Psychiatry Research 216 (2014) 432–437

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Dissociating effects of spatial learning from locomotor activity for ouabain-induced bipolar disorder-like rats

Ying-Chou Wang [a,1], En-Nan Wang [a,b,c,1], Chia-Chuan Wang [d], Chung-Lei Huang [e], Andrew Chih Wei Huang [e,*]

[a] Department of Clinical Psychology, Fu Jen Catholic University, New Taipei City 24205, Taiwan
[b] Department of Psychiatry, Shin-Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan
[c] Graduate Institute of Humanities in Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[d] School of Medicine, Fu Jen Catholic University, New Taipei City 24205, Taiwan
[e] Department of Psychology, Fo Guang University, Yilan County 26247, Taiwan

ARTICLE INFO

Article history:
Received 30 March 2013
Received in revised form
26 February 2014
Accepted 1 March 2014
Available online 12 March 2014

Keywords:
Ouabain
Bipolar disorder
Open field test
Water maze test
Locomotor activity
Spatial learning

ABSTRACT

Whether ouabain, a Na^+- and K^+-activated adenosine triphosphatase inhibitor, mimics cognitive impairments that can be dissociated from motor effects in the bipolar disorder-like animal model remains unclear. Ouabain and the vehicle aCSF were microinjected into the left lateral ventricle immediately, after 4 h, and after 24 h. The results showed that (a) locomotion responses of the Immediate group were significantly decreased compared to those of the aCSF group, particularly the first five minutes. (b) The ouabain-treated rats have longer latency and total distance traveled in the water maze task; however, the velocity was not affected for the ouabain group. (c) The analysis of covariance showed that the latency time (but not the total distance traveled and velocity) of the ouabain group was more impaired than that of the aCSF group, regardless of omitting total distance traveled and cross movement in the open field test. The latency might be more sensitive than the distance traveled and the velocity for assessing spatial learning. Dissociating the spatial learning from the movement may allow testing drug treatments of cognitive deficits independent of locomotor effects associated with bipolar disorder.

1. Introduction

Bipolar disorder patients continuously encounter episodes of mania and depression and exhibit hyperactivity during manic episodes. The occurrence of suicide in these individuals is 10%–15% (American Psychiatric Association, 2000). In addition to emotional irritability, aggression, and hyperactivity, bipolar disorder is often associated with cognitive symptoms, including flight of ideas, racing thoughts, and distractibility (Sato et al., 2006). In addition, psychomotor function in bipolar disorder has long been assessed (Greden and Carroll, 1981), and motor dysfunction and cognitive deficits were both thought to be associated with affective disruptions (Langenecker et al., 2010).

Some clinical investigations have shown that bipolar disorder may involve cognitive impairments (Glahn et al., 2007). For example, a previous study used computer-based cognitive function tests (i.e., Wisconsin Card Sorting Test, Spatial Working Memory Test, and Continuous Performance Test) in euthymic bipolar patients and normal subjects. Euthymic bipolar patients had significant deficits in executive function (Trivedi et al., 2008). One cognitive test that assessed verbal and nonverbal holistic function showed that manic-depression patients and unipolar depressive subjects had deficits in spatial and holistic tasks (Robertson and Taylor, 1985). Another study showed that these cognitive impairments involved short-term dysfunction of attention, memory, and visuospatial function and deficits in choice reaction times in major depression and mania patients (Bulbena and Berrios, 1993). In summary, patients with bipolar disorder exhibit diverse cognitive dysfunctions.

