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Atomoxetine modulates spontaneous and sensory-evoked discharge of locus coeruleus noradrenergic neurons

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ABSTRACT

Atomoxetine (ATM) is a potent norepinephrine (NE) uptake inhibitor and increases both NE and dopamine synaptic levels in prefrontal cortex, where it is thought to exert its beneficial effects on attention and impulsivity. At the behavioral level, ATM has been shown to cause improvements on the measures of executive functions, such as response inhibition, working memory and attentional set shifting across different species. However, the exact mechanism of action for ATM's effects on cognition is still not clear. One possible target for the cognitive enhancing effects of ATM is the noradrenergic locus coeruleus (LC), the only source of NE to key forebrain areas such as cerebral cortex and hippocampus. Although it is known that ATM increases NE availability overall by blocking reuptake of NE, the effects of this agent on impulse activity of LC neurons have not been reported. Here, the effect of ATM (0.1–1 mg/kg, ip) on NE-LC neurons was investigated by recording extracellular activity of LC neurons in isoflurane-anesthetized rats. ATM caused a significant decrease of the tonic activity of LC single-units, although leaving intact the sensory-evoked excitatory component of LC phasic response. Moreover, the magnitude of the inhibitory component of LC response to paw stimulation was increased after 1 mg/kg of ATM and its duration was prolonged at 0.3 mg/kg. Together, these effects of ATM produced an increase in the phasic-to-tonic ratio of LC phasic response to sensory stimulation. ATM also modulated the average sensory-evoked local field potential (LFP) and spike-field coherence in LC depending on the dose tested. The lower dose (0.1 mg/kg) significantly decreased early positive and negative components of the sensory-evoked LFP response. Higher doses (0.3–1 mg/kg) initially increased and then decreased the amplitude of components of the evoked fields, whereas the spike-field coherence was enhanced by 1 mg/kg ATM across frequency bands. Finally, coherence between LC fields and EEG signals was generally increased by 1 mg/kg ATM, whereas 0.1 and 0.3 mg/kg respectively decreased and increased coherence values in specific frequency bands. Taken together these results suggest that ATM effects on LC neuronal activity are dose-dependent, with different doses affecting different aspects of LC firing. This modulation of activity of LC-NE neurons may play a role in the cognitive effects of ATM.

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1. Introduction

Noradrenergic transmission is implicated in waking and arousal (Berridge and Foote, 1996; De Sarro et al., 1987), vigilance and attention (Aston-Jones et al., 1997, 1994; Rajkowski et al., 1994), memory (Arnsten, 2001; McGaugh, 2000; Przybyslawski et al., 1999), decision-making (Clayton et al., 2004; Nieuwenhuis et al., 2005a; Usher et al., 1999) and many other cognitive, sensory and motor processes (see Aston-Jones and Cohen, 2005b; Berridge and Waterhouse, 2003; Ramos and Arnsten, 2007; Robbins and Everitt, 1995 for reviews). A general role of norepinephrine (NE) seems to be the enhancement of neural activity in response to relevant stimuli and the suppression of interference from irrelevant ones, independently from their affective value (Aston-Jones and Bloom, 1981b; Foote et al., 1980). This is accomplished by modulating neural excitability producing an increase in gain (Servan-Schreiber et al., 1990) or 'signal-to-noise' ratios in target neurons (Foote et al., 1975; Waterhouse et al., 1980, 1988; Woodward et al., 1991), where NE suppresses spontaneous neuronal firing rates and enhances stimulus-evoked cellular responses (Berridge and Waterhouse, 2003). The largest group of brain noradrenergic neurons is in the brainstem locus coeruleus (LC), which sends widespread

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projections to forebrain areas and is the only source of NE in the hippocampus and neocortex (Berridge and Waterhouse, 2003; Foote et al., 1983; Ungerstedt, 1971).

