Single administration of ketamine and Pregnenolone-Methyl-Ether (3β-Methoxy-Pregnenolone) has antidepressant efficacy in a preclinical model of treatment resistant depression: identification of a plasma biomarker of pharmacological efficacy.

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The Innovation Hub for the Neurobiology of Cognition, Mood and Aging

INTRODUCTION

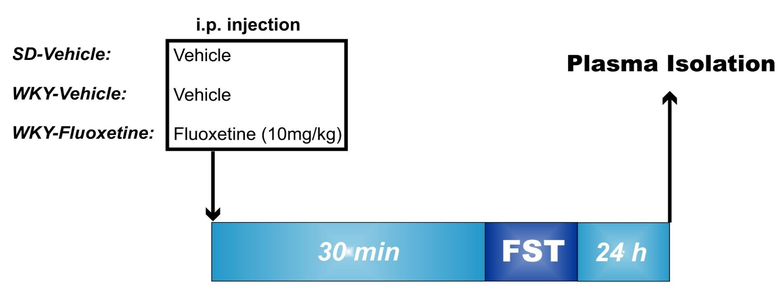
- One third of major depressive disorder (MDD) patients are unresponsive to antidepressant drugs, a subtype known as treatment resistant depression (TRD).
- Intravenous administration of the anaesthetic and recreational drug ketamine is the only effective pharmacological treatment in TRD.
- Development of preclinical assays and identification of diagnostic biomarkers is essential to facilitate drug discovery in TRD.
- Alteration in the expression of proteins associated with microtubule dynamics and neuronal plasticity has been linked with MDD pathogenesis and treatment.
- Acetylated α-Tubulin (Acet-Tub) is a marker of reduced microtubule dynamics and is elevated in the hippocampus of rodent models of depression [1].
- The classic steroid-derivative Pregnenolone-Methyl-Ether (PME; also known as 3β-Methoxy-pregnenolone) was recently assessed as a neuronal microtubule modulator showing preclinical antidepressant efficacy [1]
- The endogenous 'depressed' Wistar Kyoto (WKY) rat is unresponsive to SSRIs and was used here as a purported model of TRD.
- **Aim: To investigate the antidepressant efficacy of PME compared with ketamine in the WKY rat model of TRD and to explore the feasibility of plasma Acet-Tub as biomarker of disease or pharmacological efficacy.**

METHODS

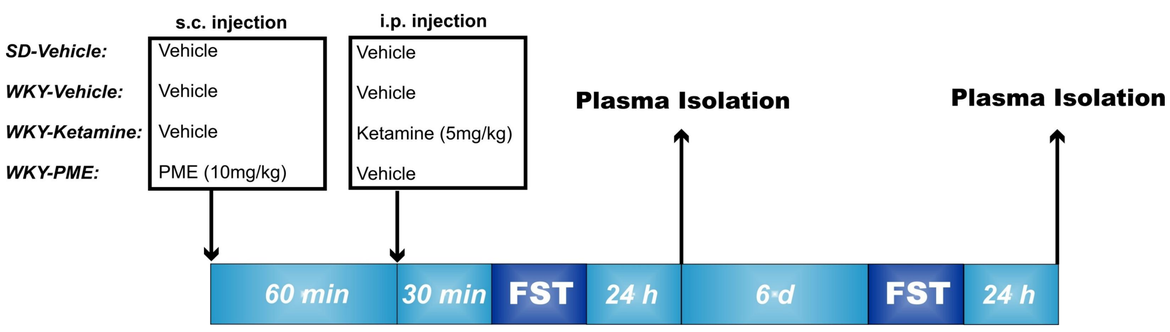
Forced Swimming Test (FST): Male WKY rats (approx. 200g) were administered either fluoxetine (10 mg/kg, i.p.), ketamine (5 mg/kg, i.p.), PME (10 mg/kg, s.c.) or corresponding vehicle solutions (0.9% saline, i.p., sesamie oil s.c.). Depressive-like behaviour (i.e. immobility) was tested in the FST to measure both rapid and long-lasting pharmacological effects (see below) and compared with 'healthy' Sprague-Dawley (SD) rats. FST was performed as previously described [2]. WKY rats demonstrate spontaneous immobility in the FST compared with SD rats [3], therefore no pre-test was required.

