Tea Rose called 'Old Blush' (Phillips & Rix, 1993). He also produced a similar hybrid named 'Félicité et Perpétue' by crossing *R. sempervirens* with another Chinese rose named 'Noisette' (Phillips & Rix, 1993), but this is described to have a strong musk fragrance and smaller flowers (Haynes, 2012c). 'Félicité et Perpétue' could be a suitable alternative where its distinction from 'Adélaide D'Orleans' is minimal, but the latter is preferred for this rose found in Gozo for lacking the musky fragrance. *Rosa* 'Blush Noisette' (*Rosa* ×*noisettiana* Thory) was also considered, but similarly this is a highly fragrant rose, and differs in being smaller shrub and its flowers are more pinkish.

Remark: This rose is found situated very close to a water pumping station built by the British at the beginning of the 20th century at the bottom of the valley of Mġarr ix-Xini. It is assumed that it has been planted by English servicemen and naturalising in the sheltered sides of this valley. Its current spread reaches some 25×15 m rambling and competing with the common and native bramble *Rubus ulmifolius* L.

Status: New, unrecorded, non-native taxon for the Maltese flora. Persisting and well established rose spreading vegetatively to the surrounding natural habitats, but not invasive. Very rare (one locality in Gozo).

10. *Rosa* 'Excelsa' (*R. luciae* × 'Crimson Rambler') [Figure 4A]
Michael H. Walsh, 1908 (United States)

Distribution: Malta: Fawwara area (! 16-Jun-2010).

Habitat: Disturbed weedy area along roadside.

Description: Trailing and rambling shrub with profusely branched stems up to 4 m long. **Stems** glabrous, finely striated, bright to pale green with few prickles. **Prickles** narrow, slightly curved, 4–6 mm long with a 4 mm wide base, yellowish green with a pinkish hue then becoming straw-coloured. Acicles absent. **Leaves** imparipinnate, 7–11 cm long, with 7(–9) leaflets, light to medium green, glabrous, slightly polished on both sides. **Leaflets** 2.0–3.0 × 1.2–1.6 cm, narrowly lanceolate with a cuneate base and an acute tip; margin uniserrate all round; teeth slightly appressed with margin, eglandular. **Stipules** 12–16 × 4–5 mm, oblong with two dentate(-lanceolate) auricles each 3–6 mm long, entirely green, margin partly raised up, entirely lined with long, linear herbaceous, red-tipped teeth up to 3 mm long and with stipitate red glands (up to 0.5 mm long) alternating between the teeth. **Rachis** with few pricklets (2 mm long) below and occasionally with few stipitate glands above. **Inflorescence** at terminal branches in 8–20(28) corymbose clusters. **Buds** pale rosy-pink developing into deep magenta flowers. **Corolla** double, mildly fragrant, 3.0–4.5 cm in diameter. Petals pink flushing abruptly to white and finally to a narrow yellow border at the base. **Sepals** 0.8–1.2 cm long, semi-reflexed in flower, lanceolate with a caudate-acuminate tip, unlobed or with one (or rarely two) pair of linear lobes about 3 mm ling; abaxial surface with numerous stipitate, red glands and sparsely minutely tomentose increasing towards the margin, adaxial surface entirely velutinose-pubescent and paler (hoary-grey) in colour. **Hypanthium** ovoid, 5 mm long, generally glabrous; pedicels about 2–4 cm long, glabrous or with few stipitate glands. **Stamens** numerous, up to 5–10 mm long, filaments white, anthers golden yellow. **Styles** free, slightly pubescent, exserted by 4–5 mm through a small orifice (1 mm wide) of a flattened conical disc; stigma green, slightly swollen-coralloid. **Hips** not observed, possibly caducous. Hypanthia and pedicels of several flowers attacked by mildew. Flowering between the end of May and beginning of July.

Taxonomy: Several characters matched with *Rosa multiflora*, where the most important similarities were the corymbose sprays of numerous flowers, the glandular and dentate stipules, the glandular sepals and subglabrous styles (Klastersky, 1968; Graham & Primavesi, 2005). However, owing to its double flowers, this individual is clearly a hybrid. The best matching hybrid was found to be the *Rosa* 'Excelsa' (also known as the Rosa

'Red Dorothy Perkins'), involving the parent *R. wichuraiana* (= *R. luciae*) from which it attains its glossy, glabrous and perennial leaves and the red-maroon petioles and pedicels. According to HMF (2016), this popular rose is a hybrid formed between *Rosa wichuraiana* × 'Crimson Rambler', the latter being a multiflora hybrid cultivar. The flowers of this hybrid has a conspicuous whitish centre and few to several white streaks at the outer parts of the petals, both of which were observed in the studied specimen. *Rosa* 'Super excelsa' (= *Rosa* 'Super Dorothy Perkins') is another hybrid involving *R. luciae* and *R. multiflora*, which is similar to the rose found at Fawwara, but it is reported to form larger flowers and hence much more conspicuous sprays and a more intense scarlet colour.

History: Michael H. Walsh in Massachusetts used both *R. multiflora* and *R. wichuraiana* in his breeding program from which he introduced the *Rosa* 'Excelsa' rambler in 1909. It set a new standard for Wichuraiana hybrids and it was soon grown everywhere, for it was healthier and easier to train than 'Crimson Rambler' but it is susceptible to mildew (Scaniello & Bayard, 1994) in HMF (2016). It has a natural weeping habit and became an important rose standard for producing weeping roses (Redell, 1998).

Status: New, unrecorded, non-native taxon for the Maltese flora. Persisting and well established individual, spreading locally and naturalizing vegetatively but not invasive. Very rare (one locality).

11. *Rosa* 'Juliana von Stolberg' (*Rosa multiflora* × *Rosa* 'Rush') [Figure 4B]
Louis Lens, 1924–2001 (Belgium, before 1999)

Distribution: Malta: Fawwara area (! 26-Jun-2016, pers. comm. R. Bartolo).

Habitat: Disturbed weedy area in dirt and rubble along roadside.

Description: Same as above in many critical characters, but more robust with thicker stems and larger **prickles**, up to 11 mm long, and leaves with larger leaflets (7–9 per leaf) measuring 3–4 × 1.8–2.2 cm, similarly glabrous, glossy and crenulate-dentate. **Stipules** up to 20 mm long with the same margin morphology bearing linear herbaceous teeth and stipitate glands, somewhat the teeth longer and the glands more proliferous, even present the margin of the teeth. **Corymb** of 8–15 flowers, which differ by being single (constantly 5-petals), slightly larger (4–5 cm across) and purple pink with a wide white centre. **Hypanthium** and **pedicels** with several stipitate glands, becoming more numerous on the sepals, also like 10, partially reflexed (diagonal orientation) to reflexed and lacking lateral teeth or projections at the margin. Hypanthium is much different, fusiform, 12 mm long rugose and glandular. **Stamens** and **styles** same as 10. **Hips** (of previous year), reddish-brown, fusiform, punctate, possibly remains of the stipitate glands, and 14–18 mm long and 10 mm in diameter. Flowering between June and beginning of July.

Taxonomy: The spray of flowers, the glandular and dentate stipules, the glandular sepals and the fused styles in a shortly exserted column were judged to be a character set of *Rosa multiflora*. However, the vivid purple-pink colour of the flowers and the glossy, evergreen leaves suggested hybridism. Amongst the multiflora hybrids, the 'Juliana von Stolberg' (also known as the 'Super Red Dorothy Perkins') was found to be the most suitable name for this rose, owing to its large, glossy evergreen leaves with shortly acuminate tips and single flowers with a white centre. Other names attributed to this rose are 'Juliana' and 'LLX 8868' (HMF, 2016).

History: Countess Juliana von Stolberg was born in 1506 and on the occasion of her 500th anniversary a rose was named after her and planted in the German town Stolberg in the Harz Mountains (HMF, 2016).

Status: New, unrecorded, non-native taxon for the Maltese flora. Could be still adventive due its location over and scrambling down a rubble wall of a cultivated field, but it appears to be completely neglected with new shoots found in the ground away from the wall making it to be more likely a persisting non-native rose. Very rare (one locality).

12. *Rosa* cf *luciae* s.l. [Figure 2A]

Distribution: MALTA: Wied id-Dis (! 1-Jun-2006).

Habitat: Abandoned cultivation in a terrace of an unused dwelling close to valley bed.

Figure 4: **A**. *Rosa* 'Excelsa' left to right: inflorescence; leaf; detail of stipule; detail of sepals (Fawwara, Siġġiewi, 22-May-2016). **B**. *Rosa* 'Juliana von Stolberg' left to right: inflorescence (Fawwara, Siġġiewi, 21-Jun-2016); sepals; stipule (Fawwara, 02-Jul-2016). **C**. *Rosa rubiginosa* L. left to right: corolla; bud showing hypanthium and sepals (Wied il-Lunzjata, Gozo, 19-May-2010); hips (Wied il-Lunzjata, Kerċem, Gozo, 17-Jan-2014). Photos by Stephen Mifsud except first photo of 4B who was taken and kindly supplied by R. Bartolo.

Description: Deciduous, sub-erect, few-branched shrub with straight to slightly curved branches. **Stems** greyish-green, glabrous, moderately prickled but rather numerous on the lower branches. **Prickles** narrow, slightly curved or almost straight, beige-brown becoming grey with age, 4–7 mm long with a 6–9 mm base. Acicles absent or very few on the pedicels. **Leaves** imparipinnate, 4.0–7.5(9.0) cm long with 5 leaflets, glabrous, bright green and glossy on both sides. **Leaflets** (15)20–30 × (12)18–25 mm, suborbicular to broadly ovate with a rounded base and an obtuse-rounded tip; margin uniserrate almost around entire leaf, eglandular. **Stipules** glabrous, 10–16 × 3–4 mm, linear-oblong; margins smooth at lower half then becoming lined with stipitate glands towards the apex, auricles divergent, narrow-deltate, 4–6 mm long. **Flowers** double, white with a hint of pink, 4–6 cm across, and faintly fragrant. **Pedicels** with stiff glandular hair about 0.5 mm long, hypanthium glabrous. **Sepals** lanceolate with an acuminate tip, reflexed at flowering, margin with 1 or 2 pairs of teeth, 15–20 × 5–7 mm, abaxial side glabrous becoming velutinose towards the margin, adaxial surface entirely finely velutinose. **Stamens** numerous, 5 mm long, filaments white, anthers yellow to tobacco brown. **Styles** free, 4 mm long, glabrous; stigma capitate-coralloid, pale-green, glabrous. **Hips** dark red subglobular to pyriform with persistent reflexed sepals, smooth, 8–12 mm across and 10–16 mm long. Flowering between May and June.

Taxonomy: This rose was first encountered in May 2009 and at the time had many mature flowers. When the specimen was re-examined more critically in May 2016, it had only one wind-damaged flower. It appeared to be another Wichuraiana rambler rose, but a concrete identification could not be reached due to lack of representative flowers.

Status: Persisting, non-native rose since no influx of human activities have been detected, but not invasive. Very rare (one locality).

4 Conclusion

This preliminary survey on wild roses has led to important updates to the native and alien flora of the Maltese Islands. Seven new taxa have been added to the Maltese alien flora, *Rosa canina* and *R. gallica* have been rediscovered, and new records of *R. sempervirens* and *R.* ×*damascena* are also reported (see Table 2). Over 24 roses have been found to occur naturally, of which many were rambler roses. Eight ramblers were Wichuraiana hybrids ('Albéric Barbier', 'Léontine Gervais' and 'Excelsa'), one Multiflora hybrids ('Juliana von Stolberg') and one Sempervirens hybrid ('Adélaide D'Orleans'). *Rosa rubiginosa* and *R.* ×*pseudorusticana* have also been discovered from Wied il-Lunzjata (Gozo) and Wied id-Dis (Malta) respectively. Only one individual from Wied id-Dis has not been identified.

This account widens the knowledge of the non-native flora of the Maltese islands and the distribution of native or historically-recorded roses. Moreover, once these roses are identified, they can easily be employed in landscaping management due to their survival and growth in the wild. This study hence provides names of roses are likely to be successful and low-maintenance, suitable for the Maltese landscape. It can be concluded that none of the reported roses are invasive and that the Wichuraiana hybrids are very successful in Malta. This species is also one of the main progenitors of many hybrid cultivars of roses in the Iberian peninsula (Silvestre & Montserrat, 1998).

Although great effort has been made to provide a concrete identification, the present author is not an experienced rhodologist. This account shall hopefully attract the attention of experts of roses to improve the taxonomic or cultivar identification reported here. Finally, this study may perhaps help international rhodologists to recover some old roses, which may have become lost or very rare in cultivation and hence can be recovered from suitable propagation methods from these naturalized refugia, given that some of these roses may have been imported many decades ago during the British rule.

Other wild populations of roses are present in the Maltese islands, for example some material collected by Edwin Lanfranco and Michael Briffa some 30 years ago (pers. comm. Edwin Lanfranco, June 2016), need to be examined and checked if they still occur in Maltese natural habitats. A few other populations found by the present author lacked flowers during this survey and this needs to be studied in an investigation planned for the coming few years, as a sequel and update to this preliminary.

Acknowledgements

I am immensely grateful to the following contributors who have informed me about the occurrence of wild roses which were examined in this account: Bartolo R., Chetcuti Carmen, Chirchop Anthony, Grech Richard, Lanfranco Edwin, Mifsud Owen, and Restall Brian. I would also wish to thank Bakay László, Briffa Michael and Lanfranco Edwin and for their personal communications which I have made reference to. Further comments by Edwin Lanfranco on the overall works was also appreciated.

Table 2: Species, bi-parental (simple) hybrids and complex hybrids (cultivars) occurring naturally in the Maltese islands, and their local distribution and status.