Psychostimulants are known to improve hyperactivity, impulsivity and inattentiveness in attention deficit/hyperactivity disorder (ADHD) by stimulating NE and dopamine (DA) neurotransmission (Biederman and Spencer, 1999; Zametkin and Rapoport, 1987), but they are also effective at improving attention and other cognitive processes in healthy individuals (Mohamed and Sahakian, 2011; Sahakian and Morein-Zamir, 2011). ADHD drugs such as methylphenidate and amphetamines have recently gained popularity as cognitive enhancers and their use by the "non-clinical" population has grown accordingly (Greely et al., 2008). Their effects on cognition are thought to be mediated in part by decreasing LC spontaneous activity (Pliszka et al., 1996; Solanto, 1998) and by fine modulation of prefrontal NE levels (Ramos and Arnsten, 2007). For instance, it has been demonstrated that methylphenidate administration decreases spontaneous discharge activity of LC neurons (Devilbiss and Berridge, 2006), while having an excitatory influence on prefrontal cortex (PFC) neuronal activity (Salek et al., 2012). Moreover, methylphenidate decreases average sensory evoked field potentials recorded from several other brain regions (Yang et al., 2006a,b). Other non-stimulant medications that have been shown to improve cognitive processes in both healthy and clinical subjects include the wake-promoting agent modafinil and NE reuptake inhibitors (Ballon and Feifel, 2006; Bidwell et al., 2011; Minzenberg and Carter, 2008). A common effect of all these putative cognition-enhancing drugs is to modulate NE transmission in the forebrain, but their exact mechanism of action is still unclear.

Atomoxetine (ATM) is a selective NE reuptake inhibitor with high affinity for the NE transporter and much lower affinity for the DA and serotonin transporters (Bymaster et al., 2002). However, ATM also enhances prefrontal DA, as well as NE, levels in the rat PFC (Bymaster et al., 2002; Swanson et al., 2006). This is because DA transporters are sparse in PFC (Sesack et al., 1998; Soucy et al., 1997) and extracellular DA is taken up by the NET there (Carboni et al., 1990). ATM, which is the first non-stimulant medication indicated for ADHD treatment (Pliszka, 2003; Spencer et al., 1998), decreases omission errors in the continuous performance task (CPT) of sustained attention and normalizes altered electroencephalographic measures in children with ADHD (Barry et al., 2009a). ATM has also been shown to improve working memory, response inhibition and other executive functions in patients with ADHD (Brown et al., 2011; Faraone et al., 2005; Gau and Shang, 2010). Moreover, ATM decreases impulsivity in high impulsive rats (Fernando et al., 2012) and improves attentional set shifting in rats with a compromised noradrenergic system (Newman et al., 2008). Recently, ATM demonstrated better efficacy than placebo in preventing relapse to drug use in animal models (Economidou et al., 2011), but has not been fully evaluated yet in clinical trials for drug addiction (Sofuoglu, 2010).

ATM improves indices of prefrontal executive functions such as working memory (Gamo et al., 2010; Tzavara et al., 2006), inhibition (Chamberlain et al., 2006; Robinson et al., 2008) and attention (Jentsch et al., 2009) in normal subjects also. In the rodent version of the CPT, the 5-choice serial reaction time task (5-CSRTT; Bari et al., 2008), ATM increased response accuracy and decreased premature responses, which is thought to reflect improved attention and impulse control, respectively (Baarendse and Vanderschuren, 2012; Blondeau and Dellu-Hagedorn, 2007; Navarra et al., 2008; Paterson et al., 2011; Robinson, 2012; Robinson et al., 2008). Using other translational tasks, researchers have shown improvements in response inhibition (Eagle et al., 2008), behavioral flexibility (Seu et al., 2009) and sustained attention (Jentsch et al., 2009) after ATM administration in animal models. Importantly, these effects seem to be mediated almost entirely by the modulation of the noradrenergic, rather than dopaminergic, system (Bari et al., 2009; Pattij et al., 2012; Pattij and Vanderschuren, 2008).

