Amino Acids (2012) 43:2285–2292
DOI 10.1007/s00726-012-1306-y

ORIGINAL ARTICLE

Modafinil improves performance in the multiple T-Maze and modifies GluR1, GluR2, D2 and NR1 receptor complex levels in the C57BL/6J mouse

Sunetra Sase · Deeba Khan · Fernando Sialana · Harald Höger · Nina Russo-Schlaff · Gert Lubec

Received: 6 April 2012 / Accepted: 17 April 2012 / Published online: 22 May 2012

Abstract Modafinil has been shown to modify behavioural and cognitive functions and to effect several brain receptors. Effects, however, were not observed at the receptor protein complex level and it was therefore the aim of the study to train mice in the multiple T-Maze (MTM) as a paradigm for spatial memory and to determine paralleling brain receptor complex levels. Sixty C57BL/6J mice were used in the study and divided into four groups (trained drug injected; trained vehicle injected; yoked drug injected; yoked vehicle injected). Animals obtained training for 4 days and were killed 6 h following the last training session on day 4. Hippocampi were dissected from the brain, membrane fractions were prepared by ultracentrifugation and were run on blue-native gels and immunoblotted with antibodies against major brain receptors. Modafinil treatment led to decreased latency and increased average speed, but not to changes in pathlength and number of correct decisions in the MTM. Drug effects were modifying receptor complexes of GluR1, GluR2, D2 and NR1. Training effects on receptor complex levels were observed for GluR3, D1 and nicotinic acetylcholine receptor alpha 7 (Nic7). GluR1 levels were correlating with GluR2 and D1 levels were correlating with D2 and NR1. Involvement of the glutamatergic, NMDA, dopaminergic and nicotinergic system in modafinil and memory training were herein described for the first time. A brain receptor complex pattern was revealed showing the concerted action following modafinil treatment.

Keywords Modafinil · Brain receptor · Glutamatergic · Nicotinergic · Dopaminergic · Spatial memory

Introduction

2-[(Diphenylmethyl) sulfinyl] acetamide, more commonly known as modafinil, is a novel wake-promoting stimulant used to treat narcolepsy. It was first marketed in the early 1990s in France for the treatment of excessive somnolence and is currently being used in the United States as a treatment for narcolepsy, shift work sleep disorder and sleep apnea (Minzenberg and Carter 2008).

Various studies have shown that modafinil has a different neurochemical profile and is structurally unrelated to classical psychostimulants such as amphetamines (Lin et al. 1992) and methylphenidate (Jasinski 2000). Catecholamine transporters that directly bind modafinil are dopamine transporters (DAT) (Madras et al. 2006; Paterson et al. 2010; Qu et al. 2008) and norepinephrine transporters (NET) (Madras et al. 2006; Mitchell et al. 2008). Administration of modafinil blocks DAT (Volkow et al. 2009) and significantly elevates extracellular serotonin, norepinephrine, dopamine (DA) (de Saint et al. 2001) and glutamate (Ferraro et al. 1998) levels and decreases GABA (Ferraro et al. 1998, 1999). Brain regions likely affected by modafinil are the prefrontal cortex (PFC), medial hypothalamus (de Saint et al. 2001) and increases in synaptic plasticity of

Electronic supplementary material The online version of this article (doi:10.1007/s00726-012-1306-y) contains supplementary material, which is available to authorized users.

S. Sase · D. Khan · F. Sialana · N. Russo-Schlaff · G. Lubec (✉)
Department of Pediatrics, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria
e-mail: gert.lubec@meduniwien.ac.at

H. Höger
Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna, Brauhausgasse 34, 2325 Himberg, Austria

the dentate gyrus in rats have been reported (Tsanov et al. 2010). Evidence suggests that the effects of modafinil are due to the activity of monoaminergic systems, implicating DA receptors D1 and D2 (Korotkova et al. 2007; Qu et al. 2008; Seeman et al. 2009; Young 2009; Young and Geyer 2010). Ferraro and coworkers have shown that the GABAergic system is involved by studies revealing that the selective $GABA_B$ receptor antagonist phaclofen and the $GABA_A$ receptor agonist muscimol are counteracting modafinil effects (Ferraro et al. 1998). Subsequently, this group confirmed modafinil effects on the GABAergic receptor system by the use of $GABA_A$ receptor agonist muscimol and $GABA_A$ antagonist bicuculline (Ferraro et al. 1999). Recent work also provides evidence for involvement of alpha1-adrenoceptors based upon the results from receptor blocking by the antagonist prazosin (Winder-Rhodes et al. 2010).

Effects of modafinil in rodents have been reported in several memory paradigms. Investigations using the Morris Water Maze task (MWM) and T-maze have demonstrated improvement in spatial memory performance upon administration of modafinil. Ward et al. tested modafinil effects on a delayed non matching to position task, a variant of the water maze. Modafinil treated rats made significantly more correct choices at two different doses (Ward et al. 2004). Berachochea et al. tested modafinil in a serial spatial discrimination reversal T-maze and observed enhanced learning in terms of faster learning rates (Beracochea et al. 2002). Tsanov et al. observed that modafinil facilitates water maze performance, and Shuman et al. reported increased performance in the MWM and in contextual fear conditioning in a dose dependent way (Shuman et al. 2009; Tsanov et al. 2010).