#	Taxon	Hybrid and group	Distribution	Status (refer to Nesom, 2000)	Fig.
1	*Rosa sempervirens* L.	NO	MALTA: Buskett Woodland, Wied Inċita, Wied Għar Dalam, Wied il-Għasel (iż-Żenqa), Wied Anġlu, Wied Ħażrun, Ta' Baldu, Santa Katarina, scree below Inquisitor's Palace, Laferla Cross (overlooking Wied Fulija), Wied Għomor, Wied Speranzaˆ, Wied il-Kbirˆ. GOZO: "Migiarro" (extinct)	Native	1A
2	*Rosa rubiginosa* L.	NO	GOZO: Wied il-Lunzjata	Persisting	4C
3	*Rosa canina* L.	NO	GOZO: Wied ir-Riħan, Nadur.	Native (?) or persisting non-native	3A
4	*Rosa gallica* L.	NO	MALTA: Wied Speranza, Mosta	Persisting and established	3B
5	*Rosa* ×*damascena* Mil.	YES Gallica	MALTA: fields below Dwejra, Mġarr; Ħal-Drago, Bidnija (cultivated?). GOZO: Wied ir-Riħan and Għajn Qasab, Nadur	Persisting, but some populations are adventive	3C
6	'Albéric Barbier'	YES Wichuraiana	MALTA: Wied il-Qlejgħa; Ħagar Qim Temples; Marsa (close to old power station); Għargħur (close to Wied il-Faħam); Fawwara; Tarġa Gap, Mosta. Also found cultivated at Santa Luċia area in Mtarfa.	Persisting and established	1B
7	'Léontine Gervais'	YES Wichuraiana	MALTA: Il-Qaws area, Dingli	Persisting and established 1C	
8	*Rosa* ×*pseudo-rusticana* Crepin ex Rogers	YES *R. stylosa* × *arvensis*	MALTA: Wied id-Dis	Persisting	2B
9	'Adélaide D'Orleans'	YES Sempervirens	GOZO: Wied Mġarr ix-Xini	Persisting and established	2C
10	'Excelsa'	YES Wichuraiana	MALTA: Road to Fawwara	Persisting and established	4A
11	'Juliana von Stolberg'	YES Multiflora	MALTA: Fawwara	Likely persisting	4B
12	*Rosa* cf *luciae* s.l.	YES Indet.	MALTA: Wied id-Dis	Persisting	2A

References

Bakay, L. B., Racek, M., Rovná, K. & Kerényi-Nagy, V. (2015). *Rosa rubiginosa* L., a new Rose species for the Flora of Malta. In *Rozsa-Es Galagonya-Konferencia A Karpat-Medenceben. Nk. Konf. 2015* (Majus 29–30, Godollo. 1).

Borg, J. (1927). *Descriptive flora of the Maltese Islands: including the ferns and flowering plants*. Malta: Govt. Print. Off.

Casha, A. (2013). *Flora of the Maltese Islands. An Introduction.* (Volume 3). Malta: Coral Print.

Chapman, A. (2012). The Barbiers and their Roses. Retrieved April 2016, from http://www.annchapman.net.nz/content/barbiers-and-their-roses

Ciantar, G. (1772). *Malta illustrata, ovvero descrizione di Malta con le sue antichitá ed altre notizie, divisa in quattro libri, del commendatore F. Giovanfrancesco Abela*. Malta.

Clapham, A. R., Tutin, T. G. & Warburg, E. F. (1962). *Flora of the British Isles* (Second). Cambridge: University Press.

Duthie, J. F. (1872). Notes on the Flora of Malta and Gozo. *The Journal of Botany British and Foreign*, *1872*, 206–210.

Giardina, G., Raimondo, F. M. & Spadaro, V. (2007). A catalogue of plants growing in Sicily. *Bocconea*, *20*, 582.

Graham, G. G. & Primavesi, A. I. (2005). Roses of Great Britain and Ireland. In Botanical Society of the British Isles (Ed.), *BSBI Handb.* (Volume 7, p. 208). London.

Grant, W. A. (2000). *Botanica's Roses*. Hong Kong: Laurel Glen Publishing.

Grech Delicata, G. C. (1853). *Flora Melitensis sistens stirpes phanerogamas in Melita insulisque adjacentibus hucusque detectas secundum systema Candolleanum digestas: Melitæ*. Valletta, Malta.

Gulia, G. (1872). *Maltese Botany: Il Barth* (Volume 1). Malta.

Haslam, S. M., Sell, P. D. & Wolseley, P. A. (1977). *A flora of the Maltese islands*. Msida: Malta University Press.

Haynes, J. (2012a). History of Roses: Damask Roses. Retrieved May 2016, from http://www.rose.org/wp-content/uploads/2012/01/History-of-Roses-Damask.pdf

Haynes, J. (2012b). History of Roses: Gallicas - Rose of Provins. Retrieved May 2016, from http://www.rose.org/wp-content/uploads/2012/01/History-of-Roses-Gallicas-doc.pdf

Haynes, J. (2012c). History of Roses: Noisette Roses. Retrieved May 2016, from http://www.rose.org/wp-content/uploads/2012/01/History-of-Roses-Noisettes.pdf

Herbs2000. (2002–2016). History of the Rose. Retrieved May 2016, from http://www.herbs2000.com/flowers/r_history.htm

HistoricRoses. (2009). A brief history of Gallicas. Retrieved May 2016, from http://historicroses.org/index.php?s=history_gallicas

HMF. (2016). Help Me Find : Roses, Clematis and Peonies and everything gardening related. Retrieved May 2016, from http://www.helpmefind.com/rose/l.php?l=2.2291.2

Huxley, A., Griffiths, M., Levy, M. & Royal Horticultural Society. (1992). *The new Royal Horticultural Society dictionary of gardening Vol.4 (R–Z)*. London.

HWR. (2009). Wichuriana ramblers. Retrieved May 2016, from http://hartwoodroses.blogspot.com.mt/2009/11/flowers-on-friday-wichuriana-ramblers.html

Klastersky, I. (1968). *Flora Europaea Volume 2 - Rosaceae to Umbelliferae* (T. G. Tutin, V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters & D. A. Webb, Eds.). Cambridge: Cambridge University Press.

Lanfranco, E. (1989). *Nature Guide Series: Wild Flowers of the Maltese Islands*.

Lanfranco, E. & Bonett, G. (2015). *Nature Guide Series Wild Flowers of the Maltese Islands*. Malta: BDL Ltd.

LN311. (2006, Dec 7). Regolamenti tal-2006 dwar il-Protezzjoni tal-Flora, Fawna u Ambjenti naturali/Flora, Fauna and Natural Habitats Protection Regulations, 2006. Laws of Malta, Subsidiary Legislation 504.73, Legal Notice 311 of 2006. Malta: Supliment tal-Gazzetta tal-Gvern ta' Malta, Nru. 18,006: 4214–4498.

Mifsud, S. (2002–2014). MaltaWildPlants.com (An online flora Maltese islands - Rosacaea). Retrieved May 2016, from http://www.maltawildplants.com/ROSA/

Mifsud, S. (2010a). MaltaWildPlants.com (An online flora Maltese islands - Rosa Gallica). Retrieved May 2015, from http://www.maltawildplants.com/ROSA/Rosa_gallica.php

Mifsud, S. (2010b). MaltaWildPlants.com (An online flora Maltese islands - Rosa rubiginosa). Retrieved May 2016, from http://www.maltawildplants.com/ROSA/Rosa_rubiginosa.php

Nesom, G. L. (2000). Which non-native plants are included in floristic accounts? *SIDA*, *19*(1), 189–193.

Phillips, R. & Rix, M. (1993). *The quest for the rose*. London: Random House.

Pignatti, S. (1982). *Flora d'Italia: Vol. 2*. Bologna: Edagricole.

Pottier-Alapetite, G. (1979). *Flore de la Tunisia - Angiosperms-Dicotyledones Apeteles-Dialypetales*. Tunis: Imprimerie Officielle.

Redell, R. C. (1998). *The rose bible*. California: Chronicle Books.

RHS. (2016). The Royal Horticultural Society website. Retrieved May 2016, from https://www.rhs.org.uk/plants/details?plantid=1658

Scaniello & Bayard. (1994). *Climbing roses*. New York: Macmillan General Reference.

Silvestre, S. & Montserrat, P. (1998). *ROSA* L. In S. Castroviejo, F. Munoz Garmendia & C. Navarro (Eds.), *Flora iberica plantas vasculares de la peninsula iberica e islas baleares - vol. 6 rosaceae* (pp. 143–195). Madrid: Real Jardín Botánico, CSIC.

Sommier, S. & Caruana Gatto, A. (1915). *Flora Melitensis nova. Firenze*. Firenze: Stabilimento Pellas.

Stace, C. (2010). *New Flora of the British Isles* (3rd). Cambridge: Cambridge University Press.

Tabone, T. J. (2008). A list of records of some rare vascular flowering plants occurring in the Maltese Islands. *Cent. Mediterr. Nat. 4*(4), 311–337.

ThePlantList. (2014). The Plant List - Version 1.1. Retrieved July 2014, from http://www.theplantlist.org/

VRA. (2016). Vivaio Rose Antiche. Retrieved May 2016, from http://www.vivaioroseantiche.it/scheda-rosa.php?idrose=670

Weber, H. C. (2008). *Ornamental plants of Malta*. Germany: Margraf Publishers.

Weber, H. C. & Kendzior, B. (2006). *Flora of the Maltese Islands. A field Guide*. Germany: Margraf Publishers.

Cutajar, A., Farrugia, P., Casha, O., and Vella, P. (2017). *Xjenza Online*, 5:57–68.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.06

Research Article

A Concurrent Engineering Approach to Develop BioMEMS Employed in a Deep Brain Stimulator Integrated with a Drug Delivery System

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Abstract. This paper presents an Integrated Product Development (IPD) based model to specifically develop bio-medical micro-electro-mechanical-systems (BioMEMS). The concurrent engineering model is based on the IPD model phases, which are presented and formulated by the Integration DEFinition (IDEF) modelling language. To evaluate the IPD model, a case study concerning the development of a BioMEMS device for a deep brain stimulation (DBS) system was investigated. By following the relevant mechanisms and controls in the model, a design concept of a wireless head-mounted DBS implant integrated with a drug delivery system (DDS) was conceived. The contribution of this paper is the IDEF model, which provides a road map to the product development team members in order to take a concurrent engineering approach to develop BioMEMS. The qualitative feedback received from the identified stakeholders, together with the quality of the case study employed, namely, an integrated DBS and DDS solution, indicate a degree of evidence that the model provides a sound basis in this direction.

Keywords: Integrated Product Development, Design Tools and Methods, Product Miniaturization, BioMEMS

1 Introduction

Physical movement and brain functioning are naturally taken for granted unless a physiological impairment restricts their function. Conditions or symptoms such as tremors, dystonia, obsessive-compulsive disorder, depression, or severe chronic pains definitely reduce the quality of life by restricting these abilities. It is reported by CBC (2010), that one of the most severe diseases which affects both motion and the brain is Parkinson's disease; a progressive neurological disorder exhibited by seven to ten million people worldwide. It is caused by a deficiency of dopamine-producing cells and affects the motor and coordination functions in a human being.

The monitoring and reduction of tremors associated with Parkinson's disease can be performed through controlled stimulation via a pulse generator device, which can be configured via an external portable programmer. The electrodes, which are embedded in the affected brain zones, receive these controlled pulses and distribute them to the nervous system in order to properly actuate the parts of the body which exhibit abnormal motor activity. This process is known as DBS. In DBS, electrodes are placed in the thalamus or in the subthalamic nucleus or globus pallidus. According to Klaubert (2005), the electrodes are connected by means of wires to the impulse generator implanted under the skin of the chest below the collarbone. A schematic diagram of the DBS setup is illustrated in Figure 1.

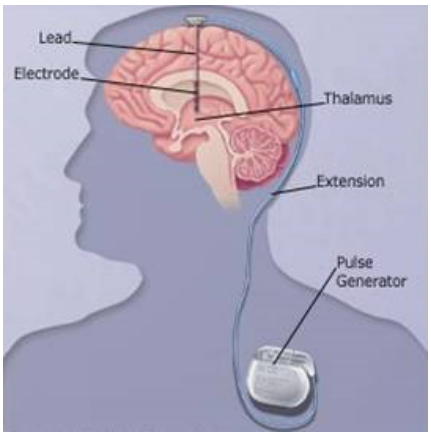

Figure 1: Schematic diagram of a Deep Brain Stimulator (WebMD, 2015).

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Once activated, the device sends continuous electrical pulses to the targeted brain areas, modifying the behaviour of the brain's neural network that is responsible for the motor symptoms. Da Silva (2013) states that this medical procedure is better than thalamotomy or pallidotomy, because DBS can be configured according to the needs of the patient and can be applied without affecting other parts of the brain. DBS is usually supported by means of a prescribed daily drug intake.

Micro-Electro-Mechanical Systems, or MEMS, is a technology that in its most general form, can be defined as miniaturized electro-mechanical elements (i.e. devices and structures) that are made using microfabrication techniques. Meanwhile, BioMEMS have emerged as a subset of MEMS devices for applications in biomedical research and medical micro-devices. They are used for various applications such as drug delivery, tissue engineering and bio-sensor development. The study carried out by Da Silva (2013) confirmed that the BioMEMS technology market has been growing extensively in the last couple of years.

In the recent years, automatic drug delivery has been a very active research field in the pharmaceutical industry. Drug intake could be better controlled if the patient has an implantable BioMEMS device that can be actuated in such a way that the drug is released periodically when needed. Although BioMEMS devices provide solutions to challenges faced in the medical sector, they also give rise to a number of issues at the micro-scale level. Alexander, Rogers, Sheehan and Willson (2004) highlighted that one of the most critical issues that has to be taken into consideration when using MEMS devices is bio-compatibility. Any device to be implanted in the body for an extended period of time should not include toxic materials or fluids or pose the risk of causing damage to any local tissue. Another important factor to be addressed is hermeticity, such that the functionality of the device is not compromised by its surroundings. For these reasons, designing BioMEMS is not always an easy task. One should consider various factors in the early stages of the product development, that might affect the final design. The product development of BioMEMS based medical devices has a complex nature since it involves multidisciplinary stakeholders such as physicians and engineers, often with conflicting views (Santos, 2013).

In addition, the development of medical devices is highly influenced by the regulatory requirements and business considerations (Santos, 2013). Despite these challenges, a study by Linehan, Paté-Cornell and Yock (2007) reveals that there was little data on the development process of medical technologies. The findings in this study have also been confirmed by an extensive literature review carried out six years later by Santos (2013). The IPD model is one proposal for the implementation of concurrent engineering in the product development process (Andreasen & Hein, 2000). This model follows the idea of parallelizing tasks which are carried out by various streams within the product development process in order to allow for concurrent consideration of the problems. In view of this context, the overall goal of this work was to develop an IPD based model specifically targeted for the development of BioMEMS. Given the complexity to develop a BioMEMS device which requires multidisciplinary expertise, modelling of the process will greatly help IPD stakeholders to visualise the information flow from the concept to the product launch. To this end, in this paper the Integration DEFinition (IDEF) modelling language is deployed to portray the various features characterising the information flow in such an IPD model.

Based upon this introduction, the rest of this paper is organised as follows. Section 2 reviews concurrent engineering approaches as well as existing products related to DBS, which are readily available on the market. The developed IPD model is then presented in Section 3, with particular emphasis made on the product design phase. The subsequent section focuses on the implementation of the developed model via a case study of an integrated DBS system equipped with a drug delivery mechanism. Results of an evaluation carried out with a sample of subjects, consisting mainly of IPD stakeholders, patients and neurosurgeons, are presented in Section 5. The strengths and weaknesses of the proposed model together with those of the case-study solution are discussed in Section 6. Further research directions are also proposed. Finally Section 7 draws key conclusions, highlighting the contribution made in this paper.

