Behavioural Brain Research 219 (2011) 98–104

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Tail-pinch stress and REM sleep deprivation differentially affect sensorimotor gating function in modafinil-treated rats

Yia-Ping Liu[a,b,*], Che-Se Tung[a], Chia-Hsin Chuang[a], Shih-Mao Lo[c], Yu-Chi Ku[c]

[a] Department of Physiology and Biophysics, National Defence Medical Centre, Taipei, Taiwan, ROC
[b] Department of Psychiatry, Tri-Service General Hospital, Taipei, Taiwan, ROC
[c] Beitou Armed Forces Hospital, Taipei, Taiwan, ROC

ARTICLE INFO

Article history:
Received 25 August 2010
Received in revised form 4 December 2010
Accepted 8 December 2010

Keywords:
Acoustic startle response
Prepulse inhibition
Modafinil
REM sleep deprivation
Tail-pinch

ABSTRACT

Prepulse inhibition (PPI) is a phenomenon in which a mild stimulus attenuates a cross-modality startle response to later intense stimulation. PPI is thought to index the central inhibitory mechanism through which behavioural responses are filtered. The present study compared the effects of two stress paradigms on the acoustic startle response (ASR) and on PPI in a rat model. The tail-pinch (TP) method produces an acute and immediate stressful condition, whereas rapid eye movement (REM) sleep deprivation (REMSD) leads to a more persistent and long-term stress. Our results demonstrated that in rats, TP stress reduced the size of the ASR, and REMSD impaired PPI. The wake-promoting agent modafinil (MOD) had no effect on PPI if given alone. However, MOD reduced the ASR and PPI under TP stress, whereas only PPI was reduced by MOD after 96 h of REMSD. These results suggest that distinct stress paradigms differentially mediated sensorimotor gating abilities in terms of either responsiveness to the stimulus or information-filtering capabilities.

1. Introduction

A startle response can be modified if the given stimulus is preceded by a milder stimulus. The phenomenon is manifested in a cross-modality nature including tactile, visual and acoustic modalities [19]. For example, when a rat receives a sudden and intense acoustic stimulus, the stimulus typically evokes an acoustic startle response (ASR) that consists of contraction of the major muscles of the body into a hunched position [31]. If the startling sound is preceded by a moderately-intense prepulse stimulus, the amplitude of the ASR is attenuated [4,25]. The reduction in the ASR as a result of a prepulse – called prepulse inhibition (PPI) – is not a learned behaviour and is believed to index central processes related to information processing and sensory gating [22,32,55]. A disruption of PPI (i.e., a prepulse that does not reduce the ASR) indicates an impairment of sensorimotor gating abilities, and occurs in many pathological conditions including stress paradigms [10,57]. Disruptions of the ASR and PPI are related to a variety of mental disorders, including generalized anxiety disorder, post-traumatic stress disorder, and schizophrenia, in which stress has been considered as a critical determinant [54]. In animal studies, stress-induced impairment of sensorimotor gating abilities is generally examined through chronic aversive treatment, for example, restraint [18] or electric shock [53]. Moreover, recent work by Choy and van den Buuse [8] revealed that the effect of rat stress paradigms on sensorimotor function would interact with the condition under which the animals' arousal level was pharmacologically altered. This finding augmented the hypothesis that sensorimotor gating ability can be modulated by level of arousal [49]. However, supportive evidences from different stress paradigm are required.

Many studies have used the tail-pinch (TP) method of inducing stress, which involves pinching the tail of a rat with a clamp. The TP method creates a mild stress and induces a state of activation [6,29], which affects many behaviours including the ASR and PPI. Brake and colleagues [5] demonstrated that multiple exposure to TP stress exaggerated the amplitude of ASR, while disrupting the sensorimotor gating function, as evidenced by a reduction in PPI at the prepulse levels of 6 and 9 dB above background. However, the effects of stress on the gating performance of rats in Brake's study resulted from a subchronic rather than an acute stress paradigm to rats [5]. In the present study, we developed a within-session testing procedure to examine the immediate effect of TP stress on the sensorimotor reactivity of acoustic startle in rats.