In humans, modafinil effects on cognitive performance are not unequivocal. Turner et al. tested cognitive enhancing effects of modafinil in healthy volunteers and observed significantly enhanced performance in the digit span, visual pattern recognition memory, i.e., spatial planning tests (Turner et al. 2003). In healthy volunteers without sleep deprivation, Müller and coworkers reported subtle stimulation effects on maintenance and manipulation processes in working memory tasks (Müller et al. 2004). Randall et al. observed an enhanced span of immediate verbal recall and short-term visual recognition memory in non-sleep deprived volunteers (Randall et al. 2005). Modafinil enhanced efficiency of PFC-related cognitive information processing using BOLD fMRI (Rasetti et al. 2010). Winder-Rhodes finally showed positive modafinil effects on performance accuracy and latency (Winder-Rhodes et al. 2010).

A few clinical trials suggest that modafinil may improve cognitive dysfunction in neuropsychiatric disorders in schizophrenia. Spence et al. reported some beneficial cognitive effects in a subset of patients with chronic schizophrenia, and Turner et al. reported improvement of cognitive function in the short-term verbal memory span test as one of the several memory paradigms (Spence et al. 2005; Turner et al. 2004). Blackwell and coworkers treated patients with Huntington's disease with modafinil to improve cognitive functions. Modafinil, however, had deleterious effects on visual recognition and working memory (Blackwell et al. 2008). Kahbazi et al. used assessment of cognitive functions by the Teacher and Parent ADHD rating scale-IV and reported beneficial effects in children and adolescents with attention deficit and hyperactivity disorder (Kahbazi et al. 2009).

Although modafinil has been shown to have some beneficial cognitive effects in animals and humans, no systematic study has been carried out to investigate the concerted action of major brain receptor systems. This lack of information on a major question formed the rationale for the current in vivo study in an animal model of memory performance.

Therefore, the aims of the study were to demonstrate the role of modafinil in modulating mouse spatial memory in a land maze along with the paralleling major brain receptor complexes.

Materials and methods

Animals

Sixty male C57BL/6J mice were used for the studies. Mice used were aged between 10 and 14 weeks, because at this age development is complete and aging processes are far from being started. All the mice were purchased from JANVIER SAS Laboratories (Le Genest Saint Isle, France) and maintained in cages made of Makrolon and filled with autoclaved woodchips in the Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna. An autoclaved standard rodent diet (Altromin, Germany) and water in bottles were available ad libitum. The room was illuminated with artificial light at an intensity of about 200 lx in 2 m from 5 am to 7 pm. All tests were performed between 8 am and 1 pm.

All procedures were carried out according to the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by Federal Ministry of Education, Science and Culture, Austria (licence number BMWF-66.009/0240-II/106/2009). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Modafinil administration

Modafinil was prepared freshly everyday by dissolving in a vehicle 10 % DMSO + 2 % Cremophor (C5135; Sigma-

Aldrich, Vienna Austria) (Qu et al. 2008). The dose of injection was 80 mg/kg weight and was administered intraperitoneally daily 30 min prior to MTM experiments. For MTM, animals were divided into four groups each containing 15 animals as Trained Drug Injected (TDI), Trained Vehicle Injected (TVI), Yoked Drug Injected (YDI) and Yoked Vehicle Injected (YVI).

Studies in the MTM

MTM was performed as described previously (Patil et al. 2009). Prior to testing, mice were deprived of food for 16 h to motivate food searching. Mice were placed in a start box in a black cylindrical start chamber. After 10 s elapsed, the chamber was lifted and the first trial was started. Mice were searching for the reward and the trial was completed when mice had reached the goal box, or if failed, after 5 min. After mice arrived in the goal box, they were allowed to consume a small piece of a food pellet provided as reward and transferred to their home cage. Immediately after each trial, the entire maze was cleaned with 1 % incidin solution. 15 mice each, drug injected and vehicle injected, were trained with 3 trials per day for 4 days. Trials were carried out using 20 min intervals. 15 mice each were used as YDI and YVI controls that spent the same time in the MTM without learning the task as there was no reward. After testing, animals were given food as per body weight (120 g/kg) into the home cage, representing the amount to preserve their body weight, but making them hungry for the following day for MTM tests. Trials were recorded using computerized tracking/image analyzer system (video camcorder: 1/3 in SSAMHR EX VIEWHAD coupled to computational tracking system: TiBeSplit). The system provided the following parameters, correct or wrong decision, path length, speed, and latency to reach the goal box. Six hours following the training on day 4, animals were deeply anaesthetized with CO_2 and killed by neck dislocation. Hippocampi were rapidly dissected and stored at −80 °C for biochemical analysis.