2 Related Work

2.1 Product Development Models

This section first reviews generic product development models, followed by specific ones, in particular those related to medical devices. Amongst several existing models, the model proposed by Eppinger and Ulrich (2011) starts by the planning phase followed by concept development, system level design, detail design, testing and refinement and production ramp-up. At each phase, marketing, design and production activities are identified. Similarly to the model presented in Andreasen and Hein (2000), these two models are characterized by a matrix which chronologically shows the different development phases in relation to the functional core disciplines (Schätz, 2006). Common to all the aforementioned models is the fact that the information flow between the different phases is not modelled formally using the IDEF modelling language but rather by means of block diagrams and flowcharts (Fenech & Farrugia, 2014). In

this work, the IDEF0, which is one class of IDEF modelling languages, was chosen to model the IPD as it is specifically designed to represent the activities, actions and decisions of a 'system'. Furthermore, apart from its simplicity, the major benefit of using IDEF0 is that it applies dynamic information into the model to handle problems involving parallel activities (Fenech & Farrugia, 2014).

As will be described later on in Section 3, the model presented in this paper is closer to the original standard IDEF0 modelling language, with the introduction of a data connectivity type to reflect the stakeholders input. An extensive literature review of product development models, specifically targeted for medical devices, was carried out by Santos (2013). Over thirty models were reviewed, none of which specifically treated BioMEMS. Watty and Binz (2005) presented a proposal for MEMS design methodology based on the VDI guideline 2206 (Beuth, 2004). Even though this methodology covers the whole life-cycle of a component, it does not specifically mention very important aspects for BioMEMS, which are the need for safety, risk assessment and quality considerations.

Due to the novelty and nature of the products, materials, risk and quality assessment and manufacturing technologies, the development of BioMEMS requires a structured development process. As explained in Smith (1997), BioMEMS need not only efficient components but also their correct and reliable interaction to fulfill the function of the entire system. Despite this, the above review suggests that there exists no definite methodology tailored specifically for the development of BioMEMS. Many disciplines use approved methodologies for product development such as the VDI-guidelines for mechanics as explained by Reichl (1994) and mechatronics as discussed by Beuth (2004), or widely automated design procedures in microelectronics. The models reviewed are aimed at serving as a guideline to implement product development related principles in certain disciplines and do not sufficiently include the interdisciplinary and system interrelations demanded in the development of BioMEMS.

2.2 Review of Deep Brain Stimulation Devices

There are various solutions available on the market for DBS systems. Current systems involve the need for DBS to use electrical impulses to stimulate a target area in the brain which affects movement by altering the activity in that region. The procedure does not destroy any brain tissue and stimulation can be changed or stopped at any time. Some of the solutions are readily available on the market, like Medtronic^{TM} devices, while others are still at a concept stage. It is vital to mention that undergoing DBS surgery does not mean that one stops daily drug intake.

Current solutions offer various advantages such as the fact that DBS is reversible and the patient can stop treatment at any time. DBS can be customized to the patient's requirements in such a way that the electrical stimulation is adjustable according to the patient's response to medications. On the other hand, failure of the battery could result in the device not working beyond its estimated time limit. As a result, this would entail the need for another minor surgery to replace the battery. Also, if the frequency and the intensity of the pulses required by the patient are increased, these could result in the need to change the battery before its expected life-time.

Most common DBS systems generally have the pulse generator system placed in the chest area and which is connected via an extension wire to the electrode region in the skull area. The literature review presented in Cutajar (2014) shows that the device is susceptible to have broken leads or wires, especially the extension wire. This is most likely to occur in slim people. Possible studies could exploit alternative ways to avoid this failure. Research has been made on ways how to extend the battery lifetime of a DBS system. For this reason, seeking to further investigate battery longevity would be a major requirement demanded by the stakeholders.

Current DBS systems are relatively modular, however, if one simply focuses on their function and their additional potential capabilities, one can easily note that it is quite limited in terms of flexibility. This can be mainly noted since its purpose is solely intended for the treatment of uncontrolled diseases or movements. In addition, through an existing literature research (Obeso et al., 2001; Parkinson's Disease Foundation, 2015) it was revealed that while medication is significantly reduced after surgery, the body still needs to be sustained by the daily medication dosage. In addition, the literature survey presented in Cutajar (2014) showed that the integration of these two systems (DBS and DDS) together is in its infancy. DBS and DDS are presented as individual solutions for different purposes.

Through quantitative data gathering from a market research, it was discovered that the currently available pulse generator devices embedded in the chest area are quite large in size (Cutajar, 2014). This possibly results in certain level of discomfort to the patient. The possibility of miniaturization of an integrated DBS system was especially highlighted by young patients (Cutajar, 2014). Based on existing research current systems the current minimum size is approximately 54 mm by 54 mm and the target size is to go down to 25 mm by 25 mm which is approximately half the size of current system. The aspect of miniaturisation combined with the lack of integration of the DBS with DDS mentioned above, provided scope and motivation for the research team to seek an innovative solution.

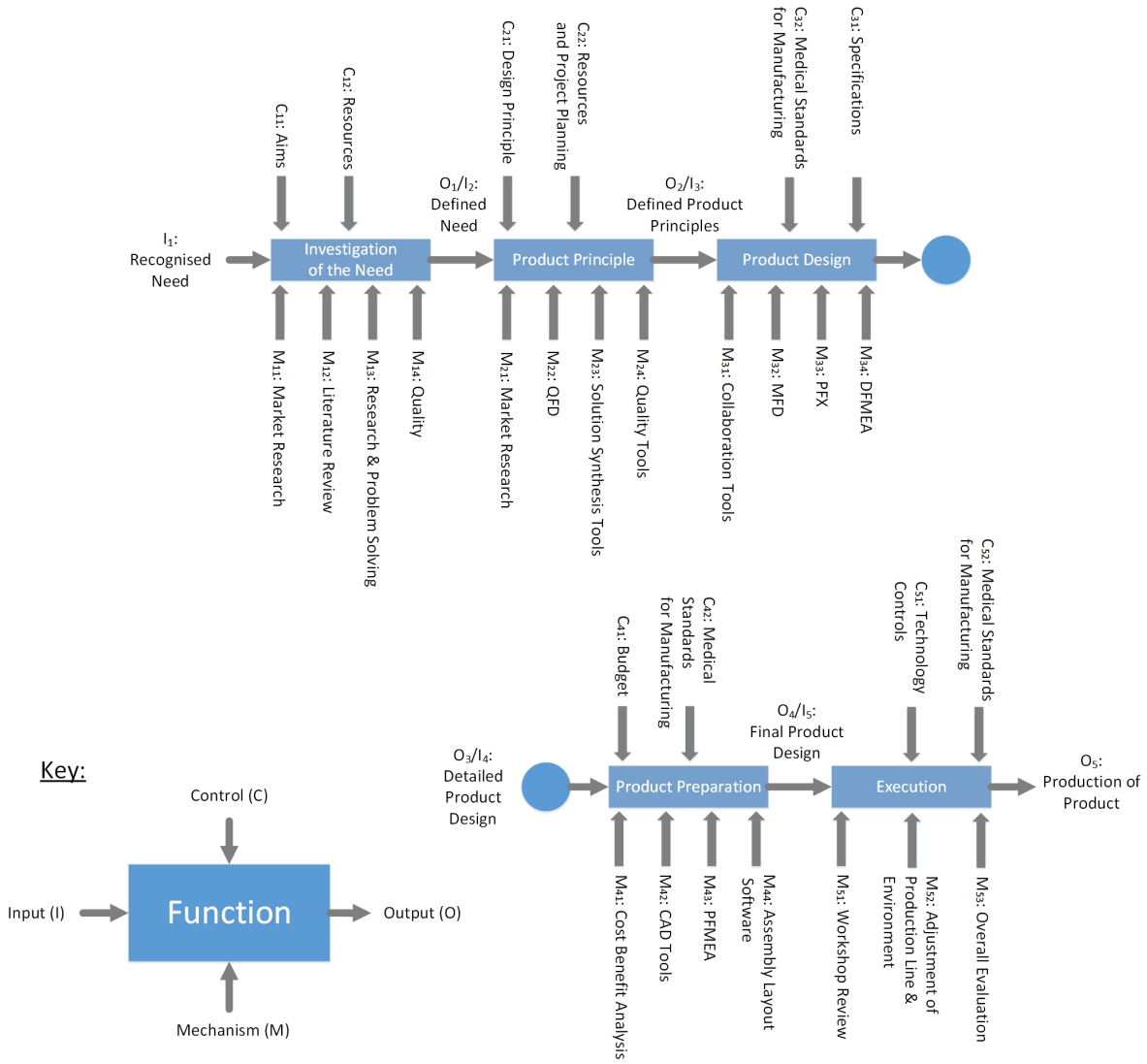

Figure 2: General block diagram of the developed IDEF0 model.

3 An IDEF0 Model for BioMEMS

From the identification of the market gap and the requirements set out by several stakeholders, it is important to formulate a concurrent engineering approach to develop BioMEMS. This new approach needs to make use of a model which provides means for new marketing and sales opportunities while resulting in a planned manufactured strategy to produce high quality products, which conform to the biomedical standards and directives set out by the medical sector.

The model being proposed (refer to Figure 2) incorporates both the IPD model phases such as the investigation of the need and product principle by Andreasen and Hein (2000) and the IDEF0 modelling language principles. It must be mentioned that the phases in Figure 2 can also be perceived as functions within an IDEF0 modelling context. Andreasen and Hein's model has been employed in this research, since it integrates all the pillars from the very initial recognition of the need to the final execution. On the other hand, a shortcoming of this model and which is addressed in this work, lies in the fact that no explicit reference is made to quality considerations. Through extensive market research it was established that quality is significantly important when developing BioMEMS (Cutajar, 2014).

IDEF0 was chosen as it offers a structured representation of the different functions of a system and it captures the activities, decisions and actions taken. The tool acknowledges that a successful systems development requires input and validation from the users. In fact, IDEF0 incorporates inputs, outputs, controls and mechanisms for a specific function (refer to legend in

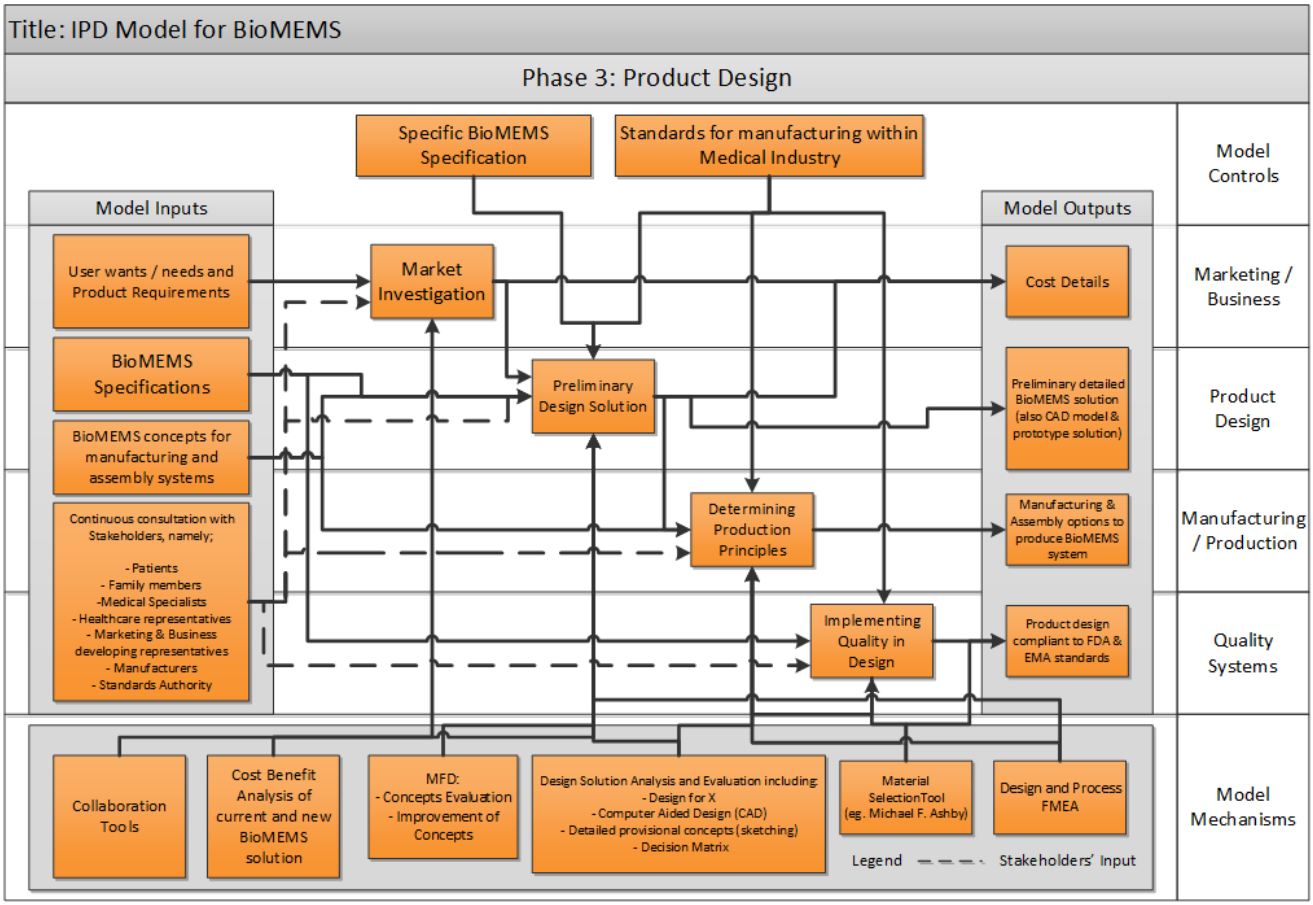

Figure 3: IDEF0 model of Phase 3: Product Design.

Figure 2). It must be pointed out that the IDEF0 model presented in this section was developed and based on the requirements to market, design and fabricate BioMEMS. Figure 2 depicts a general overview of the developed model, consisting of five phases (Phase 1 to Phase 5), whereby development flows sequentially through these phases whilst, in each of these phases, development activity will be taking place both sequentially and concurrently, involving the three IPD pillars along with quality. Since the design stage is critical in the overall IPD model, this paper focuses on Phase 3. This phase involves important decisions on the preliminary design solution of a BioMEMS device and is presented in Figure 3. Further details on the other phases are available in Cutajar (2014).