Clearly, there is much interest in the pro-cognitive effects of ATM, and a host of behavioral studies have demonstrated beneficial effects of this drug in various domains of cognitive functioning both in humans and non-human models, but little is known about the neural substrates responsible for such effects. Although the modulation of the NE-LC system by some putative cognitive-enhancing drugs such as methylphenidate (Devilbiss and Berridge, 2006; Lacroix and Ferron, 1988; Olpe et al., 1985) and modafinil (Akaoka et al., 1991) have been assessed by electrophysiological techniques, to our knowledge data are currently not available on ATM effects on the discharge activity of NE-LC neurons. Here, we investigated the modulatory effect of different doses of ATM on the spontaneous and sensory-evoked activity of LC neurons using single-unit extracellular recordings in anesthetized rats. Moreover, we recorded LC local field potentials (LFPs), and analyzed sensory-evoked LFP response (Katzner et al., 2009; Mitzdorf, 1985; Yang et al., 2006a), spike-field coherence in LC (Fries, 2005) and coherence between cortical EEG and LC LFPs before and after ATM administration. These measures may provide important information on modulation of afferent inputs to LC, and responsiveness of LC neurons to such afferents, by ATM.

2. Materials and methods

2.1. Animals

Male Long Evans rats (Charles River) were used in these experiments (350–600 g). Animals were singly housed under a 12:12 h reverse light:dark cycle with food (Harlan Teklad) and water available *ad libitum*. Every effort was made to minimize animal suffering and to use the minimum possible number of animals. All procedures followed National Institute of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee.

2.2. Surgical procedure

Rats were initially anesthetized with isoflurane in a plastic chamber and maintained at 2–2.5% throughout the experiment by nosecone administration. The animals were mounted in a stereotaxic frame (Kopf Instruments) equipped with atraumatic earbars and placed on an air table. Body temperature was maintained at ~37 °C using a thermistor-controlled heating pad coupled with a rectal probe. The skull was exposed and two jeweler's screws were implanted over frontal and contralateral parietal cortices where electroencephalogram (EEG) leads were attached. The head of the animal was tilted by 15° (nose down) and a hole was drilled for the insertion of a single barrel glass micropipette filled with 2% pontamine sky blue solution in 0.5 M sodium acetate at the following coordinates: AP, −3.7 mm; ML, +1.2 mm (relative to *lambda*).

2.3. Locus coeruleus extracellular recordings

Recording micropipettes were pulled with a Narishige vertical puller and tips broken to 2–3 μm resulting in an impedance of 3–12 MΩ. A manual hydraulic microdrive was used to slowly advance the recording pipette through the brain. LC neurons were identified according to well-established elecrophysiological and anatomical criteria (Aston-Jones and Bloom, 1981b; Aston-Jones et al., 1980; Cedarbaum and Aghajanian, 1976). These include their spontaneous discharge rate (1–3 Hz), biphasic response to contralateral foot pinch and proximity to mesencephalic trigeminal (Me5) neurons, which exhibit a characteristic response to jaw movement. The signal was pre-amplified by a neuroprobe amplifier (model 1600, A-M Systems Inc) and then split into two separate identical amplifiers (CWE, model BMA 200) for filtering at 100–3 kHz and 1–1 kHz bandpass for single-unit and LFP recordings, respectively. Both signals were digitized by a CED Micro 1401 at 5.5 kHz and stored on a computer running Spike 2 version 5 software (Cambridge Electronic Design, Cambridge, UK) for off-line spike sorting and signal processing. Single-unit traces were monitored on a dual beam storage oscilloscope and via a loudspeaker. The EEG trace was amplified by a BMA 831 (CWE), filtered (1–50 Hz), sampled at 925 Hz and monitored online on the computer screen to ensure a constant level of anesthesia (Fig. 1). Once an LC cell was isolated, data was collected for at least 2 min