Neuroscience 157 (2008) 95–104

A NOVEL ELECTROPHYSIOLOGICAL MODEL OF CHEMOTHERAPY-INDUCED COGNITIVE IMPAIRMENTS IN MICE

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Abstract—Purpose: Chemotherapeutic agents are known to produce persistent cognitive deficits in cancer patients. However, little progress has been made in developing animal models to explore underlying mechanisms and potential therapeutic interventions. We report an electrophysiological model of chemotherapy-induced cognitive deficits using a sensory gating paradigm, to correspond with performance in two behavioral tasks.

Experimental design: Mice received four weekly injections of methotrexate and 5-fluorouracil. Whole-brain event-related potentials (ERPs) were recorded throughout using a paired-click paradigm. Mice underwent contextual fear conditioning (CFC) and novel-object recognition testing (NOR).

Results: Chemotherapy-treated animals showed significantly impaired gating 5 weeks after drug treatments began, as measured by the ratio of the first positive peak in the ERP (P1) minus the first negative peak (N1) between first and second auditory stimuli. There was no effect of drug on the amplitude of P1–N1 or latency of P1. The drug-treated animals also showed significantly increased freezing during fear conditioning and increased exploration without memory impairment during novel object recognition.

Conclusions: Chemotherapy causes decreased ability to gate incoming auditory stimuli, which may underlie associated cognitive impairments. These gating deficits were associated with a hyperactive response to fear conditioning and reduced adaptation to novel objects, suggesting an additional component of emotional dysregulation. However, amplitudes and latencies of ERP components were unaffected, as was NOR performance, highlighting the subtle nature of these deficits.

Key words: chemotherapy, cognitive impairment, sensory gating, event-related potentials, animal model, post-traumatic stress disorder.

Recent data suggest that there may be a greater degree of neurotoxicity and cognitive decline following treatment of non-CNS tumors with systemic chemotherapy than was previously recognized. Over the last decade, several longitudinal clinical studies have established a strong connection between cancer chemotherapy and cognitive deficits, even when controlling for differences in gender, cancer diagnosis, drug treatment regimen, dosage administered, and related psychological comorbidities such as depression or anxiety (Schagen et al., 1999; Dietrich et al., 2006). These findings, colloquially referred to as 'chemo-brain,' have received much concern of late, with recent reviews and a 2003 conference (Tannock et al., 2004; Ahles and Saykin, 2007) stressing the need to develop an appropriate animal model, to create a sensitive neuropsychological paradigm to detect these deficits, and to probe involved neural circuitry with neuroimaging.

We addressed these gaps in the literature by investigating the effects of chemotherapy on sensory information processing and cognitive-behavioral functioning in a mouse model. We employed a sensory gating paradigm using auditory event-related potentials (ERPs) in which whole-brain electrical activity is recorded while animals are presented with pairs of identical auditory stimuli (termed first stimulus (S1) and second stimulus (S2)). Neural responses are correlated with the onset of each stimulus such that extraneous electrical activity is averaged out and the remaining waveform reflects coordinated activity of the neural generators in the auditory information processing pathway. Previously, we developed and utilized mouse models for measuring auditory ERPs following either pharmacological or genetic manipulations that are relevant to psychiatric conditions (Connolly et al., 2003; Siegel et al., 2003; Maxwell et al., 2006a,b; Metzger et al., 2006). In humans, early ERPs (1–8 ms) represent activation of brainstem structures such as the cochlear nucleus; mid-latency ERPs (8–40 ms) indicate forebrain activity including thalamus and hippocampus and long-latency ERPs (50–300 ms) represent processing at the level of the cortex, involving primary and association cortices (Picton et al., 1974; Reite et al., 1988). Early, middle, and long-latency ERPs recorded from mice resemble corresponding human components in topography and response

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Abbreviations: A2/A1, ratio of response following the second tone to the first; CFC, contextual fear conditioning; DMSO, dimethyl sulfoxide; EEG, electroencephalogram; ERP, event-related potentials; LTP, long-term potentiation; MTX, methotrexate; NE, norepinephrine; NOR, novel-object recognition; N1, first negative peak in the event-related potential; PTSD, post-traumatic stress disorder; P1, first positive peak in the event-related potential; P2, second positive peak in the event-related potential; P3, third positive peak in the event-related potential; rmANOVA, repeated measures analysis of variance; S1, first stimulus; S2, second stimulus; 5-FU, 5-fluorouracil.