Infrared Western Blotting (IFWB): Plasma was isolated after each FST exposure. The expression of Acet-Tub in plasma was measured using a protocol of IFWB adapted from previous studies [2].

Experiment 1: Timeline



Experiment 2: Timeline



Experiment 3: Timeline

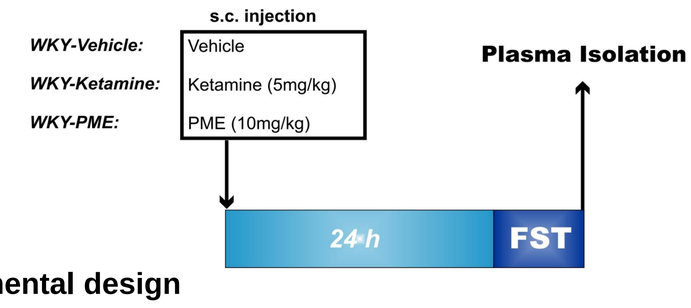


Figure 1. Experimental design

CONCLUSIONS

- WKY rats are a suitable model for screening of novel pharmacological treatments for TRD due to their sensitivity to ketamine and lack thereof to SSRIs.
- PME demonstrates rapid onset and long-lasting (24h) antidepressant efficacy in this TRD model comparable to that of ketamine.
- WKY rats exhibit consistent overexpression of plasma Acet-Tub in line with the previous observed overexpression in the WKY hippocampus [4].
- Ketamine and PME reduce plasma Acet-Tub overexpression consistent with their antidepressant efficacy.
- Plasma Acet-Tub represents a potential biomarker of pharmacological efficacy and disease progression in TRD.
- Microtubules represent a novel target for drug discovery in TRD.

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Transforming Drug Discovery in Depression to Scare Away the Dark

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RESULTS

Experiment 1: WKY rats as a model of TRD

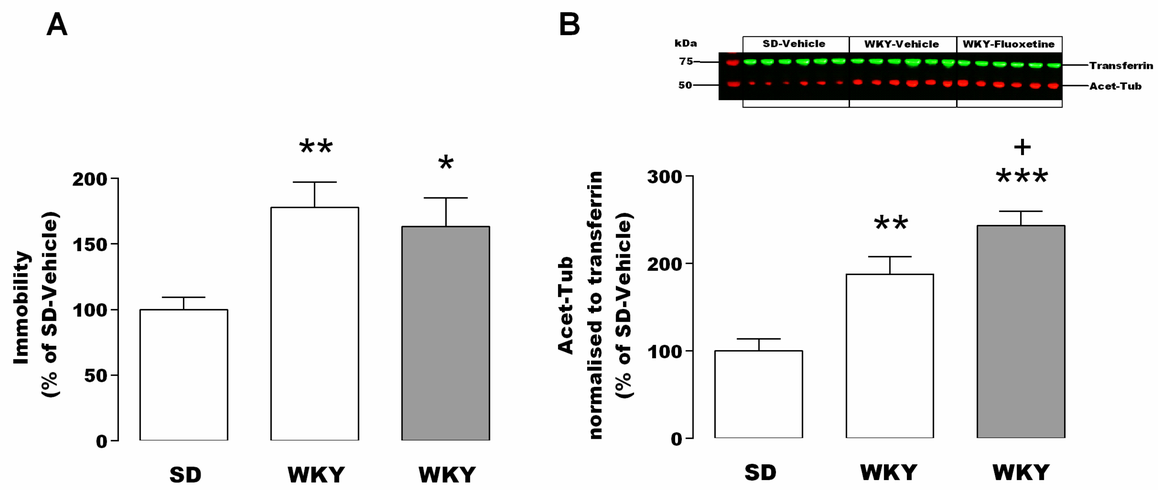

Figure 2. WKY rats exhibited increased depressive-like behaviour in the FST and overexpression of plasma Acet-Tub. Acute fluoxetine had no effect on depressive-like behaviour and augmented plasma Acet-Tub overexpression.

