Neuroscience 146 (2007) 515–524

EFFECTS OF RESTRAINT AND HALOPERIDOL ON SENSORY GATING IN THE MIDBRAIN OF AWAKE RATS

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Abstract—Deficits in sensory processing have been reported to be associated with an array of neuropsychiatric disorders including schizophrenia. Auditory sensory gating paradigms have been routinely used to test the integrity of inhibitory circuits hypothesized to filter sensory information. Abnormal dopaminergic neurotransmission has been implicated in the expression of schizophrenic symptoms. The aim of this study was to determine if inhibitory gating in response to paired auditory stimuli would occur in putative dopaminergic and non-dopaminergic midbrain neurons. A further goal of this study was to determine if restraint, a classic model of stress known to increase extracellular dopamine levels, and systemic haloperidol injections affected inhibitory mechanisms involved in sensory gating. Neural activity in the rat midbrain was recorded across paired auditory stimuli (first auditory stimulus (S1) and second auditory stimulus (S2)) under resting conditions, during restraint and after systemic haloperidol injections. Under resting conditions, a subset of putative GABA neurons showed fast, gated, short latency responses while putative dopamine neurons showed long, slow responses that were inhibitory and ungated. During restraint, gated responses in putative GABAergic neurons were decreased (increased S2/S1 or ratio of test to conditioning (T/C)) by reducing the response amplitude to S1. Systemic haloperidol decreased the T/C ratio by preferentially increasing response amplitude to S1. The results from this study suggest that individual neurons encode discrete components of the auditory sensory gating paradigm, that phasic midbrain GABAergic responses to S1 may trigger subsequent inhibitory filtering processes, and that these GABAergic responses are sensitive to restraint and systemic haloperidol.

Key words: sensory gating, stress, electrophysiology, dopamine neurons, GABA neurons, schizophrenia.

Dysregulation of midbrain circuit activity is hypothesized to contribute to cognitive deficits associated with disorders such as addiction (Wise, 1987), schizophrenia (Laruelle et al., 1999), and Parkinson's Disease (Ungerstedt and Arbuthnott, 1970). Patients with these and other neuropsychiatric diseases often have disturbances in inhibitory filtering mechanisms that are thought to contribute aberrant sensory processing and behaviors (Adler et al., 1982; Clementz et al., 1997; de Bruin et al., 2001; Franks et al., 1983; Freedman et al., 1996; Ghisolfi et al., 2004; Gillette et al., 1997; Light and Braff, 1998; Neylan et al., 1999). While activity of dopaminergic and non-dopaminergic midbrain neurons encodes salient stimuli involved with attentional processes associated with learning and reward (Pan et al., 2005; Schultz, 1998; Steffensen et al., 2001) there are few studies that have documented midbrain activity across behavioral paradigms other than reward-related paradigms. The goal of this experiment is to record activity of midbrain neurons across an auditory sensory gating paradigm in order to determine if activity of these neurons may contribute to circuit mechanisms that underlie early pre-attentional stages of information processing (Ellenbroek, 2004).

Auditory sensory gating paradigms, where electrophysiological responses to repetitive paired auditory stimuli are recorded, are used to test the integrity of neural circuits mediating inhibitory sensory filtering mechanisms (Adler et al., 1982). Responses to the first auditory stimulus within the pair theoretically evoke inhibitory mechanisms that attenuate responses to the second. In normal individuals, gated electrophysiological responses consist of reduced electrophysiological response amplitudes to the second auditory (S2 or test) stimulus as compared with the first (S1 or conditioning) stimulus so that the ratio of test to conditioning, or T/C ratio is less than 1. Gated response patterns are seen with EEG P50 evoked responses in schizophrenic patients (Light and Braff, 1998), N40 evoked responses in animal models (Moxon et al., 2003; Stevens et al., 1998) or unit activity of hippocampal pyramidal neurons (Moxon et al., 1999), as well as afferent target areas of midbrain neurons such as the amygdala (Cromwell et al., 2005) and medial prefrontal cortex (Mears et al., 2006).

Schizophrenic patients show a characteristic deficit in sensory gating paradigms that can be replicated in animal models of schizophrenia where the suppression to the second stimulus is reduced (Adler et al., 1982; Boutros et al., 1991; Clementz et al., 1997; Ellenbroek, 2004; Freedman et al., 1996). This deficit is thought to be trait-associated and linked to the cholinergic neurotransmission in the hippocampus. Response amplitudes to the second, or test stimulus can be reduced by nicotine (Adler et al., 1992, 1993; Bickford and Wear 1995) and alpha-7 agonists (Stevens et al., 1998), thus transiently reversing this form of gating deficit. Non-symptomatic relatives of schizo-

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Abbreviations: NHS, normal horse serum; S1, first auditory stimulus; S2, second auditory stimulus; T/C, ratio of test to conditioning.

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doi:10.1016/j.neuroscience.2007.01.060

phrenic patients also show increased response amplitudes to the test stimulus under baseline conditions (Siegel et al., 1984; Adler et al., 1992; Waldo et al., 1995; Stevens et al., 1998), and families with gating deficits show a dinucleotide polymorphism in the chromosomal region encoding the nicotinic alpha-7 subunit (Freedman et al., 1997). Finally, typical neuroleptics do not reverse this type of gating deficit in schizophrenic patients (Freedman et al., 1987), suggesting that while administration of typical neuroleptics may have powerful effects on schizophrenic symptoms, it may not ameliorate circuit deficits underlying this particular gating deficit.

