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Research report

Age-dependent effects of modafinil on acoustic startle and prepulse inhibition in rats

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ABSTRACT

Modafinil is a psychostimulant approved for treating excessive sleepiness in adults; off-label uses (e.g., treatment of cognitive impairment in schizophrenia, ADHD and age-related dementias) are currently being explored. The effects and mechanisms of action of modafinil have not been fully established. In the present study, the effects of modafinil were examined in young adult (7-month-old) and middle-aged (21–22-month-old) rats, using the acoustic startle response (ASR) and prepulse inhibition (PPI). In the control condition, middle-aged rats showed lower activity levels, significantly lower ASR amplitudes and significantly longer ASR latencies compared to young adult rats. The effects of modafinil differed by age: activity levels and ASR amplitudes were significantly increased in middle-aged rats, whereas activity levels were lower and ASR amplitude was significantly decreased in young adult rats. Modafinil did not significantly alter PPI or startle latencies relative to the control condition. Amphetamine, used as a positive control, significantly decreased ASR amplitude in young adult rats and significantly impaired PPI for both age groups. Amphetamine-induced PPI impairment was greater for young adult rats (34% reduction in ASR amplitude) than for middle-aged rats (24% reduction). The results offer new insights into the effects of modafinil and its mechanism of action, and are consistent with the idea that modafinil enhances vigilance and cognitive functioning in individuals with deficits but not in normal, healthy individuals.

1. Introduction

Modafinil (2-[(diphenylmethyl)sulfinyl]acetamide) is a Schedule IV psychostimulant that is currently approved for treating excessive sleepiness caused by conditions such as narcolepsy, shift work sleep disorder, and obstructive sleep apnea/hypopnea syndrome in adults. Off-label uses, particularly treatment of cognitive impairments associated with a variety of neuropsychiatric disorders, are currently being explored. In general, modafinil has been shown to increase alertness and improve cognitive abilities (learning, memory, and attention) in diverse clinical populations, including patients with schizophrenia, attention deficit hyperactivity disorder, and depression [2,39].

The ability of modafinil to enhance cognitive functioning in individuals without preexisting cognitive impairment or healthy adult animals is less clear. Some studies found that modafinil facilitated learning and memory in healthy adult mice [3–6] and enhanced sustained attention and motivation in tasks requiring spatial memory and visual attention in rats [38,55]. Other studies found no positive effects of modafinil on sustained attention, or increased impulsivity (premature responding prior to cue onset) in rats tested in multiple choice reaction time tasks [38,56]. Morgan et al. [41] offered several potential reasons for equivocal results across studies, including task difficulty, subject age, and baseline levels of cognitive performance. In studies of young, healthy subjects with no cognitive deficits, modafinil may have been unable to improve performance in simple tasks due to ceiling effects. In contrast, modafinil may have beneficial effects when subjects have preexisting cognitive impairments, such as those associated with ADHD, schizophrenia, depression, or aging. Consistent with this, Morgan et al. [41] found that modafinil improved sustained attention and decreased impulsivity in middle-aged (18–20-month-old) female rats tested in a 3-choice visual discrimination and sustained attention task. Cognitive impairment was assumed based on previous studies showing that cognitive decline is evident by middle age in rats [27,28,33]. The middle-aged rats given an oral dose of 64 mg/kg modafinil made fewer premature responses and displayed greater accuracy and faster reaction times compared to vehicle-treated controls.

The present study used the acoustic startle reflex (ASR) and prepulse inhibition (PPI) to further examine the effects of modafinil in healthy young adult and middle-aged rats. ASR and PPI are behavioral paradigms that are increasingly being used as measures of cognitive and affective processes in both animals and humans [18].