In contrast to TP stress, which causes behavioural activation by adding an acute negative stimulus, stress induced through paradoxical sleep deprivation influences a rat's arousal level by preventing rapid eye movement (REM) sleep, which is a required

* Corresponding author at: Department of Physiology and Biophysics, National Defence Medical Centre, No. 161, Section 6, Min-Chuan East Road, Taipei 114, Taiwan, ROC. Tel.: +886 2 87923100x18614; fax: +886 2 87923153.
E-mail addresses: yiaping@ms75.hinet.net, yiaping@mail.ndmctsgh.edu.tw (Y.-P. Liu).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.12.012

behaviour [33,45,59]. Previous evidence has shown that rats subjected to REM sleep deprivation (REMSD) exhibited an increase in the ASR and an impairment of PPI performance, and this was not associated with the environmental stress [21]. In the present study, we examined the ASR and PPI performance of rats in a similar REMSD paradigm. Additionally, we administered the non-stimulant wake-promoting agent diphenyl-methyl-sulfinyl-2-acetamide (Modafinil, MOD). Evidence reveals that the effect on REM sleep caused by MOD is different than that of traditional stimulants (i.e., amphetamine or cocaine) [16,58]. Increasing interest has been focused on the benefit of MOD on working performance in people with sleep insufficiency or deprivation. While its precise mechanism of action is not well identified, MOD is currently used to treat narcolepsy and idiopathic hypersomnia through maintaining wakefulness and vigilance [2,14]. The effect of MOD on sensory gating performance, particularly following sleep–wake disturbances, is therefore worth examining.

The present study aims, for the first time, to compare the effects of TP and REMSD on the ASR and PPI in a rat model. REMSD was accomplished by the water platform method described previously [23]. We further compare the effects of MOD on the ASR and PPI during the TP procedure and following 96 h of REMSD. The results of this study should allow not only a more precise description of alterations in sensorimotor gating abilities in different stress paradigms, but they should also contribute to the understanding of the functional role and clinical applications of MOD.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley (SD) rats (BioLASCO, Taiwan, ROC) weighing between 300 and 350 g were used. All animals were housed in groups of three and in a temperature- and humidity-controlled holding facility with 12-hour light/dark cycles (light on from 07:00 to 19:00). All rats received food and water *ad libitum*. Testing took place between 08:00 and 18:00, and each rat was tested at the same time every day when possible. All experimental procedures were evaluated and approved by the animal care committee of the National Defence Medical Centre. All efforts were made to reduce the numbers of animals used and to minimise animal suffering during the experiments.

2.2. ASR and PPI testing

The ASR and PPI experiments were carried out in four startle chambers (SR-LAB, San Diego Instrument, San Diego, CA) with Plexiglas cylinder (9 cm diameter). A speaker mounted 24 cm above the animal to provide the background noise, prepulse stimuli and startle stimuli, which were controlled by the SR-LAB. Startle responses were transduced by a piezoelectric accelerometer mounted below the cylinder, digitised (0-4095) and rectified. Movement of the rats was measured during 100 ms after startle stimulus onset. Rats were allowed to habituate to the background noise of 70 dB for 5 min after being placed into the chambers. For repeat testing, rats would be placed in different chamber for eliminating a possible chamber effect. The calibration of acoustic stimuli was performed every week using a digital sound level meter (RadioShack, Fort Worth, TX, USA) positioned inside the cylinder on the startle platform 24 cm beneath the speaker.

For rats following 96 h REMSD regime, a total 72 trials were delivered with an average inter-trial interval of 15 s. The first 6 (Block 1) and last 6 (Block 3) trials consisted of single 40 ms 118 dB startle stimuli (trial of pulse alone). The middle 60 trials (Block 2) consisted of the random delivery of 12 trials of startle stimuli alone, 12 no-stimuli trials during which there was no startle stimulus and only background noise was present, and 36 prepulse trials (i.e., prepulse plus pulse). The prepulse trials consisted of a 20 ms prepulse burst (3, 5, and 10 dB above background) and then a 40 ms 118 dB startle stimulus with 100 ms in between. The entire session lasted approximately 25 min. The ASR (measured in arbitrary units) was defined as the average of 100, 1-ms stabilimeter readings collected from the stimulus onset of the first 6 trials of startle alone (i.e., Block 1). PPI was determined by the data of Block 2 according to the formula: [1 − (mean value of the startle of prepulse trials/mean value of the startle of pulse alone trials)] × 100%.

For TP experiment, the protocol was adapted from the Block 2 trials mentioned above but structured as a pre-TP, TP, and post-TP design. Thus, within a session, PPI was measured before (pre-TP block), during (TP block), and after (post-TP block) exposure to TP stress, with an average of 2 min in-between allowing the experimenter to place or remove the tail clip. For each block, 24 trials were delivered and consisted randomly of 4 trials of no-stimuli, 5 trials of startle stimuli alone, and 15 trials of prepulse trials (3, 5, or 10 dB above the background noise, for each level, 5 trials). The ASR and PPI were obtained from the data of each corresponding block. The entire session for the TP experiment lasted approximately 30 min.