However, recent animal studies have only focused on the hyperactivity associated with bipolar disorder (Kim et al., 2008; Minassian et al., 2010). Little animal research has investigated the cognitive dysfunction associated with bipolar disorder. Animal models that employ drugs of abuse to elicit the hyperactivity symptoms of bipolar disorder cannot clearly discern whether the drug-induced hyperactivity is caused by the locomotor symptoms of drug addiction or the motor-like symptoms of bipolar disorder (Antelman et al., 1998; Frey et al., 2006). The present study used

* Correspondence to: Department of Psychology, Fo Guang University, No. 160, Linwei Road, Jiaosi Shiang, Yilan County 26247, Taiwan.
Tel.: +886 3 987 1000x27114; fax: +886 3 987 5530.
E-mail address: chweihuang@mail.fgu.edu.tw (A.C.W. Huang).
[1] The first and second authors contributed equally to this article.

http://dx.doi.org/10.1016/j.psychres.2014.03.003
0165-1781/

the sodium–potassium adenosine triphosphatase (Na,K-ATPase) inhibitor ouabain to block sodium–potassium pump function (El-Mallakh and Wyatt, 1995) and hypothesized that resting membrane potentials would be dysfunctional (Zugno et al., 2009). Brain neurons affected by ouabain treatment have shown strong hyperactivity and are presumed to induce the hyperactivity associated with mania-like symptoms (El-Mallakh et al., 2003; Herman et al., 2007). The present study used the open field test to assess the motor function and the Morris water maze to measure the spatial learning of bipolar disorder in ouabain-treated rats.

The present study tested the following: (*i*) whether ouabain induces bipolar disorder-like motor symptoms, (*ii*) whether an identical ouabain treatment also affects cognitive function, reflected in spatial learning, and (*iii*) if such results may indicate dissociation between cognitive function and motor activity in an ouabain-induced bipolar disorder-like animal model.

2. Methods and materials

2.1. Subjects

Seventy-two adult male Wistar rats weighing 250–300 g were purchased from the Laboratory Animal Center, National Taiwan University (Taipei, Taiwan). All rats were allowed to inhabit a temperature-controlled (approximately $22 \pm 2^{\circ}C$) colony room for 7 days before being subjected to experimentation. They were group-housed, two per cage, in a colony room with a 12 h/12 h light/dark cycle (lights on 06:00–18:00) with food and water available ad libitum. The present study was performed in compliance with the Animal Scientific Procedures Act of 1986 and received local ethics committee approval.

2.2. Apparatus

The open field consisted of a square plastic box (86 cm × 86 cm × 50 cm). Each side of the box was equally divided into four parts. The square was separated into 16 sub-squares (Decker et al., 2000). The water maze consisted of a round plastic pool (200 cm diameter, 50 cm height) of water (Liu and Bergin, 2009). Swim behavior was measured by video tracking software (Video Tracking Record System Version 1.17, SINGA Technology Corporation, Taipei, Taiwan).

2.3. Procedure

2.3.1. Experiment 1: test open field task to determine the locomotion effect

The open field test was measured for 30 min immediately, 4 h, and 24 h after aCSF vehicle or ouabain injection for one trial. Other rats were assigned into the aCSF ($n=8$), Immediate ($n=7$), 4 h ($n=7$), and 24 h ($n=8$) groups. The number of sub-square crossing for locomotor activity was assessed, serving as the motor index.

2.3.2. Experiment 2: test water maze task to determine the spatial learning effect

Immediately after aCSF vehicle or ouabain administration, rats were subjected to the water maze test and served as aCSF ($n=11$) and ouabain ($n=16$) groups. The Morris water maze had four trials over one session, and each trial was recorded at 120 s when the rats did not find out the hidden platform. When rats climbed into the hidden platform, they were allowed to stay there for 30 s. The latency to reach the platform, total distance traveled, and swimming velocity for each trial were recorded.

2.3.3. Experiment 3: test open field and water maze tasks to dissociate the locomotion from the spatial learning

All rats were subjected to the open field and the water maze tasks. Fifteen rats were assigned into the aCSF ($n=8$) and ouabain ($n=7$) groups. They were either given an aCSF or ouabain injection, and immediately encountered the open field and the water maze tests. The experimental procedures of the open field and the water maze tests in Experiment 3 followed the same procedures of Experiment 1 and Experiment 2, respectively.

