European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

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Fluoxetine, not donepezil, reverses anhedonia, cognitive dysfunctions and hippocampal proteome changes during repeated social defeat exposure

M.G. Gottschalk[a], P. Mortas[b], M. Haman[b], S. Ozcan[a], B. Biemans[b,*,1], S. Bahn[a,**,1]

[a]*Cambridge Centre for Neuropsychiatric Research, Department of Chemical Engineering and Biotechnology, University of Cambridge, UK*
[b]*Roche Pharma Research & Early Development, NORD DTA, Roche Innovation Center Basel, Switzerland*

Received 20 April 2017; received in revised form 17 September 2017; accepted 3 November 2017

KEYWORDS
Major depressive disorder;
Social defeat model;
Proteomics;
Protein interaction networks;
Intracranial-self-stimulation;
Selected reaction monitoring;
Fluoxetine;
Donepezil

Abstract
While anhedonia is considered a core symptom of major depressive disorder (MDD), less attention has been paid to cognitive dysfunctions. We evaluated the behavioural and molecular effects of a selective serotonin re-uptake inhibitor (SSRI, fluoxetine) and an acetylcholinesterase inhibitor (AChEI, donepezil) on emotional-cognitive endophenotypes of depression and the hippocampal proteome. A chronic social defeat (SD) procedure was followed up by "reminder" sessions of direct and indirect SD. Anhedonia-related behaviour was assessed longitudinally by intracranial self-stimulation (ICSS). Cognitive dysfunction was analysed by an object recognition test (ORT) and extinction of fear memory. Tandem mass spectrometry (MS^E) and protein-protein-interaction (PPI) network modelling were used to characterise the underlying biological processes of SD and SSRI/AChEI treatment. Independent selected reaction monitoring (SRM) was conducted for molecular validation. Repeated SD resulted in a stable increase of anhedonia-like behaviour as measured by ICSS. Fluoxetine treatment reversed this phenotype, whereas donepezil showed no effect. Fluoxetine improved recognition memory and inhibitory learning in a stressor-related context, whereas donepezil only improved fear extinction. MS^E and PPI network analysis highlighted functional SD stress-related hippocampal proteome changes including reduced glutamatergic neurotransmission and learning processes, which were reversed by fluoxetine, but not by donepezil. SRM validation of molecular key players involved in these pathways confirmed the hypothesis that fluoxetine acts via increased AMPA receptor

*Correspondence to: F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124, CH-4070, Basel, Switzerland.
**Correspondence to: Department of Chemical Engineering and Biotechnology, University of Cambridge, Philippa Fawcett Drive, Cambridge CB3 0AS, UK.
E-mail addresses: barbara.biemans@roche.com (B. Biemans), sb209@cam.ac.uk (S. Bahn).
[1]Joint senior authorship.

https://doi.org/10.1016/j.euroneuro.2017.11.002

Please cite this article as: Gottschalk, M.G., et al., Fluoxetine, not donepezil, reverses anhedonia, cognitive dysfunctions and hippocampal proteome changes.... European Neuropsychopharmacology (2017), https://doi.org/10.1016/j.euroneuro.2017.11.002

signalling and Ca^{2+}-mediated neuroplasticity in the amelioration of stress-impaired reward processing and memory consolidation. Our study highlights molecular mediators of SD stress reversed by SSRI treatment, identifying potential viable future targets to improve cognitive dysfunctions in MDD patients.