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doi:10.1016/j.neuroscience.2008.08.060

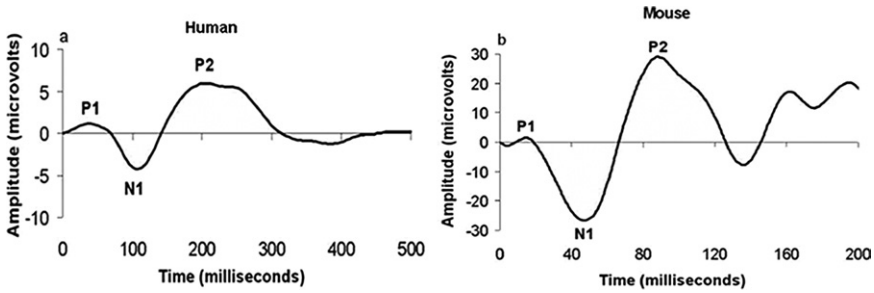

Fig. 1. Correspondence between auditory evoked potentials recorded from humans in the Cz configuration (a) and mice (b). Morphology of the waveforms is largely homologous between species with the main peaks labeled P1, N1, and P2. The latencies of ERP peaks from mice are approximately 40% of those recorded from humans. The amplitudes are smaller in humans reflecting the use of scalp EEG rather than depth electrodes in mice.

properties (Connolly et al., 2003; Umbricht et al., 2004). Rodents share many similarities with humans for specific portions of the ERP, including the first, second, and third positive peaks (termed P1, P2, and P3) as well as the first negative peak in the mouse ERP (termed N1) which share stimulus and pharmacologic response properties with the human P50, P200, P300 and N100 respectively (Fig. 1) (Siegel et al., 2003; Connolly et al., 2004). The latencies of ERP peaks from mice have been shown to be consistently 40% of those recorded from human subjects (Umbricht et al., 2004). Thus, ERPs are an especially attractive means of evaluating sensory processing in animals as there are direct human correlates for these measures.

We specifically focused on sensory gating of ERPs, in which the amplitude of the neural response to S2 is normally diminished compared with that of S1, reflecting basic sensory habituation. Sensory gating is a fundamental aspect of pre-attentional processing characterized by habituation of brain responses to a repetitive sensory stimulus, which provides individuals with the ability to filter out irrelevant or redundant information from the environment and to attend to more salient stimuli. Abnormalities in sensory gating have become an attractive target for animal models of impaired cognitive function, linked to disorders such as schizophrenia, in which gating deficits are largely accepted as an endophenotypic marker of the disease (Adler et al., 1993). This has facilitated the development of animal models which have led to an enhanced mechanistic understanding of the neural circuitry involved and identification of new targets for therapeutic interventions (Braff and Light, 2004; Ellenbroek, 2004).

In the present study, we administered a combination of methotrexate (MTX) and 5-fluorouracil (5-FU) to adult mice, which is a standard clinical regimen for treatment of human breast cancers. We employed a dosing strategy that has already been shown to produce mild cognitive-behavioral impairments in mice (Winocur et al., 2006) in order to elicit electrophysiological correlates of those deficits. In addition, we extend previous animal behavioral studies by employing novel object recognition (NOR) and contextual fear conditioning (CFC) tasks, which are sensitive to various stages of cognitive and emotional processing that may be altered with chemotherapy. NOR is a recognition memory task that has been shown to require both the hippocampus and the cortex (Carpenter and Grossberg, 1993), while CFC is also hippocampally-dependent as well as sensitive to modulation by the amygdala (Phillips and LeDoux, 1992). These tasks are especially relevant in light of recently published data which demonstrate that chemotherapeutic agents disrupt hippocampal neurogenesis and that these cellular changes may underlie the observed cognitive impairments (Seigers et al., 2008).

The significance of this work is that it furthers the development of a much-needed animal model and, for the first time, probes involved neural-circuitry with electrophysiological recording. Any observed ERP changes can be easily corroborated in the clinical populations using analogous scalp electroencephalogram (EEG) techniques. Finally, ERP animal models are ideally suited for investigation into relevant genetic and pharmacological manipulations to develop an adjuvant therapy and could prove to be a pre-clinical screening technique for new chemotherapeutic agents to evaluate their neurotoxic potential.

EXPERIMENTAL PROCEDURES

Animals

Twenty-four male C57BL/6Hsd (B6) mice were obtained at 7–8 weeks of age from Harlan (Indianapolis, IN, USA). All testing was conducted between 10 and 18 weeks of age. Mice acclimated to the Animal Facility for 7 days before experimentation began. Mice were housed four to five per cage until surgeries, after which they were single-housed for the remainder of the study. They were maintained in a standard 12-h light/dark cycle with free access to food and water. Experiments were performed during the light phase between 9:00 AM and 4:00 PM. All protocols were conducted in accordance with University Laboratory Animal Resources (ULAR) guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. All efforts were undertaken to minimize the number of animals used in the experiment and their suffering.

Surgery

Animals underwent stereotaxic implantation of tripolar electrode assemblies (PlasticsOne Inc., Roanoke, VA, USA) for non-anesthetized recording of ERPs, as previously described (Connolly et al., 2003, 2004; Siegel et al., 2003; Maxwell et al., 2004a,b). Animals were anesthetized with isoflurane and recording electrodes were placed unilaterally in CA3 of the hippocampus (1.4 mm posterior, 2.65 mm lateral, and 2.75 mm deep relative to