Sample preparation

Total membrane fraction

15 hippocampi of TDI, TVI, YDI and YVI mice each (total $n = 60$) were homogenized in ice-cold homogenization buffer [10 mM HEPES, pH 7.5, 300 mM sucrose, one complete protease inhibitor tablet (Roche Molecular Biochemicals, Mannheim, Germany) per 50 mL] by Ultra-Turrax® (IKA, Staufen, Germany). The homogenate was centrifuged for 10 min at 1,000×*g* and the pellet was discarded. The supernatant was centrifuged at 50,000×*g* for 30 min in an ultracentrifuge (Sorvall, WX 80 Ultraseries centrifuge; Thermo Fisher Scientific, Vienna, Austria). Subsequently, the pellet was homogenized in 5 mL washing buffer (homogenization buffer without sucrose), kept on ice for 30 min and centrifuged at 50,000×*g* for 30 min. All the individual 60 samples were used for the gel experiments (Heo et al. 2010).

Extraction of membrane receptor protein

Whole membrane pellets were suspended in extraction buffer [1.5 M 6-aminocaproic acid, 300 mM Bis–Tris, pH 7.0] and divided into two parts. To one part, 10 % Triton X-100 stock solution was added at a ratio of 1:4 to achieve final 2 % Triton X-100 concentration for extraction of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtype Glu1 (GluR1), AMPA receptor subtype Glu 2 (GluR2) and AMPA receptor subtype Glu 3 (GluR3) receptors. To the second part 10 % DDM stock solution was added in a ratio of 1:9 to achieve final 1 % DDM concentration for extraction of dopamine 1 (D1) and dopamine 2 (D2), nicotinic acetylcholine receptor subtype alpha 7 (Nic7), N-methyl-D-aspartate receptor NMDA (NR1) receptors. Solubilisation was done by vortexing every 10 min for 1 h followed by centrifugation at 20,000×*g* for 60 min at 4 °C for clarification. The protein content was estimated using the BCA protein assay kit (Pierce, Rockford, IL, USA). Extracted membrane receptor proteins were then aliquoted and stored at −80 °C till use.

Blue Native PAGE

20 µg for GluR1 and 25 µg each for GluR2 and GluR3 receptors, 80 µg for D1, 60 µg for D2, 50 µg for Nic7 and NR1 of prepared samples were loaded. 16 µL BN PAGE loading buffer [5 % (w/v) Coomassie G250 in 750 mM 6-aminocaproic acid] was mixed with 100 µL of the prepared sample and loaded onto the gel. BN-PAGE was performed in a PROTEAN II xi Cell (BioRad, Vienna, Austria) using 4 % stacking and 5–18 % separating gels.

The BN-PAGE gel buffer contained 500 mM 6-aminocaproic acid, 50 mM Bis–Tris, pH 7.0; the cathode buffer 50 mM Tricine, 15 mM Bis–Tris, 0.05 % (w/v) Coomassie G250, pH 7.0, and the anode buffer 50 mM Bis–Tris, pH 7.0. The voltage was set to 50 V for 1 h, 75 V for 2 h, 100 V for 2 h and was increased sequentially to 400 V (maximum current 15 mA/gel, maximum voltage 500 V) until the dye front reached the bottom of the gel (Kang et al. 2009). Native high-molecular mass markers were obtained from Invitrogen (Carlsbad, CA, USA).

Western blotting

Proteins separated on the gel were transferred onto PVDF membranes. After blocking with 5 % non-fat dry milk in

Table 1 The antibodies for the individual immunochemical detection of brain receptors is provided

Antibody	Species	Catalogue number	Company	Dilution
GluR1	Rabbit polyclonal	Ab31232	Abcam	1/20,000
GluR2	Rabbit polyclonal	AB1768-25UG	Millipore	1/10,000
GluR3	Rabbit polyclonal	#3437	Cell signaling	1/4,000
D1	Rabbit polyclonal	Ab85608	Abcam	1/5,000
D2	Rabbit polyclonal	AB5084P	Millipore	1/15,000
Nic7	Rabbit polyclonal	Ab23832	Abcam	1/3,000
NR1	Rabbit polyclonal	Ab28669	Abcam	1/5,000

0.1 % TBST (100 mM Tris–HCL, 150 mM NaCl, pH 7.5, 0.1 % Tween 20) for 1 h at 21 °C, membranes were incubated overnight at 4 °C with gentle agitation using diluted primary antibodies as per Table 1.

After six times washing with 0.1 % TBST, membranes were incubated with horseradish peroxidase-conjugated Goat polyclonal Secondary Antibody to Rabbit IgG (Abcam 6722, Cambridge, UK) and then again washed with 0.1 % TBST. Membranes were developed with the ECL plus Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK). Densities of immunoreactive bands were measured by the Image J software program (http://rsb.info.nih.gov/ij/).

Loading control

For the loading control, the procedure as described previously (Welinder and Ekblad 2010) with minor modifications was followed. After immunodetection, membranes were washed twice with 0.1 % TBST, stained for 1 min with 0.1 % coomassie R-350 (GE Healthcare) prepared in methanol:water, 1:1, destained for 5 min with aqueous solution containing 7 % methanol and 50 % acetic acid, washed with water, air dried and scanned at 600 dpi resolution (Supplementary Fig. 1).

