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Distinct behavioral phenotypes in novel "fast" kindling-susceptible and "slow" kindling-resistant rat strains selected by stimulation of the hippocampal perforant path

Tomer Langberg [1], Ryan Dashek [2], Bernard Mulvey [3], Kimberly A. Miller [4], Susan Osting, Carl E. Stafstrom [5], Thomas P. Sutula *

Department of Neurology, University of Wisconsin School of Medicine and Public Health, 1685 Highland Avenue, Madison, WI 53705, United States

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ABSTRACT

Kindling is a phenomenon of activity-dependent neural circuit plasticity induced by repeated seizures that results in progressive permanent increases in susceptibility to epilepsy. As the permanent structural and functional modifications induced by kindling include a diverse range of molecular, cellular, and functional alterations in neural circuits, it is of interest to determine if genetic background associated with seizure-induced plasticity might also influence plasticity in neural circuitry underlying other behaviors. Outbred Sprague–Dawley (SD) rats were selected and bred for ~15 generations for "fast' or "slow" rates of kindling development in response to stimulation of the perforant path input to the hippocampus. After 7–8 generations of selection and breeding, consistent phenotypes of "fast" and "slow" kindling rates were observed. By the 15th generation "fast" kindling rats referred to as Perforant Path Kindling Susceptible (PPKS) rats demonstrated a kindling rate of 10.7 ± 1.1 afterdischarges (ADs) to the milestone of the first secondary generalized (Class V) seizure, which differed significantly from "slow" kindling Perforant Path Kindling Resistant (PPKR) rats requiring 25.5 ± 2.0 ADs, and outbred SD rats requiring 16.8 ± 2.5 ADs ($p < 0.001$, ANOVA). Seizure-naïve adult PPKS and PPKR rats from offspring of this generation and age-matched adult outbred SD rats were compared in validated behavioral measures including the open field test as a measure of exploratory activity, the Morris water maze as a measure of hippocampal spatial memory, and fear conditioning as a behavioral paradigm of associative fear learning. The PPKS ("fast" kindling) strain with increased susceptibility to seizure-induced plasticity demonstrated statistically significant increases in motor exploratory activity in the open field test and reduced spatial learning the Morris water maze, but demonstrated normal fear conditioned learning comparable to outbred SD rats and the "slow" kindling-resistant PPKR strain. These results confirm that selection and breeding on the basis of responses to repeated pathway activation by stimulation can produce enduring modification of genetic background influencing behavior. These observations also suggest that genetic background underlying susceptibility or resistance to seizure-induced plasticity in hippocampal circuitry also differentially influences distinct behaviors and learning that depend on circuitry activated by the kindling selection process, and may have implications for associations between epilepsy, comorbid behavioral conditions, and cognition.

Abbreviations: SD, Sprague–Dawley; PPKS, Perforant Path Kindling Susceptible; PPKR, Perforant Path Kindling Resistant; AD, After discharge; ADT, After discharge threshold; CS, Conditioning stimulus; US, Unconditioned stimulus.

* Corresponding author at: Department of Neurology, 7158 MFCB, University of Wisconsin, 1685 Highland Avenue, Madison, WI 53705, United States.
E-mail addresses: langberg.tomer@gmail.com (T. Langberg), dashek@wisc.edu (R. Dashek), mulveyb@wusm.wustl.edu (B. Mulvey), kim_a_miller@comcast.net (K.A. Miller), smknodel@wisc.edu (S. Osting), cstafst1@jhmi.edu (C.E. Stafstrom), sutula@neurology.wisc.edu (T.P. Sutula).
[1] Neuroscience Graduate Program, University of California, Berkeley, CA 94720–3200, United States.
[2] University of Wisconsin School of Veterinary Medicine, 2015 Linden Dr., Madison, WI 53706, United States.
[3] Washington University School of Medicine, 660 S Euclid Ave., St. Louis, MO 63110, United States.
[4] University of Minnesota Medical School, 420 Delaware Street SE, Minneapolis, MN 55455, United States.
[5] Division of Pediatric Neurology, Department of Neurology, Johns Hopkins School of Medicine, 200 North Wolfe Street, Baltimore, MD 21287, United States.
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1. Introduction

Kindling, the phenomenon of seizure-induced neural circuit plasticity, was discovered nearly 50 years ago during the course of experiments investigating the effects of repeated brain stimulation on learning and memory acquisition. Kindling can be induced in species ranging from amphibians to primates by repeated episodes of network synchronization or seizures which cause a progressive, permanent increase in susceptibility to additional seizures (Goddard et al., 1969). Kindling induces progressive, permanent structural and functional circuit alterations with increasing susceptibility to additional seizures in neural circuits throughout the brain, and eventually results in emergence of recurring spontaneous seizures that define epilepsy (Wada et al., 1975; Wada and Osawa, 1976; Pinel and Rovner, 1978; Sayin et al., 2003, Bertram, 2007). The process of kindling has been widely used as an experimental model for investigation of temporal lobe epilepsy, the most common form of poorly controlled human epilepsy (Neligan et al., 2012). As a phenomenon of activity-dependent neural plasticity and circuit remodeling, kindling can also be regarded as a neurobiological process contributing to progressive features of poorly controlled epilepsy. Because activity-dependent plasticity also underlies processes of learning and memory, it was of interest to determine if genetic background influencing kindling development might also influence other behaviors dependent on neural plasticity.