2.3. The TP method

The TP method used in the present study was modified from that reported by Picone and Hall [46]. Specifically, the tail of the rat was measured to determine where its diameter was 4.3 mm, and that spot was clamped by a standard metal caliper (providing a force of 500 ± 50 g). All tails were re-measured and remarked prior to each TP session to control for possible differences in diameter due to oedema. The TP procedure in the present study occurred concomitant with the moment the rat experienced an acoustic stimulus. For doing so the ASR and PPI testing were performed according to a pre-TP, TP, and post-TP block design. Rats were placed in the centre of the startle test chamber, the length of the tail was guided through the chamber's floor, and a cotton-padded clip was applied at the previously marked diameter to avoid tail damage. Rats did not vocalize during the application of TP pressure.

2.4. REMSD

The method of REMSD used in this study was modified from Ferraz's version of the water platform technique [20] and was employed previously by the same team [36]. The technique takes advantage of the fact that an animal entering REM sleep loses its postural control due to a decrease in muscle tone. As a result, the rats would touch the surrounding water, and REM sleep would be interrupted. Specifically, a round platform (with a 6.5-cm diameter and a 2-cm height) was secured to the bottom of a water tank, which was made from a cage ($43 \times 22 \times 21\ cm^3$) identical to the ones in which the rats are normally housed. The water level was set at 1 cm, and the animal was acclimated to the water environment with access to food and water *ad libitum*. The REMSD cages were placed together, so rats could see each other through the transparent walls of the cages, thus reducing the possibility of stress developing from total social deprivation [47]. It is possible that non-REM sleep was also influenced to some degree, but earlier studies had demonstrated that a similar method mainly reduced the baseline REM sleep while slow-wave sleep remained unaffected [52]

2.5. Experimental design

Experiment 1 examined the effects of TP or REMSD on ASR and PPI. A total of 40 rats were assigned randomly as the following. (i) 16 rats were used to examine the effects of TP on the ASR and PPI by separating them into TP group and control groups ($N = 8$ per group). The control group received identical treatment to the TP group (see TP method above), except no clip was placed on the tail. (ii) 24 rats were separated into three groups (home cage control, tank control, and REMSD, $N = 8$ per group) to examine the effects of REMSD CONDITION for 96 h on the ASR and PPI. Control rats were placed in the same testing room, either in their home cages or on water tanks the same as the one used for REMSD, but with a larger platform (with 16 cm diameter), which allowed rats reach REM sleep without falling into the water, and thus can be used to eliminate the possible confounding effect of water-aversion [21,47].

Experiment 2 examined the interaction between MOD and stress paradigm (i.e., TP or REMSD). A total of 48 rats were assigned randomly to the following: (i) 8 rats were used to establish the dosing pattern of MOD (i.e., saline vehicle and 32, 64, and 128 mg/kg), based on a Latin square injection design; (ii) 16 rats (8 for MOD and 8 for saline vehicle group) were used to examine the effects of PHASE and MOD on the ASR and PPI; (iii) 24 rats were used to examine the effects of REMSD and MOD on the ASR and PPI by dividing them into four groups (home control-vehicle, REMSD-vehicle, home control-MOD, and REMSD-MOD, $N = 6$ per group). For all rats, MOD was administered (i.p.) 30 min prior to the ASR and PPI testing. Note since rats in the tank control group did not reveal any significant difference to the home cage control in either ASR and PPI, the tank control group was not used in the final experiment.

2.6. Drug

The MOD used in the current study was synthesised by the School of Pharmacy, at the National Defence Medical Centre (NDMC), Taiwan, and was employed previously by the same team [7,36]. The synthesis of MOD was based on United States Patent 4177290 (1979). Specifically, MOD (modafinil, diphenyl-methyl-sulfinyl-2-acetamide) was synthesised from bromodiphenylmethane through a reaction with thiourea and chloroacetic acid. Synthesis and functional analysis of MOD were integrative projects funded by NDMC (with the approval code DOD97-10). The drug was suspended in a 0.5% gum arabic solution and administered intraperitoneally (i.p.) in volume of 1.0 ml/kg 30 min before testing. Gum arabic is a complex mixture of polysaccharides and glycoproteins used primarily as a stabiliser. The doses of MOD employed for Experiment 2, based on doses found in the literature were 0, 32, 64 and 128 mg/kg [62]. The dose of 64 mg/kg was selected for the later use because this dose had proven effective in previously behavioural experiments (attentional set-shifting task function [24], three-choice discrimination and attention task [42], and a 5-choice serial reaction time task [36].