Statistical analysis

MTM data were analyzed by repeated measures analysis of variance (ANOVA) with subsequent unpaired Student *t* test to reveal between-group differences and the $P < 0.05$ level was considered significant. All calculations were performed using SPSS version 14.0 (SPSS, Inc., Chicago, IL). Data from the proteomic part of the study were also analysed by ANOVA followed by unpaired Student *t* test. Pearson and Spearman correlations were calculated using the SPSS version given above.

Results and discussion

As shown in Fig. 1a, latencies indicated that both groups learned the task, however, animals from the modafinil group had significantly shorter latencies on days 3 and 4. On these days, speed was also significantly increased in the modafinil group as shown in Fig. 1b. Pathlengths as well as the number of correct decisions (not shown) were comparable indicating that modafinil may not be improving spatial memory in the MTM per se as increasing speed is underlying decreased latencies. Nevertheless, modafinil is

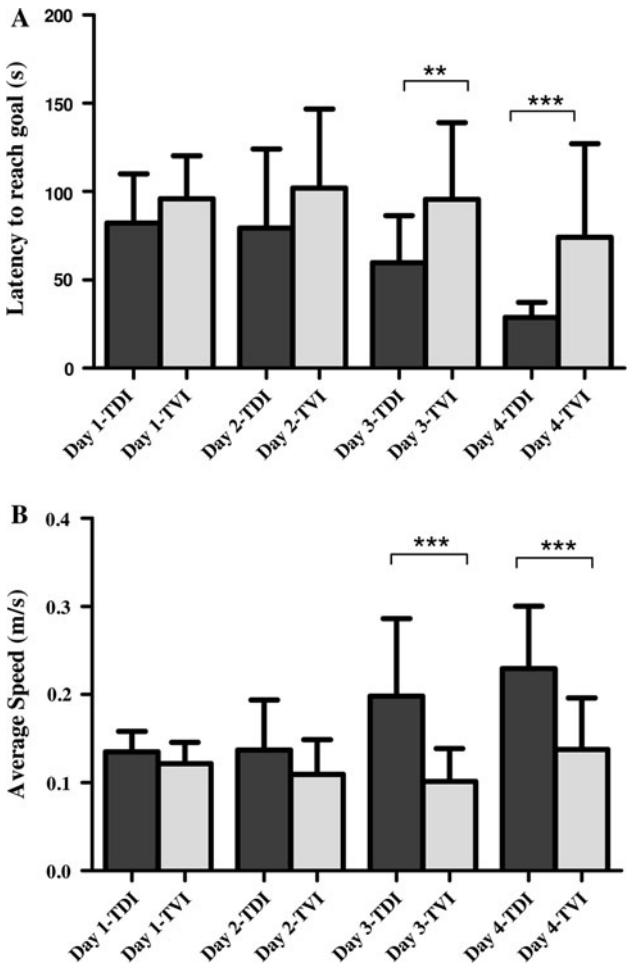

Fig. 1 Latency and speed in the MTM. Modafinil treatment improves parameters latency and speed in the MTM indicating better performance in spatial memory. $^{**}P < 0.01$, $^{***}P < 0.001$ *TDI* trained drug injected, *TVI* trained vehicle injected, *YDI* yoked drug injected, *YVI* yoked vehicle injected