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The ASR is an abrupt, transient motor reflex elicited by an intense, unexpected sound via a primary startle circuit that includes the auditory nerve, cochlear root neurons, giant neurons in the ventrolateral part of the nucleus reticularis pontis caudalis (PnC), and spinal motor neurons in the ventral horn of the spinal cord [32,49]. The amplitude of the ASR can be influenced by level of arousal and attention, as well as by the physical characteristics (e.g., intensity, duration, frequency and rise time) of the startle-evoking stimulus (SS) [7,15,23,45]. In addition, the amplitude of the ASR can be modulated by presenting a "prepulse stimulus" (PPS), such as a tone, a puff of air, or a flash of light, shortly before the SS [23]. Although the PPS is not intense enough to evoke a startle response itself, it causes the amplitude of the ASR evoked by the subsequent SS to be reduced or "inhibited." The degree to which ASR amplitude is reduced (i.e., the magnitude of PPI) serves as a measure of the behavioral salience of the PPS and the ability of the central nervous system to "gate" sensory inputs and selectively allocate processing resources to perceptually relevant stimuli [9,23]. The putative brainstem circuit that mediates PPI includes direct glutamatergic projections from the cochlear nuclei and inhibitory cholinergic projections from the pedunculopontine tegmental nucleus to PnC neurons, and indirect input from the inferior and superior colliculi in the midbrain [29]. Activity and sensitivity of the PPI circuit are regulated by diverse forebrain neural circuits that include cholinergic, GABA-ergic and dopaminergic substrates [20,29,49]. Pharmacological manipulations that have been shown to influence PPI include drugs that affect dopamine (DA), serotonin (5-HT) and NMDA gluatamate systems. PPI appears to be particularly sensitive to DA manipulations, with impaired PPI produced by indirect DA agonists such as amphetamine as well as selective DA receptor agonists [21].

Recent studies have examined the effects of modafinil on ASR and PPI in healthy adult males [46], 2–6-year-old marmosets [52], and 12–14-week-old rats [44]. In each of these studies, modafinil increased alertness or general activity levels, but had no significant effect on ASR amplitude or PPI. However, Regenthal et al. [44] also examined the effects of modafinil on ASR and PPI in 12–14-week-old rats exposed to restraint stress to create an animal model of depression. PPI was impaired in the restrained rats relative to controls, and, consistent with the idea that beneficial effects of modafinil emerge when cognitive deficits are present, oral modafinil at a dose of 50 mg/kg attenuated the PPI deficit.

Young adult (7-month-old) and middle-aged (21–22-month-old) rats were used in the present study in order to investigate potential age-related differences in the effects of modafinil on ASR and PPI. Previous studies that have examined ASR and PPI as a function of age have consistently found that the magnitude of the ASR is decreased in older subjects [25,30,42,53]. Consistent with the complexity of the neural circuits that regulate PPI, PPI results have been variable, with studies showing no difference [34] or decreased PPI in aged individuals [53], or an inverted U-shaped function with greater PPI in middle-aged individuals compared to younger or older subjects [18].

Based on the findings described above, middle-aged rats were expected to have reduced ASR amplitudes compared to young rats, and either no difference in PPI or greater PPI compared to young rats in the control condition. It was hypothesized that modafinil would enhance ASR in the middle-aged rats but not in young adult rats, consistent with the idea that modafinil enhances vigilance and cognitive functioning in individuals with deficits, but not in normal, healthy individuals. Amphetamine was included as a positive control. Because previous studies have shown that amphetamine impairs PPI (e.g., [35]), it was expected that young adult rats would show less PPI in the amphetamine condition compared to the control condition. However, given that PPI is regulated by dopaminergic substrates [21,57] and aging is commonly associated with a global loss of inhibitory function and well-documented declines in DA and other neurotransmitters in widespread regions of the rat brain (e.g., [22,37,40]), it was hypothesized that amphetamine would produce less PPI impairment in older rats compared to younger rats.

2. Materials and methods

2.1. Animals

Subjects were female Long-Evans rats bred and raised in the Western Illinois University vivarium. Rats in the Young Adult (YA) Group ($n = 9$) were 7 months old, and rats in the Middle-Aged (MA) Group ($n = 9$) were 21–22 months old when tested. There appears to be no published data on lifespan in Long-Evans rats. However, for rats in general, lifespan is approximately 2–3 years. If the lifespan of the rat is assumed to be 2.5 years and the lifespan of a human is assumed to be 80 years, then a 7-month-old rat has completed 23% of its lifespan (corresponding to a 19-year-old human) and a 21-month-old rat has completed 70% of its lifespan (corresponding to a 56-year-old human). The MA rats had previously been tested in a 3-choice visual attention task after receiving a single oral dose (64 mg/kg) of modafinil at 20 months of age [43]. YA rats weighed between 264 g and 316 g, with a median weight of 287 g; MA rats weighed between 281 g and 425 g, with a median weight of 342 g. There were no significant changes in weight for either group of rats over the course of the study.