A. Immobility in the FST. B. Plasma Acet-Tub expression Inset: representative Infrared Western Blot. Mean±SEM. *p<0.05, **p<0.01, ***p<0.001 v SD-Vehicle. +p<0.05 v WKY-Vehicle. One-way ANOVA, Fisher's LSD. *n*=6-8 per group.

Experiment 2: Rapid onset and long-lasting (7 day) efficacy

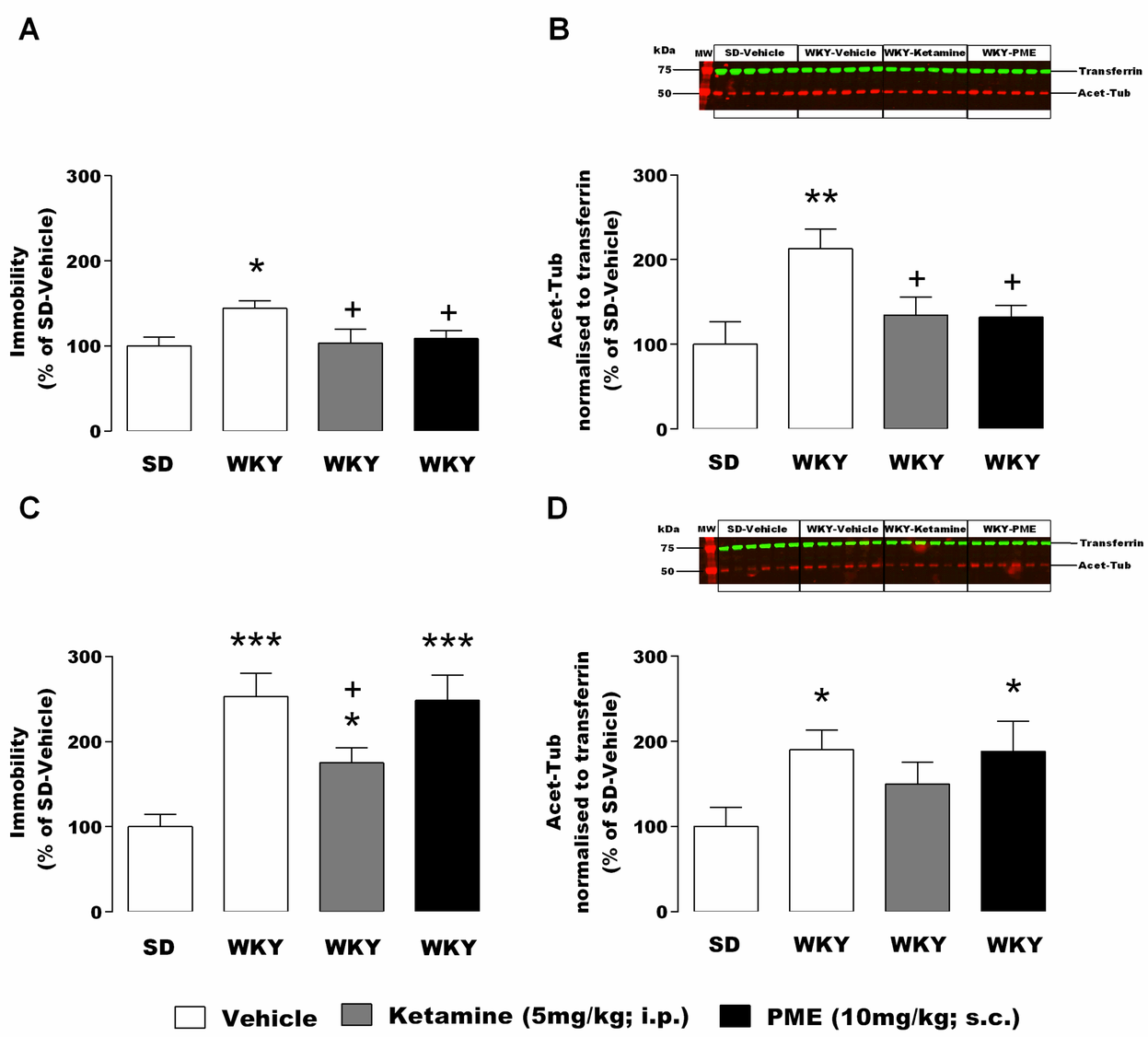

Figure 3. Ketamine demonstrates rapid onset and long-lasting antidepressant efficacy in the FST. PME demonstrates rapid onset of antidepressant efficacy. Both ketamine and PME decrease plasma Acet-Tub overexpression consistent with their antidepressant effects.