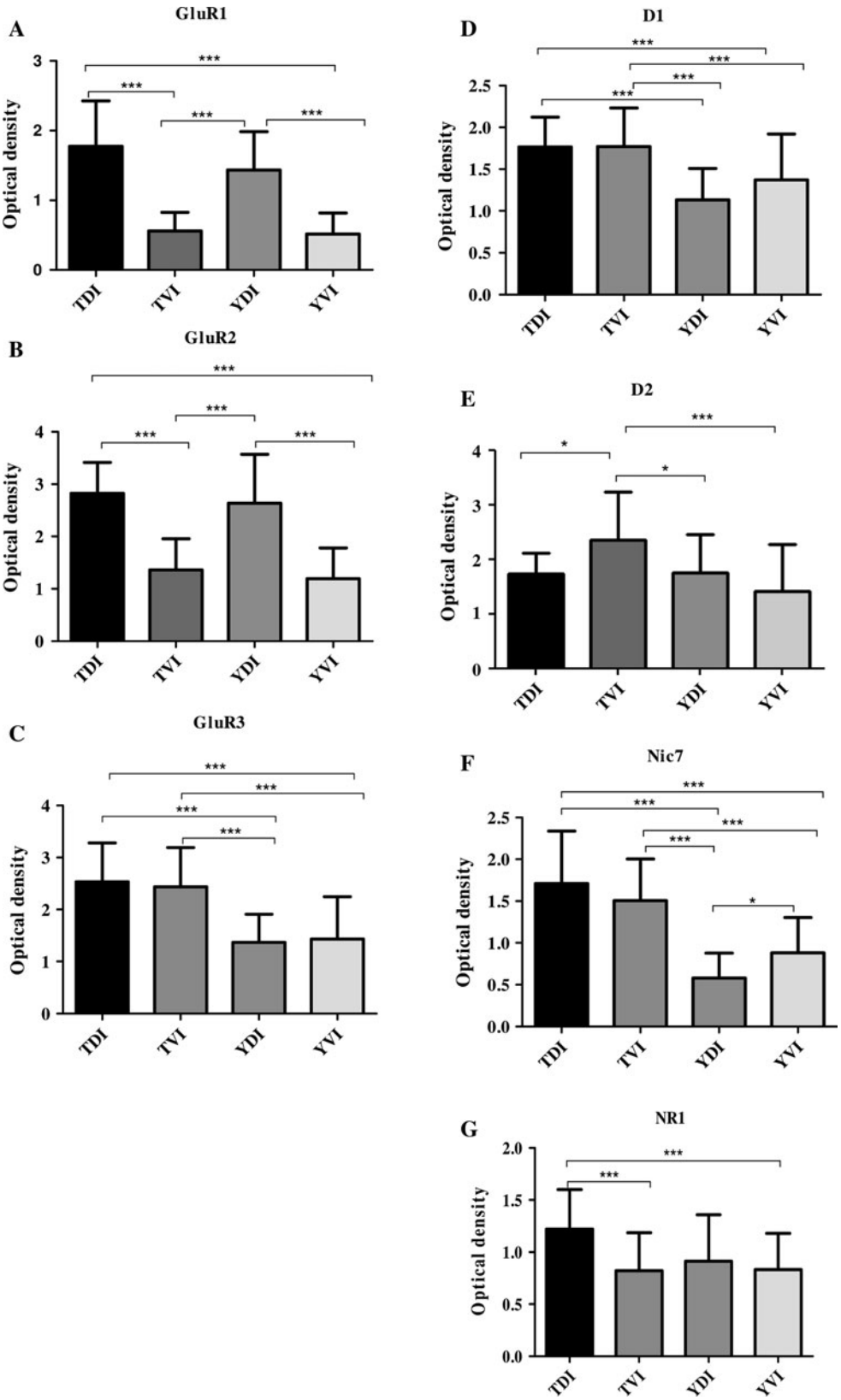

Fig. 2 Graphical demonstration of receptor complex levels. Showing the concerted action of several brain receptor systems in modafinil and vehicle treated, trained and untrained groups. $^{*}P < 0.05$, $^{***}P < 0.001$ *TDI* trained drug injected, *TVI* trained vehicle injected, *YDI* yoked drug injected, *YVI* yoked vehicle injected

improving performance in the MTM, thus partially confirming and complementing reports on the effect of modafinil observed in other paradigms for spatial memory as given above in the Introduction. It is well-known and widely accepted that spatial memory performance is different in the individual paradigms (Patil et al. 2009), and therefore, the finding that the number of correct decisions was not improved by modafinil is not contradicting previous work showing modafinil-mediated increase of correct decisions in a water maze (Ward et al. 2004).

Results of major brain receptor complex levels determinations are shown in Fig. 2 and immunoblotting results from BN-PAGEs are provided in Fig. 3. The main outcome is that modafinil was modifying receptor complexes of GluR1 (at approx. 720 kDa), GluR2 (approx. 720 kDa), D2 (approx. 720 kDa) and NR1 (approx. 242 kDa) indicating drug effects; modafinil increased receptor complex levels for GluR1, GluR2, NR1, but decreased levels of a D2 complex. The complexes may reflect dimers or oligomers, either homo- or heteromeric in nature. While there is no information about modafinil effects on AMPA receptors GluR1-3 and NR1, modification of D2 receptors and subsequently on D2 receptor complexes could have been expected (Korotkova et al. 2007; Qu et al. 2008; Seeman et al. 2009; Young 2009; Young and Geyer 2010).

Training effects on receptor complex levels were observed for GluR3 (at approx. 600 kDa), Nic7 (approx. 300 kDa) and D1 (at 550 kDa). Training was paralleled by increased receptor complex levels for GluR3, Nic7 and D1. Indeed, training in the MWM also leads to increased receptor complex levels at an apparent molecular weight of 300 kDa (Sase et al. 2011). In a previous study, GluR1 receptor complex levels were paralleling training in the MTM, but these complexes were at different apparent molecular weights and mice were un-treated (Ghafari et al. 2011).

D2 receptor complex levels in vehicle groups, trained and yoked, were significantly different, proposing involvement of this receptor complex in memory training, thus confirming the role of this receptor systems in spatial memory in humans and rodents (El-Ghundi et al. 2007; Ellis et al. 2005; Glickstein et al. 2002; Mehta et al. 2008; Mehta and Riedel 2006; Mele et al. 2004; Rinaldi et al. 2007; Wilkerson and Levin 1999).