Phase 3 focuses on the details of the developed concept and the means how to actually produce the first simulation models specifically designed for BioMEMS (refer to corresponding IDEF0 model in Figure 3). As can be observed from Figure 3, the inputs of Phase 3 are fed from the outputs of Phase 2. With reference to Figure 3, 'market investigation', 'preliminary design solution' and 'determining production principles' are illustrated as concurrent activities in the Andreasen and Hein's IPD model under the product design phase. The fourth function regarding quality was added in the proposed IPD model.The primary goal of this phase is to define further design details and ensure that the final detailed design solution is augmented with the stakeholders' feedback and requirements. This is defined in order to enhance the product specifications and set the BioMEMS concepts for the manufacturing and assembly systems. The preliminary design together with the initial considerations of manufacturing systems give rise to the possible ideas and concepts of how the system will be produced.

The mechanisms defined in Phase 3 include, the cost benefit analysis of the new BioMEMS device over current systems and the modular functional deployment (MFD) which is closely related to Quality Function Deployment (QFD). In addition, a number of design modelling tools (e.g. CAD) and design analysis tools (e.g. DFX) and evaluation tools (e.g. decision matrices and material selection tools) feature as mechanisms in Phase 3. To detect any potential weaknesses in the design and subsequently in the process, the Failure

Mode and Effect Analysis (FMEA) tool is also included as a mechanism in this phase. Similarly as in Phase 2, another important mechanism relates to the collaboration tools which aid to facilitate communication between the different IPD stakeholders. For instance, collaboration tools can be used to collectively investigate the market and to determine product principles. At the initiation of Phase 3, the market research would have been carried out, whereby this data would have been gathered and the customer's needs identified. Other controls also include the standards for manufacturing within the medical industry such as the bio-compatibility of the chosen materials which need to abide by the medical standards, such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA) standards. This shall also lead to enhanced concurrency in the development of BioMEMS. One of the major outputs is the need to set out a preliminary design which is carried out by making use of various CAD software tools. It would also be ideal if as an output of this phase, one identifies the possible technologies needed for the production of the components or systems.

4 Application Case Study

To adopt a user-centred design approach, typical end-users of the device were involved in the case study. These included five patients whose age varied between 4 to 60+ years and two neurological surgeons. Due to the difficulty in accessing BioMEMS and integrated circuit designers, it was not possible to engage such stakeholders. The study was carried out by organising different focus groups which were aimed at gathering qualitative data. The case study revolved around the development of an integrated DBS and DDS system. The need for such a system emerged after the patients and the medical specialists highlighted that the patient still needs to take a daily drug dosage after undergoing surgery. The clinical need to maintain medication is generally related only to the non-motor symptoms of the disease. As discussed in Marek and Antle (2008), specific patients have also mentioned that the daily dosage imposes a certain level of dependence on the family members and highly depends on one's health. The fact that patients need to depend on others in order to take their medication is seen as a reduction of one's quality of life. This solution would most likely aid patients who suffer from dementia and similar mental sickness. Many of the risk factors related to inadequate medication management are more prevalent in older adults, like for instance the possibility of mixing prescribed medication which could lead to serious consequences.

During the design phase it is important to determine how the product will be configured. It is also important that the designer keeps into perspective that the chosen materials conform to the medical standards, in order to ensure that the final device is bio-compatible due to the sensitivity of the environment in which it will be embedded. The various stakeholders were asked about their requirements, in order to be capable to translate these into product design specifications. To this end, a QFD was compiled during phase 2 of the IPD model (refer to Figure 2). The weight distribution of the QFD engineering requirement scores revealed that the most critical requirements of the DBS system include:

- Minimizing the probability of a defective extension wire between the pulse generator and the electrodes.
- Maximizing the patient's comfort – this is very important so as the DBS can be implanted in patients of all ages, therefore, it is crucial that the design of the DBS should aim to maximize comfort. This comes about with the possibility of miniaturizing the device.
- Maximizing battery longevity – this is one of the main critical features in the pulse generator. The better the battery's capacity, the longer the battery lifetime. The procedure to replace this requires a minor surgery. This is something one cannot do without unless the batteries are rechargeable. Thus, it is advisable that at this phase of the IPD model, the latest rechargeable batteries available on the market are sought, in order to guarantee the maximum possible lifetime of the system.
- Need for DBS system to be bio-compatible – it is one of the most important requirements of any medical device which needs to be implanted in the human body.

The results also showed that 60% of the interviewed people state that if they had to choose, they would pick a system which is aesthetically more appealing and less visible. Based on this analysis, this concept has to be unobtrusive. Meanwhile, this device also seeks to incorporate the need for automatic daily drug intake, which could ultimately allow the patients to lead a better lifestyle. A number of concepts were generated in Phase 3, by means of sketching and other synthesis tools, namely morphological charts. These concepts included a range of different configurations of the DBS implant and the DDS. Relevant stakeholders, in particular neurosurgeons and engineering personnel were involved to select the most plausible configuration, through a decision matrix. However, since in the scope of this work the actual prototype was not developed in a real industrial context, certain mechanisms such as collaboration tools, were not deployed during the evaluation exercises. It resulted that the preferred configuration would be a wireless head mounted DBS implant with a DDS. This system would be responsible for two functions, primarily

injecting a designated amount of charge into the human body by providing a precise amount of output current or output voltage for the predefined period (architecture of the DBS is shown in Figure 4(a)).

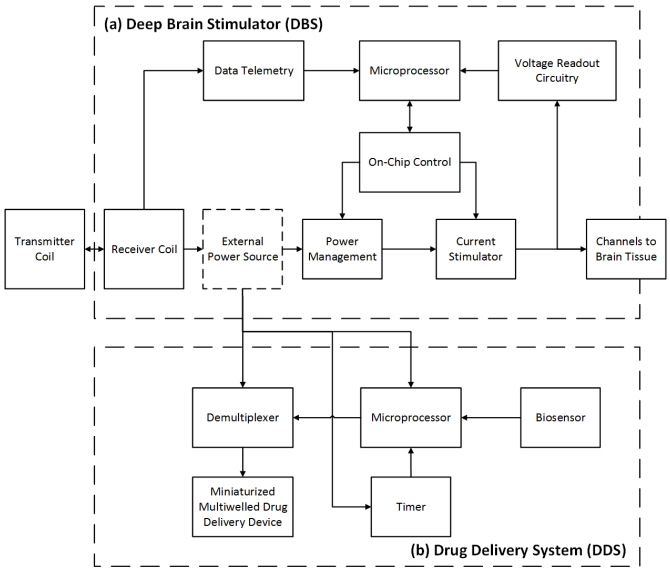

Figure 4: (a) Block diagram of the integrated DBS with adaptive power supply control and active charge balancing for both power-efficient and safe current stimulation (Lee, Park & Ghovanloo, 2013) (b) Architecture of the proposed DDS (Cutajar, 2014).

Secondly, it releases the right amount of drug at the right time needed by the body (refer to Figure 4(b)). These functions may be programmed and configured wirelessly through the use of an external device via a coil (Cutajar, 2014). The same system is used to recharge the in-packaged battery which is chosen to be a non-cytotoxic rechargeable one. An external rechargeable battery energizes the implanted device, which is placed behind the ear similarly to cochlear implants and hearing aids, via an inductive power transmission charger. This would be designed to be removable once the internal DBS system is fully charged. The external battery of the programmer and charger can then be energized 'offline' without being worn by the patient. This presents a well-designed aesthetic, safe and modular device to the end-user and it is quite unnoticeable.

5 Evaluation

As part of the evaluation strategy a number of stakeholders were questioned in relation to the validity of the IPD model as well as the developed device solution. Due to the sensitivity of the subject and the limited availability of stakeholders in the local sector, the sample of respondents was relatively small. In fact only fourteen participants took part in the evaluation process. The results focus on a qualitative rather than quantitative data collection. The participants who volunteered in the evaluation exercise consisted of four academics coming from three related fields of study (material processing, neurology and marketing), two neurosurgeons, one neurophysiologist, five patients of different age brackets, one member of the European Parkinson's Association, and one consultant in a company which supplies medical products. The participants were asked a set of questions and presented with the prototype solution and the IPD model in the case of academics. A five-point Likert scale was used to measure the participants' attitude to the posed questions. A selection of the gathered feedback together with sample comments quoted from different stakeholders coming from different sectors are provided in Table 1 (on page 8) and Table 2 (on page 9).

6 Discussion

This section discusses the strengths and weaknesses of the IDEF0 model as well as those of the case study solution which were identified from the qualitative data collected. The degree of validity of the research results obtained is also discussed in the last part of this section.

6.1 Strengths and Weaknesses of the IDEF0 Model

A number of strengths of the IPD model have been identified from the qualitative data collected, namely:

- Since the three pillars of IPD are included as activities in each phases, the proposed IDEF0 model, reflects concurrent engineering, which automatically demands an improvement in the communication between the different departments. In addition, it emphasizes the vertical integration across the three IPD pillars in conjunction with quality considerations (this also includes the risk assessment and safety considerations). This direct relationship accentuates its importance and interconnection. In addition, such integration ensures that a product of good quality is being carefully manufactured according to the exact requirements of the stakeholder.
- The investigation of the need in the early stages of development links to determine the type of product. This would then set targets for the considerations of the process type and the quality systems to be considered. This activity will aid the organisation to decide whether the project should proceed or put on hold.
- The model emphasizes the need to consider quality considerations, safety, risk assessment as well as the importance of implementing standards and guidelines since the device has to abide by a high level of medical standards.
- Activities concerning manufacturing and the possible available technologies are presented in the very

early design stages so that any defects or barriers are highlighted as soon as possible in the design cycle.
- The model provides a visual representation of the process flow along the product life-cycle from the idea initiation stage to the realization which challenges the inputs and controls.
- The feedback implemented in the model permits to revert to particular activities during the development process. Its importance comes about since a minor mistake at the development and production stage could ultimately result in challenges later on during the product's life-cycle. This may potentially result in high losses for the company.

On the other hand, one of the major criticisms highlighted by the medical team and the business representatives, was that whereas within the IDEF0 phases, a parallel flow is emphasized, reflecting concurrency of IPD, the phases themselves are sequential in nature. This is attributed to the fact that certain inputs emerge from previous output activities.

Table 1: IPD Model: Selection of the qualitative evaluation results and average ratings obtained (1:Bad – 5:Good).

Question	1	2	3	4	5	Additional Comments
Do the IPD pillars adopt a holistic approach and a level of continuity between phases?					X	Marketing and sales are the least important from the IPD pillars since if one is offering a good quality product, one does not necessarily need to market it since patients still believe in the product and need it. For this reason the sales factor is not so important when it comes to such medical products. Having said that, marketing and sales could be one of the main key barriers to certain selective patients. **Neurosurgeon** The most important pillar is design, as it is the fundamental fulcrum on which any project kick starts its process development. **Consultant at a Medical Device Company**
What is the level of importance the different solutions (current and new DBS solution) give to the three IPD pillars together with the quality factor?				X		The most important factors are the following: a very good design in the initial stages of the product's lifecycle, a good manufacturing procedure, in order to have one of the best solutions on the market, followed by a good quality product, which abides by the necessary medical standards in this field. The current solution which is available on the market does not cater for good integration between these, however the proposed system takes into account all factors, and meanwhile, one can still improve the sales execution. **Neurophysiologist**
What is the level of importance given to the vertical integration between the three IPD pillars and quality factor?					X	Vertical integration between the pillars and quality is missing in the currently available devices, and encourages the need for further parallelism between the three pillars and quality, if one needs to achieve a successful product. **Neurosurgeon**
What is the practicality of the model and does this provide enough tools to address the situation from three different IPD pillars together with quality?					X	If the mentioned tools and methodology had to be adopted during the life-cycle of the process development, the solution to the device would be based on the customers' needs and requirements while ensuring a successful sellable product. **Neurosurgeon**

Table 2: New DBS-DDS Solution: Selection of the qualitative evaluation results and average ratings obtained (1:Bad – 5:Good).

Question	1	2	3	4	5	Additional Comments
To what extent does the new design address the market gap?					X	The new design offers a safe quality product which provides a safe mindset to the customer. Its miniaturized system can make the customer instantly comfortable. Its size and charging system instantly gives a better frame of mind that it is designed for patients of all ages. This product highlights the voice of the customer. **Patient - Parkinson's Disease (Age: 35)**
To what extent do the stakeholders benefit from the new design, and how much does the design address the current limitations?					X	The main limitations are usually highlighted via the customer requirements. This was dealt with efficiently. **Neurosurgeon** Main burden is that the patient constantly needs to remember to take one's medication. This is something which was resolved through the use of the new DBS design. **Patient (45 years)**
To what extent does the new design address the importance for miniaturization and that of extended battery lifetime?				X		The system definitely presents an improvement over the current solution. It deals with the main weaknesses of the systems currently available on the market and addresses these issues in the most prominent way. **Patient (55 years)**
What is the level of importance given to the new design to address biocompatibility and abide by the regulations and standards for medical devices?					X	The new design ensures that the product is of high quality and abides by the medical standards and regulations. The material selection was carefully selected so as to opt for the best solutions. **Lecturer in Materials and Metallurgy**
How beneficial is the integration of the pulse generator device and DDS designed as a single system? To what extent does the device offers design modularity to patients to make their life easier?					X	The new system is a single device which caters for different functional solutions, thus offering a level of modularity to the patient. It would be a good idea to have the possibility of two separate solutions. **Patient (49 years)**
Does the new generated solution present a challenge to implement such device in production?				X		Manufacturing this device is not too complex. The most challenging of all is the nature of the product. The fact that it is a biomedical device, it needs to conform to certain strict requirements. **Member-European Parkinson's Association**
Do you think the new solution will present a life style improvement?					X	Confident that our lives will be improved drastically. **Patient (6 years)**
Do you believe that this solution would be cost effective?				X		It will not be a cheap solution especially because of the integration of the new DDS and working mechanism of the wireless rechargeable battery. But it offers a more flexible solution which better caters for the patient's needs. **Member-European Parkinson's Association**

6.2 Strengths and Weaknesses of the Case Study Solution

The comments gathered from the focus group shed light on a number of benefits of the proposed design when compared to the current design solutions, particularly:

- Improved comfort – a miniaturized solution which can be directly implanted and fitted into the skull area, with electrodes that are implanted in either the subthalamic Nucleus or a section of the globus pallidus. These brain sites normally play crucial roles in regulating movement. This presents various challenges due to the sensitivity and space limitation; however, one of the main benefits is that the device is much smaller and thinner than the currently available systems on the market. In addition, a miniaturised DBS-DDS system will be more appealing to young patients diagnosed with Parkinson Disease, thereby increasing the market potential of the device, given that there is more evidence pushing in the direction of an early stimulation approach (Schuepbach et al., 2013).
- Elimination of the long extension wire which connects the leads to the neuro-stimulator wire from the skull area to the chest area in the conventional devices. One of the most frequent failures is due to the breakage of this wire. This was carefully solved by omitting this wire and carefully connecting the electrodes to the DBS system via a short wire in the skull area itself. The possibility of the wire being damaged is significantly reduced.
- Increasing battery efficiency – The battery to be installed into the devices is recharged through an external charger which transmits power by means of a coupled coil system which then feeds an on-chip AC-DC converter. The external charger can be energized without being mounted on the ear, and then worn once fully charged. Once charged the external system can be removed, improving aesthetics and safety. The internal battery would then feed the internal devices.
- Less drug intake – since the drug will be delivered in the local neighbouring affected zones, the drug content can be less. When implanting the DBS device, a single surgical intervention is needed. This includes both the pulse generator and DDS in a single device. Having these two systems incorporated in a single system makes it less stressful and painful for the patient, while giving the patient the benefit of having two implanted systems.
- Throughout the design and planning, it was made sure that the stakeholders' requirements were met. The project initially focused on the miniaturization of the device and finding the ways and the means of improving battery longevity. From the market research it was highlighted that especially for older adults, the ability to remain independent depends on the ability to manage a complicated medication regime. The idea of designing a DBS system incorporated with a DDS not only results in having an integrated design through a single intervention, but also proves that the patient could be independent. The advantages of advanced drug delivery systems over traditional systems are the ability to deliver a drug more selectively to a specific site, in a more accurate way, with less frequent dosage, decreased variability in the systemic drug concentrations, absorption that is more consistent with the site and mechanism of action, and reductions in toxic metabolites.
- The new DBS system could be custom made to suit the patient's needs, and equipped with selected drugs systems. Further studies can be carried out to explore other possible drug delivery solutions. On the other hand, the DBS does not cater for all the symptoms of Parkinson's disease. While the patient will gradually reduce the drug content, the medication will not be stopped completely. For this reason, the DDS was incorporated within the DBS design in order to cater for the daily drug intake. In addition, if either of the systems fails, since the pulse generator and DDS are combined in a single device the whole device needs to be removed and re-implanted. These two systems cannot be managed separately. The major challenge for the production and manufacturing perspective is to outline the production layout of the DBS device which has the integrated DDS.