A. Immobility in the FST. B. Plasma Acet-Tub expression. C. Immobility in the FST. D. Plasma Acet-Tub expression. Insets: representative Infrared Western Blots. Mean±SEM. *p<0.05, **p<0.01, ***p<0.001 v SD-Vehicle. +p<0.05 v WKY-Vehicle. One-way ANOVA, Fisher's LSD. *n*=6-8 per group.

Experiment 3: Long-lasting (24h) efficacy

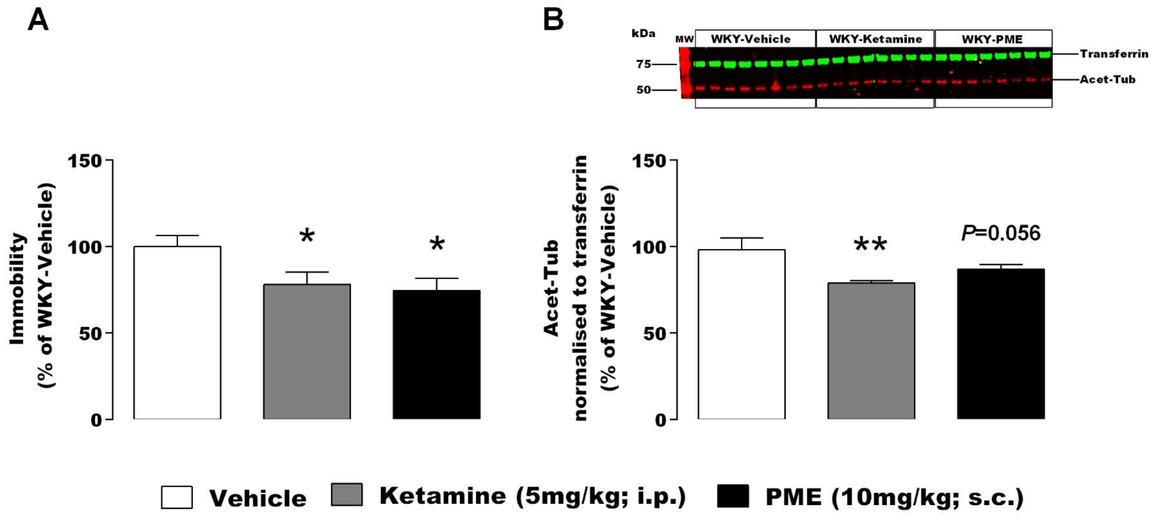

Figure 4. Ketamine and PME demonstrate long-lasting (24 h) antidepressant efficacy and decrease plasma Acet-Tub expression.

A. Immobility in the FST. B. Plasma Acet-Tub expression. Inset: representative Infrared Western Blot. Mean±SEM. *p<0.05, **p<0.01, p=0.056 v WKY-Vehicle. One-way ANOVA, Fisher's LSD. *n*=8-10 per group.

[1] Bianchi M and Baulieu EE (2012). 3β-Methoxy-pregnenolone (MAP4343) as an innovative therapeutic approach for depressive disorders. *Proceedings of National Academy of Sciences,* 109 (5): 1713-8.

[2] Ladurelle N, Gabriel C, Viggiano A, Mocaër E, Baulieu EE, Bianchi M (2012): Agomelatine (S20098) modulates the expression of cytoskeletal microtubular proteins, synaptic markers and BDNF in the rat hippocampus, amygdala and PFC. Psychopharmacology, 221, 493-509.

[3] Tejani-Butt S, Kluczynski J, Paré WP (2003). Strain-dependent modification of behavior following antidepressant treatment. *Progress in Neuro-Pschopharmacology and Biological Psychiatry,* 27, 7-14.

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