There was no correlation between parameters for spatial memory in the MTM, and the individual receptor complexes with the biological meaning that no receptor *complex* system can be linked to latency, speed, pathlength or correct decisions. Receptor complexes were, however, paralleling training in the MTM.

In trained mice with modafinil treatment (TDI), GluR1 were correlating with GluR2 complex levels ($R = 0.583$; $P = 0.03$) and D1 levels were correlating with NR1 ($R = 0.643$; $P = 0.01$) and D2 complex levels ($R = -0.543$; $P = 0.04$). In TVI, GluR1 levels were correlating with NR1 ($R = 0.569$; $P = 0.02$) and GluR2 with D2 ($R = 0.527$; $P = 0.04$). In YDI, Nic7 levels were correlating with D2 ($R = 0.579$; $P = 0.02$) and NR1 ($R = -0.616$; $P = 0.01$). No correlations were found in YVI. These data may suggest receptor interactions, a link between receptors, or even the concerted action of the above mentioned receptors under the conditions of modafinil or vehicle treatment. The correlation between the receptors may reflect either physical or functional interaction. Receptor–receptor interactions are key elements of neural transmission and integration of signals between different transmission lines at the membrane level (Agnati et al. 1995) and may be even of relevance in molecular medicine (Fuxe et al. 2007). Moreover, correlation among receptors may even represent heteromeric complexes and these are well-known for dopamine receptors

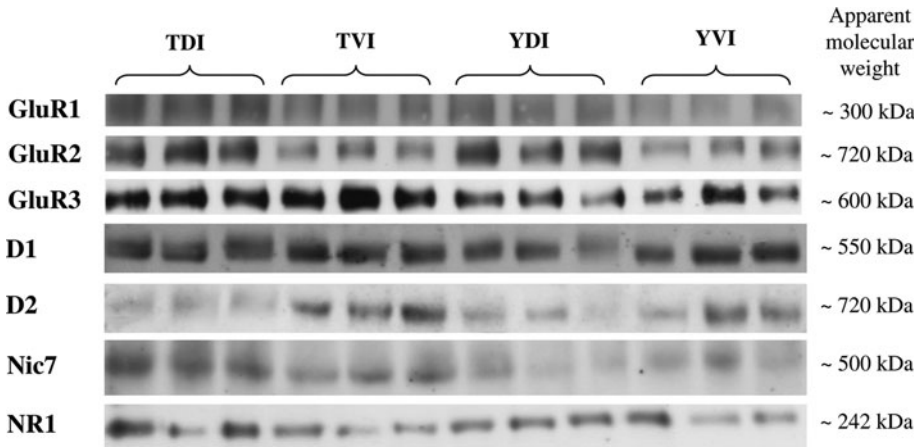

Fig. 3 Immunoblotting results. Fifteen animals were used for immunoblotting and bands representative for the individual groups are shown. *Native markers* indicate the approximate apparent molecular weight

(Borroto-Escuela et al. 2010, 2011; Ferrada et al. 2008; Marcellino et al. 2008).

Conclusion

Taken together, we have shown that modafinil increases latency and speed in the MTM along with involvement of the glutamatergic, dopaminergic and nicotinergic system. Drug effects were shown for the glutamatergic, NMDA and dopaminergic system. In addition, links between the dopaminergic receptor complexes D1 and D2 and the NMDA receptor system as well as the link between GluR1 and GluR2 have been revealed.

The findings revealing receptor complex levels rather than levels of receptor subunits are useful for design of future and interpretation of previous work on receptor systems in spatial memory per se or pharmacological studies using modafinil.

Conflict of interest The authors have declared that no conflict of interest exist.

References

Agnati LF, Ferre S, Cortelli P, Fuxe K (1995) A brief appraisal on some aspects of the receptor–receptor interaction. Neurochem Int 27:139–146

Beracochea D, Celerier A, Borde N, Valleau M, Peres M, Pierard C (2002) Improvement of learning processes following chronic systemic administration of modafinil in mice. Pharmacol Biochem Behav 73:723–728

Blackwell AD, Paterson NS, Barker RA, Robbins TW, Sahakian BJ (2008) The effects of modafinil on mood and cognition in Huntington's disease. Psychopharmacology 199:29–36

Borroto-Escuela DO, Romero-Fernandez W, Tarakanov AO, Marcellino D, Ciruela F, Agnati LF, Fuxe K (2010) Dopamine D2 and 5-hydroxytryptamine 5-HT (A) receptors assemble into functionally interacting heteromers. Biochem Biophys Res Commun 401:605–610

Borroto-Escuela DO, Van Craenenbroeck K, Romero-Fernandez W, Guidolin D, Woods AS, Rivera A, Haegeman G, Agnati LF, Tarakanov AO, Fuxe K (2011) Dopamine D2 and D4 receptor heteromerization and its allosteric receptor–receptor interactions. Biochem Biophys Res Commun 404:928–934

de Saint Hilaire Z, Orosco M, Rouch C, Blanc G, Nicolaidis S (2001) Variations in extracellular monoamines in the prefrontal cortex and medial hypothalamus after modafinil administration: a microdialysis study in rats. NeuroReport 12:3533–3537