6.3 Validity of the Research Results

In order to ensure that the obtained results were reliable and realistic it was important to involve as many respondents as possible. Despite the efforts made, the number of stakeholders who contributed to the study was relatively limited. This was mainly due to the sensitive nature of the project. Also, local expertise in this area is quite limited. On the other hand, the involvement of the main team of professionals coming from different sectors gave an added value throughout the course of the research. The fact that the patients, who have undergone the DBS surgery, were involved, contributed to a broad and detailed evaluation. This was heavily supported by the opportunity to meet a limited number of neurosurgeons, whose contribution proved to be highly beneficial. Further discussions with the sales and production representatives, including both local and foreign organisations would have made the analysis more reliable. The core team was relatively limited to a small sample of professionals. In future research, it would be a good potential input to consider increasing the amount

of team members coming from the different IPD pillars and the quality sector. This ensures that the degree of completeness of the model is also validated.

In addition, the research presented in this paper was focused primarily on the application of the proposed IPD model on a case study of an integrated DBS-DDS system from an engineering perspective. Having said that, to test further the validity of the integrated BioMEMS system from a medical perspective, clinical tests are required. For instance, the type of drug (e.g. GABA or L-DOPA) to be delivered and the location of delivery with respect to the electrical stimulation must be investigated. This shall shed light on the medical feasibility of having a system, which is able to deliver both medication and electrical stimuli.

7 Conclusion

BioMEMS technology deals with the integration of diverse micro-technologies in complex and highly integrated systems. Thus, BioMEMS require special attention with respect to their product development and the wide range of manufacturing technologies which are constantly being developed and updated. The current state and the requirements of BioMEMS technology, collectively led to the need of a methodology, specifically designed and adopted for it.

In conclusion, the key contribution of this paper lies in the proposed IPD model aimed at developing BioMEMS while taking a case study of an integrated DBS-DDS system as an application. Compared to the state-of-the-art, the proposed integrated system provides a number of remarkable benefits. Nevertheless, this is an on-going process which requires further work in order to validate the developed IPD model and to assess the effectiveness of the proposed device solution in a practical scenario.

Acknowledgements

The authors would like to acknowledge the Neurosurgical Unit at Mater Dei Hospital Malta for providing the data sources, Prof. Giuseppe Di Giovanni for his contribution in the study and all the evaluators who provided their feedback.

References

Alexander, A., Rogers, L., Sheehan, D. & Willson, B. (2004). *Microelectromechanical Drug Delivery Systems* (ME 381 Final Project Report, Northwestern University).

Andreasen, M. & Hein, L. (2000). *Integrated Product Development*. IPU.

Beuth, B. (2004). *VDI: Design Methodology for Mechatronic Systems*. VDI 2206, Berlin.

CBC. (2010). Deep brain stimulation tested for Alzheimer's. Retrieved September 22, 2015, from http://www.cbc.ca/news/technology/deep-brain-stimulation-tested-for-alzheimer-s-1.951614

Cutajar, A. (2014). *Applying an integrated product development approach for biomems* (M.Sc. Dissertation in Integrated Product Development, University of Malta).

Da Silva, M. (2013). Design for manufacturability for 3d microdevices. *NSF Workshop on 3D Nanomanufacturing: Partnering with the Industry, Birmingham, Alabama.*

Eppinger, S. & Ulrich, K. (2011). *Product Design and Development*. New York, McGraw-Hill.

Fenech, G. & Farrugia, P. (2014). An integrated product development model for aircraft food dispensing machines. *Proceedings of the 13th International Design Conference, DESIGN 2014, Dubrovnik, Croatia*, 801–810.

Klaubert, H. (2005). *Tiny Design: A Study of the Design of MEMS systems* (Ph.D. Thesis, University of Cambridge).

Lee, H., Park, H. & Ghovanloo, M. (2013). A power-efficient wireless system with adaptive supply control for deep brain stimulation. *IEEE Journal of Solid-State Circuits*, *48*(9), 2203–2216.

Linehan, J., Paté-Cornell, M. & Yock, P. (2007). *Medical Device Development Models – Final Report*. Stanford, CA: Stanford University.

Marek, K. & Antle, L. (2008). *Medication Management of the Community-Dwelling Older Adult, Patient Safety and Quality: An Evidence-Based Handbook for Nurses*. Rockville (MD).

Obeso, J., Olanow, C., Rodriguez-Oroz, M., Krack, P., Kumar, R. & Lang, A. (2001). Deep-Brain Stimulation of the Subthalamic Nucleus or the Pars Interna of the Globus Pallidus in Parkinson's Disease. *The New England Journal of Medicine*, *345*(13), 956–963.

Parkinson's Disease Foundation. (2015). Retrieved February 4, 2015, from http://www.pdf.org

Reichl, H. (1994). Einführung in das Verbundprojekt. In *VDI/VDE-Technologiezentrum Informationstechnik GmbH (Hrsg): Untersuchungen zum Entwurf von Mikrosystemen. Abschlussbericht zum Verbundprojekt 1992–1994*. Teltrow.

Santos, C. (2013). *Product Development Methodologies: the case of medical devices* (Ph.D. Thesis, University of Porto).

Schätz, C. (2006). *A Methodology for Production Development - The Body of Knowledge Approach* (Ph.D. Thesis, Norwegian University of Science).

Schuepbach, W., Rau, J., Knudsen, K., Volkmann, J., Krack, P., Timmermann, L., ... Deuschl, G. (2013). Neurostimulation for Parkinson's Disease with Early Motor Complications. *New England Journal of Medicine*, *368*(7), 610–622.

Smith, M. (1997). *Application-Specific Integrated Circuits*. Addison Wesley, Berkeley.

Watty, R. & Binz, H. (2005). Design for manufacturing and integration of microelectromechanical systems. *Proceedings of the 15th International Conference on Engineering Design, ICED 2005, Melbourne*, 3898–3911.

WebMD. (2015). Deep Brain Stimulation for Parkinson's Disease. Retrieved September 22, 2015, from http : / / www . webmd . com / parkinsons - disease / guide/dbs-parkinsons

Coleiro Preca, H. M.-L. (2017). *Xjenza Online*, 5:69–71.

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DOI: 10.7423/XJENZA.2017.1.07

Commentary

Opening Speech by President of Malta Marie-Louise Coleiro Preca at the 6th Mediterranean Neuroscience Society Conference, Organized by the Mediterranean Neuroscience Society

H.E. Marie-Louise Coleiro Preca
President of Malta

It is my pleasure to welcome you to Malta, for the 6th Mediterranean Neuroscience Conference. While I augur you a most constructive and successful conference, I do hope you will also have some time to enjoy the rich cultural and historical heritage of our islands.

Let me begin by congratulating the Mediterranean Neuroscience Society, and the Malta Neuroscience Network of the University of Malta, along with your collaborators, for organising this important conference.

I am informed that there approximately 450 delegates, participating in this conference, including many students.

I feel very privileged to be among the brains of the Mediterranean region, and the world, while you are focusing your attention on the incredible power of the human brain!

It is reassuring to note that, thanks to these conferences over the years, you have gone above and beyond sharing good practices. In fact, you have also developed strong friendships, and created new opportunities for dialogue and connection.

In today's world, where conflicts and uncertainty seem to be of constant concern, especially within the Mediterranean region, this kind of relationship building is very essential.

I would like to take this opportunity to urge all our authorities to ensure that the benefits of your work are made accessible to all their members of society, irrespective of who they are, and where they come from.

I am pleased to note that through your engagement, at this conference, you are openly sharing and building upon profound scientific knowledge, by which, you are strengthening the global scientific community.

When you, as scientists, experts, and academics, share your learning, you send a strong message, that cooperation is more important than competition; that your connections are stronger than divisions; and that your commitment, to collaborative processes, is more powerful than the threat of conflict.

In this way, you are contributing to a culture of inclusive and participatory knowledge-building, which is a vital component of a healthy and sustainable culture of peace.

I believe that these collaborative processes must continue to take place so that they value the unique contributions of stakeholders, from the Global North and the Global South.

I must note that, your collaboration is a strong contribution, to the implementation of the United Nations' Agenda 2030, and its Sustainable Development Goals. In particular, Goal Number 3, which prioritise health and wellbeing for all humanity.

In order to achieve this essential goal, your collaborations must embrace all experiences, expertise and good practices, from both shores of our Mediterranean region, and beyond.

Therefore, I urge you to do more to encourage greater synergies, between our respective universities and research centres. In doing so, we can be secure, in the knowledge that we are working together, for the greater good of all.

Undoubtedly, when we work together, our potential to achieve positive results grows.

When we work together, we show that, no matter our differences, we are united in the pursuit of the common good, and that we are united by common values, to reach a common goal.

These should be the values that define us - values that represent our determination, to be of service to others.

I urge you to be aware of the influence that our values

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Opening Speech by President of Malta Marie-Louise Coleiro Preca at the 6th Mediterranean Neuroscience Society Conference, organized by the Mediterranean Neuroscience Society at the Radisson Blu, St Julian's on the 12th of June 2017. Right, H.E. the President of Malta; Left, from the right Prof. Marc Landry President of the MNS, Dr Liana Fattore, Vice-President of the MNS and Prof. Giuseppe Di Giovanni President of the Local Organizing Committee.

have, and the effects that they have on patients and service-users, while receiving your support and care.

I believe that, in all sectors of human life, it is the dignity of the individual that must be our primary concern.

Respect for the dignity of one another must be the cornerstone of a global culture of peaceful collaboration, and the ethical foundation of all scientific and social development.

The level of dignity and respect we show to the vulnerable is a direct reflection of the solidarity that we need to continue to strengthen, within our communities and societies.

Our focus on the dignity of others must also include wider strategies, within our nations, but also internationally. This approach will prioritise the holistic wellbeing of individuals and their families, who are living with chronic or life-long conditions.

Let me take an example. The ways that we respond to autism, and other neurodevelopmental conditions, should be a reminder that we need to be mindful of the ways that our societies are responding to the needs of these people. In this context, some questions come to my mind:

- In what ways are these people being included within society?
- How is their participation being promoted and safeguarded?
- How are we ensuring that they can enjoy a good quality of life and holistic wellbeing?

I urge you to discuss and evaluate our commitment to ensure full dignity to all, by emphasizing the need for effective social strategies, which promote the dignity of all people, and in this case, particularly people living with mental and neurological conditions.

Let me take this opportunity to emphasize that, the contributions of civil society, to safeguard human dignity, are essential.

By giving greater visibility for issues of mental and neurological health in your respective ways, you are be-

ing true activists of human dignity.

I urge you to continue to strengthen the connections you have created, as professionals and practitioners, with civil society organisations.

I believe that, it is vital that different stakeholders continue to come together, linking different countries, diverse disciplines, and specific sectors, together.

In this way, we shall be promoting a holistic approach to mental and neurological health.

Such a holistic approach leads to processes of reviewing, and potentially improving, legislation and policy, for the benefit of the 450 million people currently living with mental and neurological conditions.

Before concluding, let me share a few words with the students present at this conference.

I am sure that this conference will open up many opportunities for you. You will meet leading figures in the field of neuroscience. You will also have the chance to network with your peers and educators.

This is how dialogue among professionals begins.

It is important to make the most of these opportunities, even while you are still students.

I truly believe that, by working together, you will continue to promote safe spaces for scientific disciplinary. I am convinced that your cooperation and collaboration can be a driving force, to move science to new pastures, for the benefit of our global society.

I urge you to continue to commit yourselves to the wellbeing of all humanity, to ensure that the dignity of a human person will always be at the centre of all endeavours.

I urge you also to continue to value the lived experiences, and the specific narratives, of vulnerable individuals, through an inclusive and participative approach to healthcare.

Thank you for your attention, and I look forward to the outcomes of this conference.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.08

Commentary

The Endocannabinoid System – A Look Back and Ahead

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Over the last few decades research on the cannabinoids has gone through several distinct phases:

A. Research on plant cannabinoids (mostly on tetrahydrocannabinol (THC) and cannabidiol (CBD))
B. Research on endogenous cannabinoids (mostly on anandamide and 2-arachidonoyl glycerol (2-AG))
C. Research on endogenous anandamide-like endogenous fatty acid amides with amino acids and ethanol amines.

Plant cannabinoids While many dozens of plant cannabinoids are known today, most research is still on THC and CBD (Fig. 1).

CBD was isolated in the late 1930s, but its structure was elucidated only in 1963 (Mechoulam & Shvo, 1963). In 1964, when its structure was elucidated, pure THC was isolated (Gaoni & Mechoulam, 1964) and later synthesized. The psychoactivity of cannabis preparations (marijuana, hashish etc.) is mostly due to THC, but the other constituents may affect the activity of THC. Some of its metabolites are also psychoactive.