There is evidence that the dopaminergic system may also be involved in sensory gating mechanisms. Systemic amphetamine and cocaine (Adler et al., 1986; Stevens et al., 1991; Boutros et al., 1994) and microinjections of quinpirole in the nucleus accumbens (de Bruin et al., 2001) decrease sensory gating in normal subjects and/or in animal models. Gating deficits are also correlated in other biobehavioral pathologies that may be linked to altered dopaminergic transmission such as compulsive gambling (Stojanov et al., 2003) post-traumatic stress disorder (Ghisolfi et al., 2004; Gillette et al., 1997; Neylan et al., 1999); however, unlike those regulated by the cholinergic system, these deficits are due to decreased responses to the first, or conditioning stimulus. Furthermore, unlike trait-associated gating deficits seen in schizophrenic patients, manifestation of gating deficits can be transient and state-dependent. Patients with bipolar disorder show gating deficits during psychotic episodes but not during remission (Franks et al., 1983; Baker et al., 1990). Acute stress, known to increase extracellular dopamine (Abercrombie et al., 1989; Imperato et al., 1992; Morrow et al., 1999), reduces sensory gating in control individuals (Johnson and Adler, 1993; White and Yee, 1997) and in animal models (Suer et al., 2004). Finally, manipulation of the dopaminergic system disrupts other gated behaviors associated with schizophrenia such as prepulse inhibition (Mansbach et al., 1988).

While it is clear that pre-attentive mechanisms as measured by sensory gating paradigms are sensitive to changes in dopaminergic neurotransmission, responses of individual midbrain neurons across repetitive stimuli are unknown. The goal of the present study was to use multi-unit recording techniques in freely moving rats to determine whether or not dopaminergic and non-dopaminergic midbrain neurons respond to paired auditory stimuli in a gated fashion. Furthermore, we wished to examine the effects of restraint and systemic haloperidol, conditions known to activate and block dopaminergic neurotransmission respectively, on midbrain responses to paired auditory stimuli.

EXPERIMENTAL PROCEDURES

Animals

Twenty-five experimentally naïve, male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) initially weighing 350–400 g were used for these experiments. This study was designed to minimize the number of animals used and pain related to experimental procedures. Animals were housed under a normal 12-h light/dark cycle (lights off 18:00 h). All behavioral testing was performed in the light phase of their light/dark cycle. All animals had free access to food and water at all times. All animals were maintained within the standards set forth for the care and use of laboratory animals by the National Institutes of Health. All protocols used to complete this study were reviewed and approved by the Animal Care and Use Committee at Wake Forest University Health Sciences.

Surgery

After the 3-day acclimation period, animals were handled daily for at least 1 week to habituate them to experimental manipulation and then implanted with microwire arrays. Animals were anesthetized with isoflurane and a prophylactic dose of antibiotics was administered prior to surgery. Body temperature was maintained between 35 and 37 °C and aseptic technique observed. After placing the rat in a stereotaxic apparatus, the scalp was shaved, swabbed with iodine and a central incision made to expose the skull. Small holes were drilled in the skull and two arrays of eight, stainless steel, Teflon-coated microwires (45–62 um in diameter) were lowered bilaterally into the midbrain using the following coordinates: AP, +3.5 mm from lambda; ML ±1.8 from Bregma; DV, −8.3 from skull surface. Uncoated stainless steel ground wires were positioned 2–3 mm ventral to the cortical surface and wrapped around skull screws. The recording head stage was then secured to the cranium with dental cement using skull screws as anchors.

Recording procedure and experimental paradigms

All experimental procedures followed a 10- to 14-day post-surgical recovery period that allows for stable midbrain recording sessions across days. Neuroelectric signals were amplified and filtered (0.5 and 5 kHz, 3 dB cutoffs) under software control. Signals were digitized 50 kHz per channel and spikes sorted using movable windows-based parameters for time and voltage discriminators using software and instrumentation from Spectrum Scientific (Dallas, TX, USA). Temporal records of extracellular spike activity were superimposed on digitized behavioral records using Magsort software (Biographics, Inc., Winston-Salem, NC, USA) with a time resolution of 3 ms.

On days 1 and 2, baseline activity across the paired auditory stimulus paradigm was recorded. Animals were placed in the experimental chamber and tethered to the recording system. In all cases, lightweight cabling connected the recording head stage to a freely rotating commutator, allowing the animals to move freely within the recording chamber unless otherwise noted. Midbrain activity was recorded across a sensory gating paradigm used in previous studies (Cromwell et al., 2005; Mears et al., 2006). In this paradigm, a pair of 100-ms auditory tones (65 dB; 2.5 kHz) separated by a 500-ms delay was presented every 10 s.

After the second baseline session, animals showing phasic neural responses to auditory stimuli were divided into two groups. The effect of restraint on midbrain activity was explored in the first group ($n=8$). Neural activity was recorded across a third 30-min baseline session after which the animal was removed from the recording chamber and placed in a padded, hemi-cylindrical tube in which he could not turn and from which he could not escape. The restraint apparatus and the animal were immediately placed back in the recording chamber, and activity of the same neuron population was recorded for another 30-min session across the sensory gating paradigm. This restraint paradigm has been shown to increase firing rate of VTA neurons putatively identified as dopamine neurons through electrophysiological, pharmacological and anatomical criteria (Anstrom and Woodward, 2005).

In the second group ($n=13$), the effect of a 1 mg/kg dose of systemic haloperidol on midbrain gating responses was exam-