El-Ghundi M, O'Dowd BF, George SR (2007) Insights into the role of dopamine receptor systems in learning and memory. Rev Neurosci 18:37–66

Ellis KA, Mehta MA, Wesnes KA, Armstrong S, Nathan PJ (2005) Combined D1/D2 receptor stimulation under conditions of dopamine depletion impairs spatial working memory performance in humans. Psychopharmacology 181:771–780

Ferrada C, Ferre S, Casado V, Cortes A, Justinova Z, Barnes C, Canela EI, Goldberg SR, Leurs R, Lluis C, Franco R (2008) Interactions between histamine H3 and dopamine D2 receptors and the implications for striatal function. Neuropharmacology 55:190–197

Ferraro L, Antonelli T, O'Connor WT, Tanganelli S, Rambert FA, Fuxe K (1998) The effects of modafinil on striatal, pallidal and nigral GABA and glutamate release in the conscious rat: evidence for a preferential inhibition of striato-pallidal GABA transmission. Neurosci Lett 253:135–138

Ferraro L, Antonelli T, Tanganelli S, O'Connor WT, Perez de la Mora M, Mendez-Franco J, Rambert FA, Fuxe K (1999) The vigilance promoting drug modafinil increases extracellular glutamate levels in the medial preoptic area and the posterior hypothalamus of the conscious rat: prevention by local GABAA receptor blockade. Neuropsychopharmacology 20:346–356

Fuxe K, Canals M, Torvinen M, Marcellino D, Terasmaa A, Genedani S, Leo G, Guidolin D, Diaz-Cabiale Z, Rivera A, Lundstrom L, Langel U, Narvaez J, Tanganelli S, Lluis C, Ferre S, Woods A, Franco R, Agnati LF (2007) Intramembrane receptor–receptor interactions: a novel principle in molecular medicine. J Neural Transm 114:49–75

Ghafari M, Falsafi SK, Hoeger H, Lubec G (2011) Hippocampal levels of GluR1 and GluR2 complexes are modulated by training in the multiple T-Maze in C57BL/6J mice. Brain Struct Funct. doi:10.1007/s00429-00011-00335-00428 (in press)

Glickstein SB, Hof PR, Schmauss C (2002) Mice lacking dopamine D2 and D3 receptors have spatial working memory deficits. J Neurosci 22:5619–5629

Heo S, Patil SS, Jung G, Hoger H, Lubec G (2010) A serotonin receptor 1A containing complex in hippocampus of PWD/PhJ mice is linked to training effects in the Barnes maze. Behav Brain Res 216:389–395

Jasinski DR (2000) An evaluation of the abuse potential of modafinil using methylphenidate as a reference. J Psychopharmacol 14:53–60

Kahbazi M, Ghoreishi A, Rahiminejad F, Mohammadi MR, Kamalipour A, Akhondzadeh S (2009) A randomized, double-blind and placebo-controlled trial of modafinil in children and adolescents with attention deficit and hyperactivity disorder. Psychiatry Res 168:234–237

Kang SU, Fuchs K, Sieghart W, Pollak A, Csaszar E, Lubec G (2009) Gel-based mass spectrometric analysis of a strongly hydrophobic GABAA-receptor subunit containing four transmembrane domains. Nat Protoc 4:1093–1102

Korotkova TM, Klyuch BP, Ponomarenko AA, Lin JS, Haas HL, Sergeeva OA (2007) Modafinil inhibits rat midbrain dopaminergic neurons through D2-like receptors. Neuropharmacology 52:626–633

Lin JS, Roussel B, Akaoka H, Fort P, Debilly G, Jouvet M (1992) Role of catecholamines in the modafinil and amphetamine induced wakefulness, a comparative pharmacological study in the cat. Brain Res 591:319–326

Madras BK, Xie Z, Lin Z, Jassen A, Panas H, Lynch L, Johnson R, Livni E, Spencer TJ, Bonab AA, Miller GM, Fischman AJ (2006) Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. J Pharmacol Exp Ther 319:561–569

Marcellino D, Ferre S, Casado V, Cortes A, Le Foll B, Mazzola C, Drago F, Saur O, Stark H, Soriano A, Barnes C, Goldberg SR, Lluis C, Fuxe K, Franco R (2008) Identification of dopamine D1-D3 receptor heteromers. Indications for a role of synergistic D1-D3 receptor interactions in the striatum. J Biol Chem 283:26016–26025

Mehta MA, Riedel WJ (2006) Dopaminergic enhancement of cognitive function. Curr Pharm Des 12:2487–2500