Thousands of publications have been published on the plant cannabinoids and some of them are already in use as therapeutic drugs. THC has been approved as a drug (named Marinol) for enhancement of appetite, and is also used to prevent vomiting due to cancer chemotherapy (Abrahamov & Mechoulam, 1995).

Of particular interest is CBD, which does not cause the typical cannabis psychoactivity, but is a potent antiepileptic drug (Cunha et al., 1980) and is used in many countries in pediatric epilepsy. It is being evaluated in other therapeutic areas (graft versus host disease, Yeshurun et al., 2015, schizophrenia, Leweke et al., 2012, and auto-immune diseases, for example Weiss et al., 2006).

The endogenous cannabinoids Anandamide (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Mechoulam et al., 1995) were discovered in the 1990s. Both compounds bind to the cannabinoid receptors CB1 and CB2. They are involved in a very large number of human diseases, mostly as neuroprotective entities (Pacher & Kunos, 2013).

Endogenous fatty acid amides with amino acids and ethanol amines A large number of compounds of these types have been discovered in the brain and other tissues, and some of them have been shown to be of major importance in a large spectrum of biological functions and diseases. Thus, oleoyl serine is an anti-osteoporotic molecule (Smoum et al., 2010) and arachidonoyl serine is a vasodilator and lowers brain damage (Cohen-Yeshurun et al., 2011).

Numerous pharmaceutical companies are now involved in research in all the above areas.

Acknowledgements

This commentary is a summary of the paper presented by the author at the 6th Mediterranean Neuroscience Society Conference held in Malta from 12th to the 15th of June.

References

Abrahamov, A. & Mechoulam, R. (1995). An efficient new cannabinoid antiemetic in pediatric oncology. *Life Sci. 56*, 2097–2102.

Cohen-Yeshurun, A., Trembovler, V., Alexandrovich, A., Ryberg, E., Greasley, P. J., Mechoulam, R., ... Leker, R. R. (2011). N-Arachidonoyl-L-serine is neuroprotective after traumatic brain injury by reducing apoptosis. *J. Cereb. Blood Flow Metab. 31*, 1768–1777.

Cunha, M., Carlini, E. A., Pereira, A. E., Ramos, O. L., Pimentel, G., Gagliardi, R., ... Mechoulam, R. (1980). Chronic administration of CBD to healthy volunteers and epileptic patients. *Pharmacologia, 21*, 175–185.

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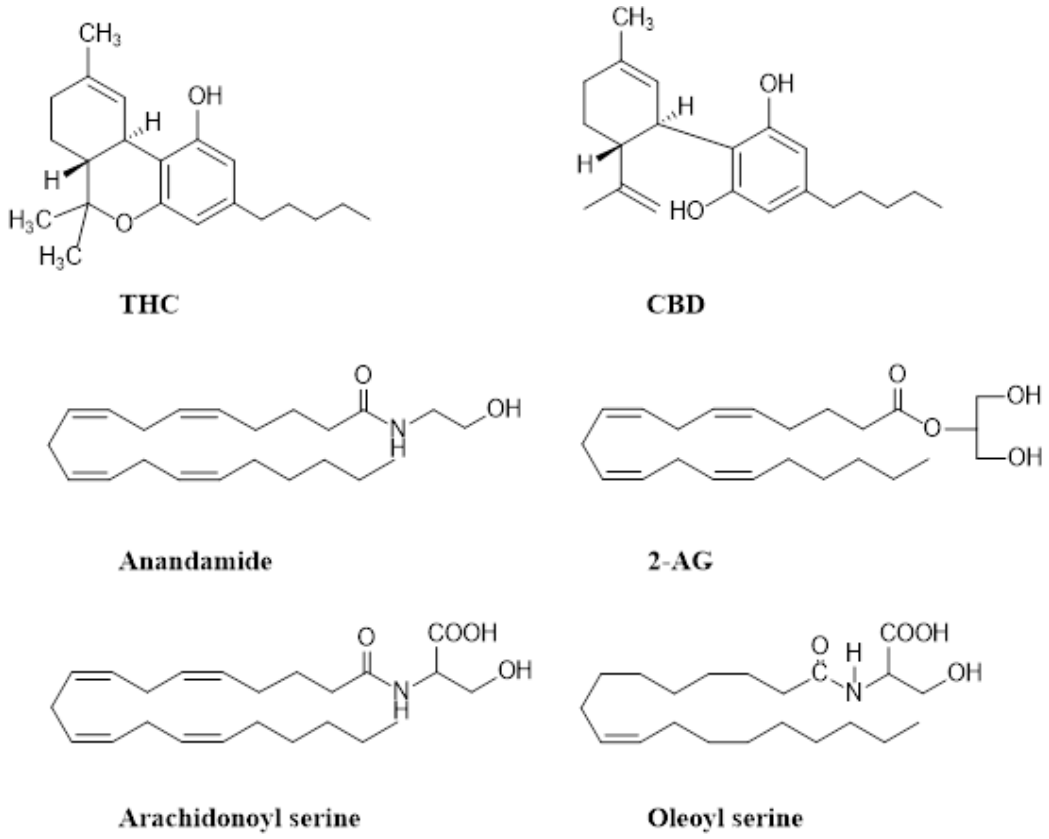

Figure 1: Cannabinoid compounds.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., ... Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science (80-.). 258*, 1946–1949.

Gaoni, Y. & Mechoulam, R. (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *J. Amer. Chem. Soc. 86*, 1646–1647.

Leweke, F. M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C. W., Hoyer, C., ... Koethe, D. (2012). Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl. Psychiatry*, *2*, 94.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., ... Vogel, Z. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol. 50*, 83–90.

Mechoulam, R. & Shvo, Y. (1963). The structure of cannabidiol. *Tetrahedron*, *19*, 2073–2078.

Pacher, P. & Kunos, G. (2013). Modulating the endocannabinoid system in human health and disease – successes and failures. *FEBS J*, *280*, 1918–1943.

Smoum, R., Bar, A., Tan, B., Milman, G., Attar-Namdar, M., Ofek, O., ... Mechoulam, R. (2010). Oleoyl serine, an endogenous regulator of skeletal mass. *Proc. Nat. Acad. Sci. 107*, 17710–17715.

Weiss, L., Zeira, M., Reich, S., Har-Noy, M., Mechoulam, R., Slavin, S. & Gallily, R. (2006). Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. *Autoimmun. 39*, 143–151.

Yeshurun, M., Shpilberg, O., Herscovici, C., Shargian, L., Dreyer, J., Peck, A., ... Ram, R. (2015). Cannabidiol for the prevention of Graft-Versus-Host-Disease after allogeneic hematopoietic cell transplantation: results of a phase II study. *Biol. Blood Marrow Transpl. 21*, 1770–1775.

Gozes, I. (2017). *Xjenza Online*, 5:74–76.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.09

Commentary

Autism, Schizophrenia and Alzheimer's Disease: A Common Thread from Neuropeptides to Brain Regulating Genes

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Our original cloning of the gene coding for vasoactive intestinal peptide (VIP) (Bodner, Fridkin & Gozes, 1985), led to the identification of VIP's involvement in synapse formation and neuroprotection, through our discoveries of activity-dependent neurotrophic factor (ADNF) (Brenneman & Gozes, 1996) and activity-dependent neuroprotective protein (ADNP) (Bassan et al., 1999; Zamostiano et al., 2001). To precisely delineate VIP and ADNP activities in the whole animal, we established transgenic animals, showing that manipulating VIP content impacts cognition in the mouse (Gozes et al., 1993). As for mouse ADNP, complete knockout results in severe neuronal tube closure defects and embryonic death at the time of neural tube closure (Pinhasov et al., 2003). ADNP haploinsufficient mice survive and show cognitive and social deficiencies, with pathologies resembling autism (Malishkevich et al., 2015) and Alzheimer's disease (Vulih-Shultzman et al., 2007). Delineating the mechanism of action of ADNP, we discovered binding to the SWI/SNF chromatin remodeling complex and heterochromatin protein 1 alpha, and direct interaction with specific gene promoters (e.g. the major risk gene for Alzheimer's disease, apolipoprotein E) (Mandel & Gozes, 2007; Mandel, Rechavi & Gozes, 2007). We have further discovered interactions with proteins associated with RNA splicing (Schirer et al., 2014), as well as with proteins regulating translation, like eukaryotic initiation factor 4E (Eif4e) (Malishkevich et al., 2015). In the cell cytoplasm, ADNP further interacts with the autophagy mechanism, binding to microtubule associated protein 1 light chain 3 (LC3) (Merenlender-Wagner et al., 2015) and to microtubule end binding proteins (EBs) (Oz et al., 2014). These multiple interactions, with key regulatory proteins, was further associated with the fact that ADNP regulates $>$ 400 genes during embryonic development (Mandel et al., 2007) and thousands of genes postnatally, with age and sex differences (Amram et al., 2016). Importantly, ADNP was recently identified as one of the major genes mutated de novo, leading to autism (short review and case report, Gozes et al., 2015). Furthermore, blood borne ADNP levels correlate with IQ tests in elderly individuals (Malishkevich et al., 2016). To try and combat ADNP deficiencies, we have designed and synthesized an ADNP – derived peptide, drug candidate, NAP (NAPVSIPQ) (Bassan et al., 1999), also known as davunetide, CP201. Containing the EB1,3 interacting domain SIP, NAP directly interacts with microtubules to induce the formation of dendritic spines (Oz et al., 2014) and brain synaptic plasticity. While enhancing ADNP interaction with microtubules as well as the autophagosome, NAP provided enhanced microtubule dynamics and active autophagy (Esteves, Gozes & Cardoso, 2014; Merenlender-Wagner et al., 2014). In animals, NAP provided protection against neuronal toxicities and genetic manipulations associated with autism, schizophrenia (Vaisburd, Shemer, Yeheskel, Giladi & Gozes, 2015) and Alzheimer's disease (Matsuoka et al., 2008). Based on the NAP binding site, a novel drug candidate was developed, namely SKIP, enhancing axonal transport and protecting cognition (Amram et al., 2016). While SKIP development is still at the preclinical stage, NAP has shown clinical efficacy and is now planned for further clinical development at Coronis Neurosciences (http://www.coronisns.com/) (see Fig. 1).

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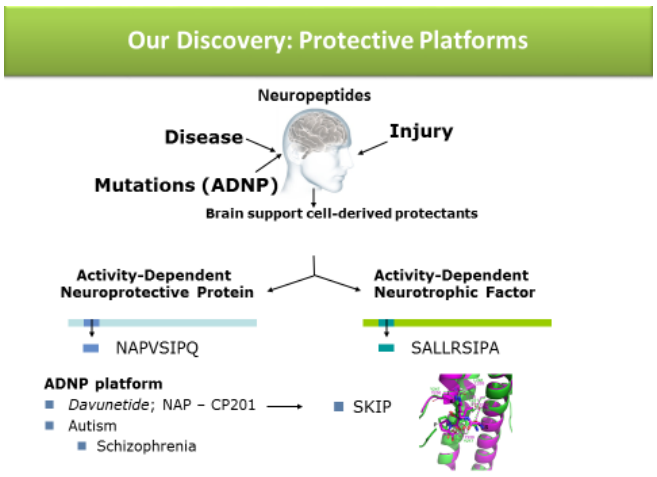

Figure 1: The figure describes our discoveries from neuropeptides (VIP) through the identification of ADNF and ADNP and novel protective peptides with a defined mechanism of action (docking on the microtubule end protein is shown) and clear clinical development path.

Acknowledgments

Support: ERA-NET Neuron, ISF, AMN

This commentary is a summary of the paper presented by the author at the 6th Mediterranean Neuroscience Society Conference, held in Malta from 12th to the 15th of June.

References

Amram, N., Hacohen-Kleiman, G., Sragovich, S., Malishkevich, A., Katz, J., Touloumi, O., ... Gozes, I. (2016). Sexual divergence in microtubule function: the novel intranasal microtubule targeting SKIP normalizes axonal transport and enhances memory. *Psychiatry*, *21*, 1467–1476.

Bassan, M., Zamostiano, R., Davidson, A., Pinhasov, A., Giladi, E., Perl, O., ... Gozes, I. (1999). Complete sequence of a novel protein containing a femtomolar-activity-dependent neuroprotective peptide. *J Neurochem*, *72*, 1283–1293.

Bodner, M., Fridkin, M. & Gozes, I. (1985). Coding sequences for vasoactive intestinal peptide and PHM-27 peptide are located on two adjacent exons in the human genome. *Proc Natl Acad Sci U S A*, *82*, 3548–3551.

Brenneman, D. E. & Gozes, I. (1996). A femtomolar-acting neuroprotective peptide. *J Clin Invest*, *97*, 2299–2307.

Esteves, A. R., Gozes, I. & Cardoso, S. M. (2014). The rescue of microtubule-dependent traffic recovers mitochondrial function in Parkinson's disease. *Biochim Biophys Acta*, *1842*, 7–21.

Gozes, I., Glowa, J., Brenneman, D. E., McCune, S. K., Lee, E. & Westphal, H. (1993). Learning and sexual deficiencies in transgenic mice carrying a chimeric vasoactive intestinal peptide gene. *J Mol Neurosci*, *4*, 185–193.

Gozes, I., Helsmoortel, C., Vandeweyer, G., Van der Aa, N., Kooy, F. & Sermone, S. B. (2015). The Compassionate Side of Neuroscience: Tony Sermone's Undiagnosed Genetic Journey–ADNP Mutation. *J Mol Neurosci*, *56*, 751–757.

Malishkevich, A., Amram, N., Hacohen-Kleiman, G., Magen, I., Giladi, E. & Gozes, I. (2015). Activity-dependent neuroprotective protein (ADNP) exhibits striking sexual dichotomy impacting on autistic and Alzheimer's pathologies. *Psychiatry*, *5*, e501.

Malishkevich, A., Marshall, G. A., Schultz, A. P., Sperling, R. A., Aharon-Peretz, J. & Gozes, I. (2016). Blood-Borne Activity-Dependent Neuroprotective Protein (ADNP) is Correlated with Premorbid Intelligence, Clinical Stage, and Alzheimer's Disease Biomarkers. *J Alzheimers Dis*, *50*, 249–260.

Mandel, S. & Gozes, I. (2007). Activity-dependent neuroprotective protein constitutes a novel element in the SWI/SNF chromatin remodeling complex. *J Biol Chem*, *282*, 34448–34456.

Mandel, S., Rechavi, G. & Gozes, I. (2007). Activity-dependent neuroprotective protein (ADNP) differentially interacts with chromatin to regulate genes essential for embryogenesis. *Dev Biol*, *303*, 814–824.