1. Introduction

Major depressive disorder (MDD) is a multi-faceted condition with several diagnostic key features (American-Psychiatric-Association, 2013). Many patients do not, or only partially respond to the standard of care (Rush et al., 2006). Additionally, common side effects cause patients to withdraw from their medications, demonstrating the medical need for developing better and safer treatments (Rush et al., 2006). Preclinical models are used to offer insight in the disease mechanism and discover novel drug targets, and ultimately to increase confidence in clinical efficacy of these novel mechanisms. However, some key symptoms of MDD are challenging to assess in a preclinical setting, such as depressed mood or loss of energy. Others are ambiguous to interpret, as they are clinical opposites (weight loss/gain, insomnia/hypersomnia, psychomotor agitation/retardation), or are arguably not present in animals (feelings of worthlessness, suicidal thoughts) (Nestler and Hyman, 2010). Despite these hurdles, other symptoms are translatable and can indeed be modelled in animals. Anhedonia (diminished interest or pleasure in reward stimuli) is a well-known core symptom of depression which can be reliably assessed in rodents (Koob, 1989). Cognitive dysfunction in MDD has been demonstrated to be equally as common and debilitating (Marazziti et al., 2010), and is linked to longer episode duration, worse functional recovery and reduced treatment response (Jaeger et al., 2006; Papakostas, 2014). In addition to impairments of working and episodic memory, patients suffering from depression have shown marked deficits in social cognition (Zobel et al., 2010) and processing speed (Castaneda et al., 2008). These different cognitive domains can also be assessed in rodents (Millan and Bales, 2013).

Cognitive impairment cannot always be improved by current psychopharmacological interventions and various compounds show differences in their efficacy treating them (Millan, 2006). Fluoxetine has for example been shown to ameliorate short term memory recall in MDD patients to a greater extent than amitriptyline (Keegan et al., 1991) and desipramine (Levkovitz et al., 2002). However, it has been argued that cognitive deficits in the course of depression are independent of an affective component (Austin et al., 2001) and that in general SSRIs elicit a modest secondary effect on mnemonic function following mood elevation, potentially also explaining findings of reduced cognitive functioning following SSRI treatment (Millan, 2006). Based on neuropsychological studies outlining the importance of cognitive remodelling in depression (Roiser et al., 2012) and molecular reports highlighting abnormalities in synaptic plasticity (Cheung and Ip, 2011), cognitive enhancers have been suggested as novel tools in antidepressant pharmacotherapy. Two randomized control trials (RCTs) of donepezil have reported improvements of neuropsychiatric symptoms in dementia, including significant benefits on depression and anxiety scales (Feldman et al., 2001; Holmes et al., 2004). Furthermore, MDD patients benefited from donepezil augmentation in a RCT following treatment with monoaminergic antidepressants and displayed increased neuropsychological functioning, including improved memory after a 1-year follow-up (Reynolds et al., 2011). Despite some of these promising findings, no drug is approved for the treatment of cognitive impairment in MDD.

Here, we tested the effects of a selective serotonin reuptake inhibitor (SSRI, fluoxetine) and an acetylcholinesterase inhibitor (AChEI, donepezil) in behavioural paradigms reflective of emotional-cognitive endophenotypes of depression (Pryce and Seifritz, 2011). The increase in serotonergic and cholinergic transmission which these drug classes aim for, was shown to influence various cellular signalling cascades linked to neuroplasticity and altered network synchronicity between prefrontal and limbic regions (e.g. the hippocampus or amygdala) (Millan et al., 2012). The chronic social defeat (SD) model has been shown to reliably elicit depression-related behaviour in rodents (Koolhaas et al., 1997) and to closely represent the molecular pathophysiology of MDD on the proteome level (Cox et al., 2016). As of yet, psychopharmacological paradigms have taken two standard routes. Either animals have been treated while experiencing chronic SD and treatment was continued after the SD exposure (Der-Avakian et al., 2014), or animals have been treated after a chronic SD protocol without exposure to further stressors (Berton et al., 2006). The first option might not be fully representative of the clinical presentation of depression, because animals are treated while a behavioural phenotype is established. The second option neglects the persistence and re-occurrence of negative life events and experiences while treatment has already started. We used a SD stress paradigm in which chronic SD was followed up with “reminder” sessions of direct or indirect exposure to SD. The development of anhedonia over the SD procedure and continued SD exposure during pharmacological treatment was monitored via intracranial self-stimulation (ICSS) (Moreau et al., 1992) of the ventral tegmental area (VTA). An object recognition test (ORT) was conducted to measure memory functioning in a non-emotional context (Ennaceur and Delacour, 1988). A fear extinction paradigm was used to assess inhibitory learning in the context of a traumatic stressor (Myers and Davis, 2007). Label-free tandem mass spectrometry (MS^E) was performed to characterise proteomic changes in the

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