Mehta MA, Montgomery AJ, Kitamura Y, Grasby PM (2008) Dopamine D2 receptor occupancy levels of acute sulpiride

challenges that produce working memory and learning impairments in healthy volunteers. Psychopharmacology 196:157–165
Mele A, Avena M, Roullet P, De Leonibus E, Mandillo S, Sargolini F, Coccurello R, Oliverio A (2004) Nucleus accumbens dopamine receptors in the consolidation of spatial memory. Behav Pharmacol 15:423–431
Minzenberg MJ, Carter CS (2008) Modafinil: a review of neurochemical actions and effects on cognition. Neuropsychopharmacology 33:1477–1502
Mitchell HA, Bogenpohl JW, Liles LC, Epstein MP, Bozyczko-Coyne D, Williams M, Weinshenker D (2008) Behavioral responses of dopamine beta-hydroxylase knockout mice to modafinil suggest a dual noradrenergic-dopaminergic mechanism of action. Pharmacol Biochem Behav 91:217–222
Müller U, Steffenhagen N, Regenthal R, Bublak P (2004) Effects of modafinil on working memory processes in humans. Psychopharmacology 177:161–169
Paterson NE, Fedolak A, Olivier B, Hanania T, Ghavami A, Caldarone B (2010) Psychostimulant-like discriminative stimulus and locomotor sensitization properties of the wake-promoting agent modafinil in rodents. Pharmacol Biochem Behav 95:449–456
Patil SS, Sunyer B, Hoger H, Lubec G (2009) Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the Multiple T-maze and in the Morris water maze. Behav Brain Res 198:58–68
Qu WM, Huang ZL, Xu XH, Matsumoto N, Urade Y (2008) Dopaminergic D1 and D2 receptors are essential for the arousal effect of modafinil. J Neurosci 28:8462–8469
Randall DC, Viswanath A, Bharania P, Elsabagh SM, Hartley DE, Shneerson JM, File SE (2005) Does modafinil enhance cognitive performance in young volunteers who are not sleep-deprived? J Clin Psychopharmacol 25:175–179
Rasetti R, Mattay VS, Stankevich B, Skjei K, Blasi G, Sambataro F, Arrillaga-Romany IC, Goldberg TE, Callicott JH, Apud JA, Weinberger DR (2010) Modulatory effects of modafinil on neural circuits regulating emotion and cognition. Neuropsychopharmacology 35:2101–2109
Rinaldi A, Mandillo S, Oliverio A, Mele A (2007) D1 and D2 receptor antagonist injections in the prefrontal cortex selectively impair spatial learning in mice. Neuropsychopharmacology 32:309–319
Sase A, Khan D, Hoger H, Lubec G (2011) Intraperitoneal injection of saline modulates hippocampal brain receptor complex levels but does not impair performance in the Morris Water Maze. Amino Acids. doi:10.1007/s00726-00011-01130-00729 (in press)
Seeman P, Guan HC, Hirbec H (2009) Dopamine D2High receptors stimulated by phencyclidines, lysergic acid diethylamide, salvinorin A, and modafinil. Synapse 63:698–704
Shuman T, Wood SC, Anagnostaras SG (2009) Modafinil and memory: effects of modafinil on Morris water maze learning and Pavlovian fear conditioning. Behav Neurosci 123:257–266
Spence SA, Green RD, Wilkinson ID, Hunter MD (2005) Modafinil modulates anterior cingulate function in chronic schizophrenia. Br J Psychiatry 187:55–61
Tsanov M, Lyons DG, Barlow S, Gonzalez Reyes RE, O'Mara SM (2010) The psychostimulant modafinil facilitates water maze performance and augments synaptic potentiation in dentate gyrus. Neuropharmacology 59:9–19
Turner DC, Robbins TW, Clark L, Aron AR, Dowson J, Sahakian BJ (2003) Cognitive enhancing effects of modafinil in healthy volunteers. Psychopharmacology 165:260–269
Turner DC, Clark L, Pomarol-Clotet E, McKenna P, Robbins TW, Sahakian BJ (2004) Modafinil improves cognition and attentional set shifting in patients with chronic schizophrenia. Neuropsychopharmacology 29:1363–1373
Volkow ND, Fowler JS, Logan J, Alexoff D, Zhu W, Telang F, Wang GJ, Jayne M, Hooker JM, Wong C, Hubbard B, Carter P, Warner D, King P, Shea C, Xu Y, Muench L, Apelskog-Torres K (2009) Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. JAMA 301:1148–1154
Ward CP, Harsh JR, York KM, Stewart KL, McCoy JG (2004) Modafinil facilitates performance on a delayed nonmatching to position swim task in rats. Pharmacol Biochem Behav 78:735–741
Welinder C, Ekblad L (2010) Coomassie staining as loading control in Western blot analysis. J Proteome Res 10:1416–1419
Wilkerson A, Levin ED (1999) Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats. Neuroscience 89:743–749
Winder-Rhodes SE, Chamberlain SR, Idris MI, Robbins TW, Sahakian BJ, Muller U (2010) Effects of modafinil and prazosin on cognitive and physiological functions in healthy volunteers. J Psychopharmacol 24:1649–1657
Young JW (2009) Dopamine D1 and D2 receptor family contributions to modafinil-induced wakefulness. J Neurosci 29:2663–2665
Young JW, Geyer MA (2010) Action of modafinil-increased motivation via the dopamine transporter inhibition and D1 receptors? Biol Psychiatry 67:784–787