2.4. Intracerebroventricular cannulation

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and guide cannulae were inserted into the left lateral ventricle (1 mm caudal to bregma, 2.5 mm lateral to midline, and 3.5 mm ventral to skull; Paxinos and Watson, 2007) using a stereotaxic apparatus. After a 7-day recovery period, they received intracerebroventricular injection of 5 μl of aCSF or 10^{-3} M ouabain (Wang et al., 2013).

2.5. Drug preparation and administration

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The artificial cerebrospinal fluid (aCSF) contained 149 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 0.27 mM NaH_2PO_4, pH 7.4. Ouabain was dissolved in aCSF at 10^{-3} M, as previously described (Herman et al., 2007).

2.6. Statistical analysis

In Experiment 1, one-way analysis of variance (ANOVA) was analyzed to test mean ($\pm$SEM) total distance traveled and mean ($\pm$SEM) cross numbers in the aCSF, Immediate, 4 h, and 24 h groups. Moreover, a 4 × 6 mixed two-way ANOVA for groups and each 5 min was used to test mean ($\pm$SEM) distance traveled for each 5 min and mean ($\pm$SEM) cross numbers. When appropriate, post hoc tests were conducted using Tukey's Honestly Significant Difference (HSD) test, values of $p < 0.05$ were considered statistically significant.

One-way ANOVA and one-way analysis of covariance (ANCOVA) were used to analyze the mean ($\pm$SEM) of latency time, total distance traveled, and swim velocity in the water maze test in Experiment 2 and Experiment 3, respectively.

3. Results

3.1. The motor activity of a motor function for ouabain-treated and control rats in the open field test

Fig. 1(A) depicts mean ($\pm$SEM) total distance traveled during 30 min for the aCSF, Immediate, 4 h, and 24 h groups in the open field test. One-way ANOVA indicated that a group factor was a significant difference ($F_{3,26}=4.67$, $p < 0.05$). Moreover, the post hoc test with Tukey indicated that only the total distance traveled path of the 24 h group was significantly increased than those of the Immediate group ($p < 0.05$).

Fig. 1(B) depicts mean ($\pm$SEM) distance traveled for each 5 min for the aCSF, Immediate, 4 h, and 24 h groups in the open field test. A 4 × 6 mixed two-way ANOVA indicated significant differences occurred at groups ($F_{3,26}=4.73$, $p < 0.05$), minutes ($F_{5,130}=31.58$, $p < 0.05$), and the interaction of groups and minutes ($F_{15,130}=3.14$, $p < 0.05$). Further, a post hoc with the Tukey test revealed that distance traveled for each 5-min period of the Immediate group was significantly decreased in the first five minutes compared to the aCSF group ($p < 0.05$). However, the 4 h and the 24 h groups were not significantly different from the aCSF group in the first five minutes (ps > 0.05), but these two groups showed significant increases compared to the Immediate group in the first five minutes (ps < 0.05). From 10 to 20 min, significant differences of the distance traveled path occurred between the Immediate and the 24 h groups (ps < 0.05). Over 25 min and 30 min, significant differences occurred at the 4 h and the 24 h groups when compared to the Immediate group (ps < 0.05). Therefore, the major finding suggested that the distance traveled during the first five minutes in the Immediate group, but not the 4 h and the 24 h groups, was significantly decreased when compared with the aCSF control group (see Fig. 1B).

Fig. 2(A) depicts mean ($\pm$SEM) cross numbers for the aCSF, Immediate, 4 h, and 24 h groups in the open field test. A one-way ANOVA indicated that the group had a significant difference ($F_{3,26}=3.37$, $p < 0.05$). The post hoc with the Tukey test indicated that a significant decrease of total cross numbers only occurred in the Immediate group when compared to the 24 h group ($p < 0.05$).

Fig. 2(B) depicts mean ($\pm$SEM) distance traveled for each 5 min for the aCSF, Immediate, 4 h, and 24 h groups in the open field test. A 4 × 6 mixed two-way ANOVA indicated significant differences occurred in group ($F_{3,26}=3.37$, $p < 0.05$), minutes ($F_{5,130}=23.74$, $p < 0.05$), and the interaction of groups and minutes