Animals were housed in pairs in a low-noise environment (ambient levels typically below 40 dB SPL) with a 12 h/12 h light/dark cycle; rat chow (LabDiet, Purina Mills, St. Louis, MO) and tap water were available *ad libitum* except on evenings prior to testing, when food was removed to increase the animal's incentive to consume a flavored gelatin cube on the following day. All procedures were approved by the Western Illinois University IACUC and complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.2. Drug preparation and administration

All rats received modafinil (Provigil®, Cephalon, Inc., Frazer, PA; 64 mg/kg), amphetamine (d-amphetamine sulfate, Sigma–Aldrich Co., St. Louis, MO; 1 mg/kg) and placebo, in counterbalanced order. Drugs were dispersed into warm raspberry flavored gelatin, then portions containing the appropriate amount of drug (approximately 3 ml each) were transferred with a syringe into miniature ice cube trays and chilled to form firm gelatin cubes. Rats were introduced to the gelatin cubes without drug over a period of several weeks, until all the rats readily consumed their portions. On test days, testing commenced approximately 2 h after the rat had consumed its portion of gelatin. A 2 h delay was imposed because peak plasma concentration occurs 2–4 h after ingestion of modafinil in humans, and the half-life of the drug is 8–18 h (NDA 2007). Recently, Regenthal et al. [44] analyzed blood samples collected from male Wistar rats following a single oral dose of modafinil (50 mg/kg) suspended in distilled water. Modafinil was rapidly absorbed and reached peak plasma levels between 30 min and 45 min post-drug administration, with a linear decrease thereafter and a mean approximated elimination half-life of 12.3 h. Testing in their study commenced 2–3 h after oral administration of modafinil.

2.3. ASR and PPI testing

ASR testing utilized commercial hardware and software (Med Associates, Inc., Georgia, VT). The rat was placed in a wire mesh holder that restricted movement, and the holder was placed on a startle platform in a sound-attenuating chamber. Stimulus generation and response recording were under computer control. The startle stimulus (SS) was a burst of white noise (20 ms duration, 0 ms rise/fall) at a level of 115 dB peak SPL. The prepulse stimulus (PPS) was a 10 kHz tone (20 ms duration, 2 ms rise/fall) presented 100 ms before the SS on PPS + SS trials. Each test session began with a 5 min habituation period, followed by three blocks of trials. Block 1 consisted of 5 trials with the startle stimulus (SS) alone; these orienting trials were not included in any subsequent analyses. Block 2 consisted of 10 trials with the SS alone interspersed in pseudorandomized order with 10 PPS + SS trials, using a PPS level of 70 dB SPL. Block 3 consisted of 40 trials, presented in pseudorandom order: 10 trials with the SS alone, 10 with the PPS at 70 dB SPL, 10 with the PPS at 75 dB SPL, and 10 with the PPS at 80 dB SPL. The 70 dB PPS was used in blocks 2 and 3 in order to examine habituation of ASR and PPI across blocks. Different levels of the PPS were used in block 3 in order to determine the PPS level that produced the most PPI in our experimental setup, where animals were tested in low level ambient noise rather than continuous background noise [11]. Intertrial interval (ITI) varied from 10 s to 30 s, with an average of 20 s between trials. Each rat was tested four times, with at least one week between tests. The first test session was for the purpose of introducing the animals to the wire holder and sound chamber and familiarizing them with the stimuli; data from this initial session were not included in subsequent analyses.

Voltages produced by movement of the rat on the startle platform were recorded over a 500 ms time window beginning 300 ms before the onset of the SS. Activity occurring during the first 200 ms (null period) provided an estimate of background levels of activity in the absence of the PPS or SS; activity during the 200–300 ms