Temporal aspects of cyclic messenger signaling in object recognition memory: a pharmalogical approach

VALORISATION ADDENDUM

Amnesia and cognitive impairment are becoming more common, especially in the aging western societies. Although cognitive decline is the hallmark symptom of Alzheimer's disease (AD) there is a host of other neuropsychiatric disorders that are associated with it. These include epilepsy, posttraumatic stress disorder (PTSD), depression, schizophrenia, Korsakoff's syndrome, Parkinson's disease and Huntington's disease. In addition, it may also be induced by malnutrition, sleep deprivation, severe stress, traumatic brain injuries (TBI) and stroke.

The tremendous burden that cognitive impairments pose on the quality of life of those who suffer from these afflictions is obvious. However, the psychological impact of cognitive impairment goes far beyond the individual, as patients become heavily dependent on relatives and professional caregivers. Furthermore, the economic burden on society is enormous. It is estimated that 5.2 million Americans currently have AD and this number is expected to grow to 13.8 million around 2050. Worldwide, the number of people suffering from dementia in 2050 is estimated to be 115 million. In the US alone, the total costs in 2014 for healthcare for people aged 65 years and older with dementia are calculated to be $214 billion. In 2013, more than 15 million family members and other unpaid caregivers were estimated to have provided 17.7 billion hours of care to people with AD and other dementias, an additional indirect cost valued around $220 billion in man hours. These examples underline the magnitude of the social and economic costs that cognitive impairment has on our society and the urgent need to develop effective treatments.

The paradoxical issue about the current pharmacological treatments for Alzheimer's disease is that they are all focused on the manipulation of a single neurotransmitter system while it is known that there are numerous other molecules involved in memory formation. At present, the only approved drug treatments are either focused on enhancing cholinergic neurotransmission by inhibiting acetylcholinesterase, like donepezil, or glutamatergic receptor blocking. The effects of these treatments are largely symptomatic and have no significant impact on the long term-progression of

the disease. In order to develop more effective treatments, there is a great need to explore alternative neurobiological targets, for instance the different PDE families.

PDEs are modulatory enzymes that regulate the intracellular levels of cyclic guanosine monophosphate (cGMP) and/or cyclic adenosine monophosphate (cAMP), which are important molecules in signal transduction related to memory formation. Several PDE families have been implicated in memory processes; amongst these are the PDE2, PDE4 and PDE5 families, for which specific pharmacological inhibitors (PDE-Is) have been developed. Two of these drugs are the PDE4 inhibitor rolipram and the PDE5 inhibitor vardenafil (Levitra). Although these PDE-Is were initially developed for diseases like chronic obstructive pulmonary disease (COPD) and erectile dysfunction, respectively, evidence from animal studies indicates that these drugs also have cognition enhancing properties. On the other hand, in human studies evidence for cognition enhancing properties of PDE-Is still has not been convincingly confirmed yet. These translational difficulties are not specific for PDE-Is, but are characteristic for the development of cognition enhancers in general. In order to achieve a better predictive validity we need to develop a more profound understanding of animal cognition, the way it is influenced by environmental factors and how to properly interpret the results from cognitive assessments. In addition, we need to invest more resources in basal scientific research into the biological processes underlying cognition so that we are better equipped to determine viable cognition enhancing treatments.

The former Nobel Prize winner Eric Kandel underlined the importance of behavioral research in his book 'In search of Memory: The Emergence of a New Science of Mind'. As he stated; "*The results of that work had shown that different patterns of stimulation alter the strength of synaptic connections in different ways. But Tauc and I had not examined how an actual behavior is changed and therefore had no evidence that learning really relies on changes in synaptic strength*." It is crucial to translate molecular findings into behavioral observations and also, to correctly interpret these behavioral changes in a meaningful way.

In this thesis, I structurally used the Object Recognition Task (ORT) to measure cognitive effects of drugs on Rats. Many different variants of the ORT are used and

there is little consensus about the correct way to analyze the readout parameters or even what exact type of memory is being measured in the ORT. We extensively investigated a large data set to establish the most appropriate way to perform the experiments and interpret the results. This way we optimized the scientific revenue of our experiments. By publishing these results in specialized journals, we contributed to the general understanding of this behavioral task and the standardization of this task across laboratories around the world.

Another important factor to take into account when translating animal studies to humans is the difference in drug pharmacokinetics between animal species. This is important because the target molecules of cognition enhancing drugs have different active time windows during the formation memories. Therefore, it is of the utmost importance to accurately determine the pharmacokinetic (PK) properties of these drugs, especially when experimenting with a one-trial learning task (like the ORT). Furthermore, these PK properties are dependent on the administration route that is used. In the case of rolipram and vardenafil we are the first group which published the PK properties of orally administered vardenafil and rolipram administered intra peritoneally, in the rat, over the first hour post-administration. These PK data helped us determine the active windows of the PDE-regulated cGMP and cAMP signaling during memory formation, which gave us information about possible interactions between acquisition and (early) consolidation processes during memory formation. Our observations confirmed previously reported molecular findings. Thus, we are the first to reveal the complete active windows of cAMP and cGMP in vivo.

A better understanding about the pharmacokinetic properties of a drug helps us to also better understand the characteristics of its target(s). In the same way it is very relevant to properly understand the expression of its behavioral effects. In this thesis we show that the expression of memory is not stable over time, but disappears for a certain amount of time before re-appearing again. Hopefully, this knowledge will assist in the development better test protocols, so that promising effects of new treatment candidates will not be overlooked in a pre-clinical phase. The expression

gap may also be present in human memory, and therefore it would be interesting to further investigate this possibility.

Finally, there is a growing concern about animal welfare. This is a valid concern as stress and discomfort are certain to affect the outcome of behavioral studies. A great deal of discomfort applied to laboratory animals is due to the large extent of impoverishment of their housing conditions due to standardization. We demonstrate that animals living in discomfort and unnatural circumstances have poorer cognitive capabilities compared to their counterparts living in more natural circumstances. We also demonstrate that these living conditions greatly affect the cognition enhancing effect of vardenafil. An effect that is likely caused by more basic underlying physiological processes and hence affects the translational value of pre-clinical studies towards cognition enhancing drugs in general. Considering enriched housing for cognition studies in animals will greatly increase animal welfare as well as enhance the predictive validity of the results found. Together with the refinements of the ORT methodology and analyses we hope to contribute to a qualitatively better and more humane way of performing animal research.