To pursue these questions, we selected and bred Sprague–Dawley rats for susceptibility or resistance to development of kindled seizures evoked by stimulation of the perforant path input to the hippocampus, a region of the brain implicated in memory formation and epilepsy. In a previous series of studies, rats selectively bred for "fast" or "slow" rates of kindling through the amygdala (McIntyre et al., 1999; Racine et al., 1999) demonstrated significant differences in several behavioral measures including more intense reaction to stressors such as fear, decreased habituation of exploratory behavior on the open field test, and impaired learning in hippocampus-dependent tasks such as the Morris water maze and a delayed alternation task (Mohapel and McIntyre, 1998; Anisman et al., 2000; Anisman and McIntyre, 2002; Kelly et al., 2003; Runke and McIntyre, 2008). These behavioral abnormalities were interpreted to suggest that enhanced seizure susceptibility is associated with attentional, emotional, and learning abnormalities potentially implicated in epilepsy and comorbid behavioral conditions (McIntyre and Gilby, 2007). To consider the possibility that there might be interesting differences between the plasticity and phenotypes of rats selected on the basis of perforant path versus amygdala stimulation, outbred Sprague Dawley rats selected for "fast" or "slow" rates of kindling development were bred for ~15 generations, and seizure-naïve adult rats from the "fast" and "slow" strains were examined for behavioral phenotypic differences in validated behavioral measures including the open field test (Prut and Belzung, 2003), the Morris water maze (Morris et al., 1982; Morris, 1984), and fear conditioning (Tronson et al., 2012). The "fast" kindling and "slow" kindling strains selected in response to stimulation of the perforant path input to the hippocampus exhibited statistically significant differences in these behavioral measures, suggesting that genetic background influencing seizure-induced plasticity also influences functional properties of neural circuitry underlying fear conditioning, motor activity, and spatial learning. Preliminary results of these experiments have been published in abstract form (Langberg et al., 2012).

2. Materials and methods

2.1. Surgical procedures

Outbred Sprague–Dawley (SD) male and female rats (Harlan, Inc.) were anesthetized with isoflurane (1–5% for induction, 1–2% for maintenance) and implanted through a craniotomy with a bipolar stimulating and recording electrode in the angular bundle of the perforant path (8.1 mm posterior, 4.4 mm lateral, 3.5 mm ventral to bregma). Bupivacaine (0.125%) was injected for analgesia around the incision and operative site prior to the procedure, and lidocaine was applied at stereotaxic pressure points. The electrode was fixed to the skull with acrylic. Flunixin 2 mg/kg IM was administered following the procedure for postoperative analgesia. All procedures were approved by the University of Wisconsin Institutional Animal Care and Use Committee and were in conformity with institutional and national guidelines.

2.2. Kindling procedures

After a 1–2 week recovery period, the afterdischarge threshold (ADT) in response to 1-second trains of 60-hz biphasic constant current stimulation was determined by application of a graded series of stimulus intensities (100, 200, 300, 400, 500, 700, 900, 1000, 1100, 1300, 1500 μamp). The ADT was defined as the lowest current intensity that evoked an afterdischarge (AD). On subsequent days, rats received twice-daily (5 days per week) electrical stimulation at the determined ADT to evoke electrographic and behavioral seizures classified according to a modified Racine scale (Racine, 1972a,b; Sutula and Steward, 1986; Cavazos et al., 1991; Cavazos et al., 1994): Class I — arrest of motion or "freezing", Class II — freezing and automatisms (blinking, facial twitching, drooling), Class III — unilateral extremity clonus, Class IV — bilateral extremity clonus, and Class V — bilateral extremity clonus and loss of postural tone. If a rat experienced 3 ADs at a given stimulus intensity, the intensity for subsequent stimulations was then reduced to the next lowest intensity in the above sequence. This protocol was intended to evoke ADs at the lowest possible (i.e., threshold) intensity. Stimulation was repeated until 3 Class V seizures were evoked. The kindling rate was defined as the number of ADs required to evoke the first secondary generalized (Class V) seizure.

2.3. Breeding

Using the above procedures, outbred SD rats were selected for "fast" or "slow" rates of kindling development in response to stimulation of the perforant path, which is the major afferent input to the hippocampus. SD rats typically develop the first Class V seizure after ~14–16 ADs using the above protocol of perforant path stimulation (Sutula and Steward, 1986; Cavazos et al., 1991; Cavazos et al., 1994). Rats experiencing the first Class V seizure with ≤10 ADs were regarded as "fast" or kindling-susceptible. Rats that experienced the first Class V seizure after ≥20 ADs were regarded as "slow" or kindling-resistant. Pairs of male and female "fast" (<10ADs) or "slow" (>20ADs) rates of kindling to the first Class V seizure were mated. Offspring were implanted with a perforant path electrode after maturation into adulthood, and received kindling stimulation according to the above protocol. Progeny with "fast" or "slow" rates of kindling in response to perforant path stimulation based on the above criteria were then bred in successive generations to select for the phenotype of "fast" perforant path kindling susceptibility or "slow" perforant path kindling resistance.

2.4. Behavioral testing

After ~15 generations, approximately equal numbers of adult male and female "fast" kindling, "slow" kindling, and outbred SD control rats which did not undergo kindling procedures were compared in validated behavioral measures including the open field test (Prut and Belzung, 2003), the Morris water maze (Morris et al., 1982; Morris, 1984), and fear conditioning (Tronson et al., 2012).

2.4.1. Open field

Exploratory motor activity was assessed on 4 consecutive days in a standardized open field consisting of a 5 × 5-foot tiled floor area divided into 25 equal-sized squares enclosed by a 3-foot-high opaque, plexiglass wall. Each trial session was initiated by placing the rat into the field