Matsuoka, Y., Jouroukhin, Y., Gray, A. J., Ma, L., Hirata-Fukae, C., Li, H. F., ... Aisen, P. S. (2008). A neuronal microtubule-interacting agent, NAPVSIPQ, reduces tau pathology and enhances cognitive function in a mouse model of Alzheimer's disease. *J Pharmacol Exp Ther*, *325*, 146–153.

Merenlender-Wagner, A., Malishkevich, A., Shemer, Z., Udawela, M., Gibbons, A., Scarr, E., ... Gozes, I. (2015). Autophagy has a key role in the pathophysiology of schizophrenia. *Mol Psychiatry*, *20*, 126–132.

Merenlender-Wagner, A., Shemer, Z., Touloumi, O., Lagoudaki, R., Giladi, E., Andrieux, A., ... Gozes, I. (2014). New horizons in schizophrenia treatment: autophagy protection is coupled with behavioral improvements in a mouse model of schizophrenia. *Autophagy*, *10*, 2324–2332.

Oz, S., Kapitansky, O., Ivashco-Pachima, Y., Malishkevich, A., Giladi, E., Skalka, N., ... Gozes, I. (2014). The NAP motif of activity-dependent neuroprotective protein (ADNP) regulates dendritic spines through microtubule end binding proteins. *Mol Psychiatry*, *19*, 1115–1124.

Pinhasov, A., Mandel, S., Torchinsky, A., Giladi, E., Pittel, Z., Goldsweig, A. M., ... Gozes, I. (2003). Activity-dependent neuroprotective protein: a novel gene essential for brain formation. *Brain Res Dev Brain Res*, *144*, 83–90.

Schirer, Y., Malishkevich, A., Ophir, Y., Lewis, J., Giladi, E. & Gozes, I. (2014). Novel marker for the onset of frontotemporal dementia: early increase in activity-dependent neuroprotective protein (ADNP) in the face of Tau mutation. *PLoS One*, *9*, e87383.

Vaisburd, S., Shemer, Z., Yeheskel, A., Giladi, E. & Gozes, I. (2015). Risperidone and NAP protect cognition and normalize gene expression in a schizophrenia mouse model. *Sci Rep*, *5*, 16300.

Vulih-Shultzman, I., Pinhasov, A., Mandel, S., Grigoriadis, N., Touloumi, O., Pittel, Z. & Gozes, I. (2007). Activity-dependent neuroprotective protein snippet NAP reduces tau hyperphosphorylation and enhances learning in a novel transgenic mouse mode. *J Pharmacol Exp Ther*, *323*, 438–449.

Zamostiano, R., Pinhasov, A., Gelber, E., Steingart, R. A., Seroussi, E., Giladi, E., ... Gozes, I. (2001). Cloning and characterization of the human activity-dependent neuroprotective protein. *J Biol Chem*, *276*, 708–714.

Austen, S. (2017). *Xjenza Online*, 5:77–80.

Xjenza Online - Journal of the Malta Chamber of Scientists
www.xjenza.org
DOI: 10.7423/XJENZA.2017.1.10

Commentary

Native Language (L1) Transfer in Second Language Learning: From Form to Concept, the Implications

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1 The Role of L1 Transfer

The influence that a student's first language (L1) can have on their acquisition of a second language (L2) has been frequently noted by language teachers (Swan, 1997; Jarvis, 2007) and documented in the literature for decades. However, thinking has gradually evolved in terms of the form that influence could take. Early research work focussed on transfer of syntax or form, but recently the role that L1 conceptual information plays in transfer has come to the fore.

The 1960s saw a plethora of contrastive studies inspired by the work of Robert Lado (1957), where languages were analysed using the prevailing structuralist approaches to language description. These contrastive studies were conceived with the view to predicting the types of errors speakers of one language would make while learning another, and this became known as the Contrastive Analysis Hypothesis (CAH) (Lado, 1957). This view was based in the behaviourist paradigm of the time which saw language learning as habit formation. This implied that learning a new language meant the transfer of elements and features from the first language to the target language, and that old 'habits' may interfere with second language acquisition (Aarts, 1982). Pairs of languages were compared in terms of their similarities and differences looking at linguistic units in relation to the overarching system to which they belonged (see Vinay & Darbelnet, 1960; Agard & Di Pietro, 1965, for examples). However, the CAH was severely criticised in the late 1960s, as it did not seem to be able to predict any classroom errors that language teachers had not already noticed, and was not able to offer any solutions with regard to how to deal with these errors (Corder, 1967).

What emerged from this debate in the late 1960s in relation to second language acquisition was the notion that what should be invested in was not the prediction of errors, but instead the investigation of noticeable errors in L2 production and their cause, or *error analysis* (EA) (Corder, 1967). The principal aim of EA was to establish whether or not L2 production errors were a result of L1 transfer; or creative construction: the creation of an independent linguistic system through cognitive processes much like those used to acquire L1. Researchers investigating this question cited results from morpheme studies which showed that participants from various L1 backgrounds acquired features of the same language in the same order, and argued, that this went against the strong L1 transfer hypothesis (Van Pattern & Benati, 2010). This indicated, they maintained, that instead of relying solely on L1 habits, learners develop an independent system subject to other factors which has become known as *interlanguage* (Selinker, 1972).

At this point, the strong form of the CAH hypothesis was rejected in favour of a weaker form which saw L1 transfer as one of the five processes which influenced interlanguage: language transfer, transfer of training, strategies of learning, strategies of communication and overgeneralisation (Selinker, 1972). Currently, while diminished in intensity, the hypothesis that L1 transfer is a factor in interlanguage is still very much alive and this is reflected in renewed interest in it, and in particular in the form that this transfer might take.

2 What Sort of Influence does L1 Transfer have on Interlanguage?

Consider the following error:

I know him for a long time.

This is a common error in Romance language speakers of English. In Romance languages such as French,

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Italian and Spanish the concept of *knowing someone for a long time* is expressed using the Present Simple tense, in English the Present Perfect is used: *I have known him for years*. At first glance, this would seem an error at the level of syntax or form; that the L1 Present Simple tense is translated into L2 English resulting in *I know him*. This purely syntactical approach was the approach taken in the 1960s and detailed above. The two forms are directly substitutable as the prototypical meaning of each tense is the same, in this case *a present state*. However, this is not the meaning which fits the concept – *knowing someone for a long time* in English, that an L1 English speaker would wish to convey, and distinguishes this learner production from that of a native speaker. In English the past to present feature of the state of *to know someone for a long time* is crucial and conveyed using the Present Perfect which denotes this past to present time span.

With more of a focus on language as a meaning making system and the advent of communicative approaches to language teaching in the 1980s, the notion of semantic transfer as an alternative emerged (Gass, 1983; Odlin, 2005; Pavlenko & Jarvis, 2008). Semantic transfer involves the transfer of the L1 form and the meaning that it holds for the speaker in to L2. Semantic transfer holds that the L2 speaker conceptualises the event in the same way as is provided for by the L1, but extends the use of the L1 form-function pairing to cover the concept to be denoted, in to the L2. Interference occurs at the level of connecting concepts with the correct L2 semantic representations and forms (Pavlenko & Jarvis, 2008). In terms of our example, this would mean that the L2 speaker takes the *I know him* form and its meaning of *present state* and uses it in L2, but understands the situation in the same terms as an English L1 speaker – as a situation which starts in the past and continues into the present.

More recently, research has been focussed on the possibility that L1 conceptual knowledge formed through socialisation into an L1 language system could play a part in L2 acquisition in the form of Conceptual Transfer (CT) (Odlin, 2005; Pavlenko & Jarvis, 2008). Jarvis (2007) describes CT in the following way:

> Conceptual transfer can be characterised as the hypothesis that certain instances of cross-linguistic influence in a person's use of one language originate from the *conceptual knowledge* and *patterns of thought* that the person has acquired as a speaker of another language.

CT holds that the speaker takes his/her L1 conceptualisation of the event, formed through L1 inductive learning processes (Croft, 2001; Pavlenko & Jarvis, 2008), and transfers this along with the form-function pairing into the L2. This implies that the perception of the event differs qualitatively cross-linguistically. This could account for some of the more persistent errors which seem to have a base in L1 and affect attainment of native speaker competence (Gruhn & Reshöft, 2014). Under the umbrella term of Conceptual Transfer, Jarvis (2007) distinguishes between two further types of conceptual transfer: *concept* and *conceptualisation transfer* (Jarvis, 2007). The former relates to the transfer of concepts stored in long-term memory and the latter to patterns of conceptualisation which are necessarily influenced by stored conceptual content.

In recent years, there has been an increased interest in Conceptual transfer and its influence on L2 interlanguage. A number of studies in both SLA and Bilingualism have shown that L1 conceptual categories have a significant effect on language production (Carroll & von Stutterheim, 2003; Bylund, 2009; Bylund & Jarvis, 2011; Schmiedtová, von Stutterheim & Carroll, 2011; Schmiedtová, 2013; Türker, 2015; Sharpen, 2016). Research has centred on conceptual transfer with its origin in cross-linguistic difference in the grammaticalisation of various concepts such as motion events (Negueruela, Lantolf, Jordan & Gelabert, 2004) and aspectual distinctions (Bylund and Jarvis, 2011). Investigators have used a variety of techniques often combining verbal elicitation tasks with co-verbal measures of behaviour such as eye-tracking and recording speech onset time (SOT) (see Schmiedtová et al., 2011; Schmiedtová, 2013). Such combinations of verbal production data with consistent measures of behaviour have produced compelling evidence for cross-linguistic differences in L1 conceptual information impacting on L2 production. In a notable study, Schmiedtová (2013) established that cross-linguistic differences in the grammaticalisation of aspect were evident in the L2 German of English L1 speakers. English encodes aspect in the form of the progressive (+*ing* form of the verb), whereas German does not. They found that this lead to English L1 speakers of German L2 mentioning fewer end points when describing an event in progress, than native German speakers. The SOT times also resembled those of native English speakers, suggesting that the L1 English speakers were maintaining L1 conceptual patterns when speaking in L2.

3 L1 Transfer in the Classroom

It is evident then, that L1 conceptual transfer is a factor in interlanguage and the effect that it has on L2 production does considerably differentiate the language of learners from native speakers. Enabling learners to overcome or circumnavigate the conceptual constraints of their native language could play an important role in helping them to achieve native-like competence in L2 (Gruhn & Reshöft, 2014).

Conceptual transfer research shows us that language as a meaning making system represents a diverse range of concepts, and triggers varying conceptualisations cross-linguistically. Focussing on linguistic meanings, and not forms, could help to raise students' awareness of inter-lingual diversity in conceptual information, and also how their own L1 impacts upon their use of L2. It falls then to language teachers to help students to develop a metalinguistic awareness (Cook, 1995) which enables them to separate form and meaning and consider language as having a potential which goes beyond the meanings that are held in speakers' minds, and on to the power that there is in the world view created by these meanings and how their manipulation can create different meanings.

Gruhn and Reshöft (2014), for example, aimed to increase students' metalinguistic awareness by introducing some psycholinguistic tests into the second language classroom (Gruhn & Reshöft, 2014). The participants in the Gruhn and Reshöft study (German L1 High School students of English L2) were introduced to a number of psycholinguistic tests such as *Endpoint* (Von Stutterheim & Nuse, 2003) and *Frog story* (Slobin, 1996) which have been used to investigate cross-linguistic conceptual influence. Participants were then facilitated in replicating the tests and reporting their results. The authors argue that by becoming aware of findings in conceptual transfer research and engaging with the experimental design, the students gained increased metalinguistic awareness. Replicating psycholinguistic tests in the classroom is certainly not possible for all language teachers who are often faced with limited resources and time. However, the study raises some interesting points with regard to raising language students' awareness of conceptual transfer effects through presentation of relevant research, and increasing their metalinguistic awareness through classroom activities targeted to do just this.

As detailed above, conceptual transfer is a cognitive phenomenon with its origins in the socialisation of a subject into a particular L1, where language is learnt through interaction with the physical world and experience of it. This implies that successful learning of an L2 should involve similar processes, where the language is experienced as meaning. This is the view taken by proponents of the embodied approach to language who maintain that humans employ the same neural mechanisms to experience the world as they do to process and understand language (Buccino & Mezzadri, 2015). This relatively new approach is grounded in neurophysiological evidence which suggests a connection between, for example, the motor system and language, through mirror neurons. Mirror neurons discharge both when a physical action is performed and when the same action is observed as being performed by another. These neurons have also been revealed to activate when the actions are described verbally (Rizzolatti & Craighero, 2004). This leads Buccino and Mezzadri (2015) to conclude:

> When a content has to be expressed and learned in a second language, it should refer to something which has been experienced sensori-motorically and emotionally by the learner.

This has a number of implications for the classroom. It means that language should be tied to experience, so that i) students should not be expected to learn language to refer to events or situations which they have not yet experienced (particularly relevant when teaching children); ii) that learning should be tied to sensorimotor experience with the world where possible and iii) that learning should start from the student's real world experience of the target language referents and build on from there (Buccino & Mezzadri, 2015). This approach may be instrumental in building new conceptual categories and conceptualisations from the outset of language learning, reducing reliance on L1.

4 Conclusion

The study of L1 transfer has evolved considerably over the last half-century from a concentration on syntax and form through to current interest in the transfer of L1 constrained conceptual content and conceptualisations. It is clear that Conceptual Transfer is a significant factor in inter-language and could account for errors which persist even at higher levels of proficiency in the target language. Gaining a deeper understanding of the way that L1 concepts and conceptualisations impact on L2 production can help up to develop strategies to help students overcome these constraints.

References

Aarts, F. (1982). The contrastive analysis debate: problems and solutions. *Stud. Anglica Posnaniensia*, *14*, 47–68.

Agard, F. B. & Di Pietro, R. J. (1965). *The Grammatical Structures of English and Italian*. Chicago: University of Chicago Press.

Buccino, G. & Mezzadri, M. (2015). Embodied language and the process of language learning and teaching. In U. M. Lüdtke (Ed.), *Emotion in Language: Theory - Research - Application*. Amsterdam: John Benjamins.

Bylund, E. (2009). Effects of age of L2 acquisition on L1 event conceptualization patterns. *Biling. Lang. Cogn. 12*(3), 305–322.

Bylund, E. & Jarvis, S. (2011). L2 effects on L1 event conceptualization. *Biling. Lang. Cogn. 14*(1), 47–59.

Carroll, M. & von Stutterheim, C. (2003). Typology and information organisation: perspective taking and language – specific effects in the construal of events. In A. Giacalone-Ramat (Ed.), *Typology and Second Language Acquisition* (pp. 66–97). Berlin: Mouton de Gruyter.

Cook, V. (1995). Multicompetence and the learning of many languages. *Lang. Cult. Curric. 8*, 93–98.

Corder, S. P. (1967). The significance of learner's errors. *Int. Rev. Appl. Linguist. 5*, 161–170.

Croft, W. (2001). *Radical Construction Grammar.* Oxford: Oxford University Press.

Gass, S. (1983). Language Transfer and Universal Grammar Relations. In S. Gass & L. Selinker (Eds.), *Language transfer in language learning* (pp. 69–84). Massachusetts: Newbury House.

Gruhn, M. & Reshöft, N. (2014). Getting closer to native speaker competence: How psycholinguistic experiments can enrich language learning and teaching. In M. Pawlak, J. Bielak & A. Mystkowska-Wiertelak (Eds.), *Classroom-oriented Research: Achievements and Challenges* (pp. 203–216). Second Language Learning and Teaching. Zurich: Springer.

Jarvis, S. (2007). Theoretical and methodological issues in the investigation of conceptual transfer. *Vigo Int. J. Appl. Linguist. 4*, 43–71.

Lado, R. (1957). *Linguistics across cultures: Applied linguistics for language teachers.* University of Michigan Press.

Negueruela, E., Lantolf, J. P., Jordan, S. R. & Gelabert, J. (2004). The "private function" of gesture in second language speaking activity: a study of motion verbs and gesturing in English and Spanish. *Int. J. Appl. Linguist. 14*(1), 113–145.

Odlin, T. (2005). Crosslinguistic Influence and Conceptual Transfer: What are the concepts? *Annu. Rev. Appl. Linguist. 25*, 3–25.

Pavlenko, A. & Jarvis, S. (2008). *Cross-linguistic influence in language and cognition.* London: Routledge.

Rizzolatti, G. & Craighero, L. (2004). The Mirror-Neuron System. *Annu. Rev. Neurosci. 27*(1), 169–192.

Schmiedtová, B. (2013). Traces of L1 patterns in the event construal of Czech advanced speakers of L2 English and L2 German. *Int. Rev. Appl. Linguist. Lang. Teach. 51*(2), 87–116.

Schmiedtová, B., von Stutterheim, C. & Carroll, M. (2011). Language specific patterns in event construal of advanced second language learners. In A. Pavlenko (Ed.), *Thinking and Speaking in two Languages* (pp. 66–107). Bristol: Multilingual Matters.

Selinker, L. (1972). Interlanguage. *Int. Rev. Appl. Linguist. 10*, 209–241.

Sharpen, R. (2016). L1 Conceptual Transfer in the acquisition of L2 motion events in Spanish and English: The Thinking-for-Speaking Hypothesis. *Open Linguist. 2*, 235–252.

Slobin, D. I. (1996). From "thought and language" to "thinking for speaking". In J. J. Gumperz & S. J. Levinson (Eds.), *Rethinking Linguistic Relativity* (pp. 70–96). Cambridge: Cambridge University Press.

Swan, M. (1997). The influence of the mother tongue on second language vocabulary acquisition and use. In N. Schmitt & M. McCarthy (Eds.), *Vocabulary: description, acquisition and pedagogy* (pp. 156–180). Cambridge: Cambridge University Press.

Türker, E. (2015). The role of L1 conceptual and linguistic knowledge and frequency in the acquisition of L2 metaphorical expressions. *Second Lang. Res. 32*(1), 25–48.

Van Pattern, B. & Benati, A. G. (2010). *Key Terms in Second Language Acquisition.* London: Continuum.

Vinay, J. P. & Darbelnet, J. (1960). Stylistique comparée du français et de l'anglais. *Rev. Belge Philol. e d' Hist. 38*(2), 451–452.

Von Stutterheim, C. & Nuse, R. (2003). Processes of conceptualisation in language production: language – specific perspectives and event construal. *Linguistics*, *41*(5), 851–881.

Bower, C. (2017). *Xjenza Online*, 5:81–82.

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News and Views

Science in the Citadel

Colleen Bower*

On a sunny Saturday afternoon, the 22nd of April, *Science in the Citadel* was held in Gozo's iconic medieval Citadel. It provided an atmospheric venue for the first science festival on the island. The festival was sponsored by Eco-Gozo, and inaugurated by Minister of Gozo, Dr Anton Refalo. The aim of the festival was to engage the public with science, to inspire the next generation towards scientific endeavour and to showcase Gozo as a potential future hub for scientific discussion and development.

Figure 1: The citadel located in Victoria (Rabat), Gozo.

As a historical venue, the Citadel presented a number of logistical challenges. The organisers were clear from the outset that, if you want to engage local people, the Citadel was the place to start. The centre of the island since before medieval days, the citadel provided a secure refuge against attack. It could not have done so were it not for the foresight and growing specialisation of the military scientists and engineers of the day. The latest in thinking regarding construction, food and water storage and defence were all incorporated into its structure. Moreover, the first Hospital on Gozo was built within its walls. Advancements were made in disease control, medicine and pharmacy, with the influx of physicians, surgeons and apothecaries under the rule of the Knights of St John, the Hospitaller Order.

Hence the Citadel and its internal structures provide tangible testimony to the fact that scientific inquiry was as relevant to the lives of the people of the past as it is today. It also presented a concrete symbol of the continuity of scientific endeavour though the centuries; the truth that, to paraphrase Newton, today's scientists might see further ahead, but only because they can stand on the shoulders of giants. And its physical situation allowed us to truly situate the various strands of science presented in what was known and perceptible to the visitors.

A walk around the Citadel reveals an archaeology museum, perfect as a venue for discussing the chemical transformations involved in turning clay into pottery; a nature museum, its exhibits the backdrop to talks about the relevance of geology to everyday life and the science attached to the flora, birds and bees of the island; an Old Prison, the context for forensic science demonstrations and an Old Hospital, the cue for biomedical and anatomical research displays and talks. The imposing bastions offer panoramic views of the skies, seas and layout of the island, inviting further investigation and interest in astrophysics with telescopes, geoscience with seismology stories and marine sciences offering results of underwater explorations. Thus the subject matter of the festival was truly embedded in its situation.

Furthermore, the festival offered tiered engagement opportunities. Whilst adults and the already science-savvy could take in a talk, meet the researcher or debate with peers in a Café Scientifique style discussion, children from kindergarten upwards were the target audi-

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ence of a range of theatrical science demonstrations, busking and shows provided by Esplora and Kids Dig Science in the equally theatrical surroundings of the Ditch (moat), and workshops in some of the arched internal spaces. The schools on the island took part in a challenge to use the Citadel as inspiration for a piece of scientific investigation with the wide-ranging results on topics from the physics of sound, inspired by the ancient bells to camouflage in nature. It is estimated that over 3000 visitors attended the festival. The results of the exit survey and the anecdotal evidence suggest that the participants were highly engaged.

Figure 2: Adults and children bubbling with enthusiasm for science.

Science in the Citadel was organised by Colleen Bower, coordinator of Café Scientifique in Gozo and a small team of volunteers in association with Esplora, the Malta Chamber of Scientists and EcoGozo. Contributions to the event included personnel from several departments of the University of Malta, researchers from Malta College for Science & Technology (MCST) and Malta Life Sciences Park projects, and special interest organisations such as BirdLife Malta, SharkLab and the National Aquarium.

Figure 3: Young scientists experimenting with bubbles and the phenomena of surface tension.

Di Giovanni, G. and Magri, D. C. (2017). *Xjenza Online*, 5:83–85.

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News Article

Xjenza Online at MNS2017 Malta

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The 6th conference of the Mediterranean Neuroscience Society (MNS), MNS2017, was organized by Prof. Giuseppe Di Giovanni, Co-ordinator of Malta Neuroscience Network (MNN) of the University of Malta and held at the Radisson Blu Hotel in St. Julian's during the week of Monday 12 June to Thursday 15 June.

The MNS2017 is a part of the effort of MNS to bring its contribution to the development of neuroscience in the Mediterranean region facilitating the exchanges with scientists from the rest of the world.

The previous MNS Conferences were organized in June 2015 in Pula (Italy), Istanbul (Turky) 2012, Alexandria (Egypt) 2009, Marrakech (Morocco) 2006, Montpellier (France) 1997.

These meetings have proved to be highly beneficial, not only for the scientific exchanges between Mediterranean neuroscientists, but also in terms of training opportunities for students and young researchers.

More than 450 delegates, including neurologists, psychiatrists, psychologists, basic and clinical researchers from 41 Countries attended the MNS2017 in Malta.

HE Marie Louise Coleiro Preca, President of Malta, opened the Conference (see her speech in this volume) together with Prof. Alfred J. Vella, the Rector of the University of Malta, Prof. Marc Landry, Mediterranean Neuroscience Society (MNS) President (University of Bordeaux, FR) and Prof. Giuseppe Di Giovanni.

The meeting gathered scientists, not only from the broad Mediterranean area, offered a rich program, spanning from molecular and cell biology to behaviour under normal and pathological conditions. This included 9 main lectures, over 60 symposia, poster sessions and social events. The meeting has been highly beneficial, not only for the scientific exchanges, but also in terms of training opportunities for students and young researchers.

Nine special keynote speakers were in the programme!

Two University of Malta Plenary Lectures by Giacomo Rizzolatti (IT/MT), and Vincenzo Crunelli (UK/MT). The others were given by:

- Juan Lerma from Spain, Secretary General of the Federation of European Neuroscience Societies (FENS);
- Raphael Mechoulam, from Israel. He discovered and isolated the molecule of THC, the main active principle of cannabis, in the 60s (see his commentary in this issue);
- Michela Matteoli, from Italy, member of the European Molecular Biology Organization (EMBO);
- Pierre J. Magistretti from Switzerland, President of International Brain Research Organization (IBRO);
- Rosa Cossart from France;
- Carmen Cavada from Spain;
- Ilana Gozes from Israel (see her commentary in this issue).

Xjenza Online was a major supporter of the MNS2017, we published a SI on the Proceedings of the Conference that was distributed to the 500 attendees (Xjenza Online Vol. 5 (SI), 2017), organized a Workshop on how to publish and gave a prize for the best Maltese posters.

Three Poster prizes were given to the best posters presented at MNS2017

1. Best Poster Prize Xjenza/RIDT For the best Maltese Posters presented at MNS2017.
2. Best Poster Prize Elsevier/J Neurosci Methods-Neuropharmacology For the Best Methodological and Pharmacological Posters
3. Best Poster Prize Springer Nature/The Receptors

The awarded received the prizes at the ceremony held at the end of the conference on the 15 June.

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Xjenza/RIDT Best Poster Prizes @ MNS2017

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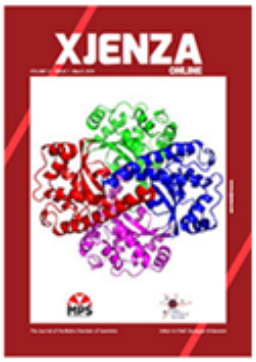

Ms Theresia Dalli. Abstract Title "Mycoplasma pneumonia associated encephalopathy: a case report"

Ms Rebecca Borg. Abstract Title "Phenotypic overlap between disruption of SNRNP biogenesis and SMN-GEMINS complex perturbation: implications for motor neuron disease"

Mr Norbert Abela. Abstract Title "The long term brain effects of binging on alcohol and marijuana in adolescent tobacco users: a study on motivation in operant food-reinforced responding"

Mr Francis Delicata. Abstract Title "Serotonin-2A AND -2C receptor modulation of the lateral Habenula activity in the context of nicotine addiction: a neuroanatomical and electrophysiological study"

The meeting had 2 free workshops on Wednesday 14 June, "How to get your paper published", and "Statistics for neuroscientists".

Xjenza and Elsevier Workshop on How to Write and Publish an Academic Paper at MNS2017 Malta

Journals exist for the purpose of disseminating new research findings and the latest scholarly thinking by professional communities worldwide. This workshop presented an opportunity for authors and would-be authors to gain insight into journal publishing from the editors of *Journal of Neuroscience Methods*, *Neuropharmacology* and *Xjenza Online*. The event covered a number of publishing related topics including how to:

- Choose the right journal;
- Tips on writing a manuscript;
- The editorial process;
- The peer-review process;
- Publishing ethics: plagiarism and data fabrication;
- Open access and funding policies.

The workshop was sponsored by Elsevier, the Malta Chamber of Scientists and the University of Malta. Around 40 researchers were in attendance for the workshop, which was held on Wednesday afternoon from 1:00 pm to 4:00 pm in room Marie Louise 1.

Figure 1: The Xjenza/RIDT poster prize awardees Rebecca Borg, Norbert Abela, Francis Delicata and Vincenzo Crunelli of the University of Malta & Cardiff University and Editor-in-Chief of the Journal of Neuroscience Methods (UK), Giuseppe Di Giovanni, Coordinator of Malta Neuroscience Network (MNN) of the University of Malta (MT), Liana Fattore, Mediterranean Neuroscience Society (MNS) Vice-President (CNR, IT), Bruno Frenguelli of the University of Warwick (UK) and Editor-in-Chief of *Neuropharmacology*, Prof. Marc Landry, Mediterranean Neuroscience Society (MNS) President (University of Bordeaux, FR) (from the left).

Figure 2: RIDT's CEO Wilfred Kenely and Prof. Giuseppe Di Giovanni.

Presentations were given by Shamus O'Reilly (Senior Publisher Elsevier), Natalie Farra, (Senior Acquisitions Editor, Neuroscience), Ryan Scicluna (University of Malta Library), Vincenzo Crunelli (Editor Journal of Neuroscience Methods), Bruno Frenguelli (Editor Neuropharmacology), Giuseppe Di Giovanni and David Magri (Editors Xjenza Online) on various aspect of publishing a research paper. The workshop opened with introductory presentations by Shamus O'Reilly on neuroscience journals and Natalie Farra on neuroscience books.

Vincenzo Crunelli of the University of Malta & Cardiff University and Editor-in-Chief of the *Journal of Neuroscience Methods*, discussed the importance of authorship and guidelines for deciding who should be listed as an author, and in what order, on a research paper. Bruno Frenguelli of the University of Warwick and Editor-in-Chief of *Neuropharmacology*, gave a presentation on editorship and how an editor goes about deciding whether a submitted manuscript is suitable for consideration, and the considerations an editor must make on deciding whether a manuscript submitted for peer-review is acceptable or not for publication.

The workshop concluded with presentations by Giuseppe Di Giovanni of the University of Malta and Editor-in-Chief of *Xjenza Online* and Associate Editor of *Journal of Neuroscience Methods*, on the peer-review process followed by David Magri of the Malta University and an Associate Editor of *Xjenza Online* on publishing ethic, from many dos and don'ts, with a brief mention of example case studies.

Further information about the conference can be obtained from the website: http://www.mnsociety.net/ and http://www.mcs.org.mt/index.php/xjenza.

Giuseppe Di Giovanni, Ph.D., Editor-in-Chief, invites you to

submit your research to Xjenza Online!

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XJENZA ONLINE
VOLUME 5 ISSUE 1, 2017

Table of Contents

Articles