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Research report

AMPA receptor mediated behavioral plasticity in the isolated rat spinal cord

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HIGHLIGHTS

- We further examine the role of the glutamatergic system on instrumental learning.
- The role of the AMPA Receptor on instrumental learning is highlighted.
- Extends our hypothesis that the plasticity of the spinal cord can be saturated.

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ABSTRACT

Previous research has demonstrated that the spinal cord is capable of a simple form of instrumental learning. Spinally transected rats that receive shock to a hind leg in an extended position quickly learn to maintain the leg in a flexed position, reducing net shock exposure whenever that leg is flexed. Subjects that receive shock independent of leg position (uncontrollable shock) do not exhibit an increase in flexion duration and later fail to learn when tested with controllable shock (learning deficit). The present study examined the role of the ionotropic glutamate receptor α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) in spinal learning. Intrathecal application of the AMPA receptor antagonist CNQX disrupted performance of a spinal instrumental learning in a dose dependent fashion (Experiment 1). CNQX also disrupted the maintenance of the instrumental response (Experiment 2) and blocked the induction of the learning deficit (Experiment 3). Intrathecal application of the agonist AMPA had a non-monotonic effect, producing a slight facilitation of performance at a low dose and disrupting learning at a high concentration (Experiment 4). Within the dose range tested, intrathecal application of AMPA did not have a long-term effect (Experiment 5). The results suggest that AMPA-mediated transmission plays an essential role in both instrumental learning and the induction of the learning deficit.

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1. Introduction

Neurons within the spinal cord are plastic and can support a range of behavioral phenomena [1]. Using traditional learning tasks, the isolated spinal cord has been found to support single stimulus learning [2], Pavlovian conditioning [3,4], and instrumental learning [5]. Our laboratory has focused on the last form of learning, in part because instrumental learning contributes to the recovery of function after spinal cord injury (SCI) [6,7].

Evidence that spinal neurons are sensitive to instrumental (response–outcome) relations has been obtained using animal subjects that have undergone a complete transection of the thoracic spinal cord, which blocks all communication between the brain and neurons below the injury. In a typical experiment [5,8], a response–outcome relationship is instituted by administering shock to the tibialis anterior muscle of one hind leg whenever that leg is extended (controllable shock). Over time, subjects in this condition exhibit a progressive increase in flexion duration that minimizes net shock exposure. Other subjects receive shock at the same time and for the same duration, but independent of leg position (uncontrollable shock). Uncontrollably shocked subjects do not exhibit an increase in flexion duration and later fail to learn when given controllable shock to either the pretreated (ipsilateral) or opposite (contralateral) leg. Further work has shown that just 6 min of intermittent, uncontrollable, shock to the leg or tail impairs learning for up to 48 h [9].

Both the acquisition of spinal instrumental learning and the induction of the learning deficit depend on a glutamatergic signal [10,11]. The N-methyl-D-aspartic acid receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) are part of the same family of ionotropic glutamate receptors [12]. Engaging the AMPAR, through the binding of glutamate, results in rapid depolarization of the cell and delayed activation of the NMDAR [13]. Activation of the NMDAR allows

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Ca^{2+} ions to flow freely into the cell [14,15]. A strong Ca^{2+} influx initiates intracellular mechanisms that modify synaptic communication, altering components thought to contribute to learning and memory [16,17], including the open probability of NMDARs, activating AMPARs and AMPAR trafficking at the synaptic cleft [12,18]. Prolonged high frequency stimulation has been linked to an NMDAR-dependent enhancement of synaptic function (long-term potentiation [LTP]) mediated by an up-regulation of AMPARs [12,16]. Conversely, stimulation parameters that lead to an overall reduction in synaptic efficacy (long-term depression [LTD]) produce a reduction of AMPAR function [12].

Enumerable studies have examined the role of the NMDAR in behavioral plasticity, both within the brain and spinal cord [15,16,19]. In contrast, while much is known about the AMPAR at the cellular level [20,21,42], relatively few studies have examined how pharmacological manipulations that target this receptor impact behavior (excluding studies designed to examine AMPA-mediated neurotoxic effects). At the level of the spinal cord, the limited work that has been done indicates that manipulations that impact AMPAR function affect nociceptive reactivity [22,23]. Also as found within the brain, chronic spinal administration of AMPAR agonists can induce a lasting effect (excitotoxicity) that results in tissue damage and a loss of plasticity [46].

In prior studies, we have assumed that alterations in AMPAR function play a pivotal role in both instrumental learning and the induction of the learning deficit [8,24,25], but this has not been tested. Instead, we have assumed that evidence that the NMDAR plays an essential role implicates, through association, the AMPAR. From this perspective, instrumental learning reflects an NMDAR/AMPAR-dependent alteration in a spinal circuit that increases flexion duration. The learning deficit was then explained by positing uncontrollable stimulation saturates glutamate-dependent plasticity [24,26]. While this reasoning seems sound given the standard view, derived from studies of hippocampal LTP, NMDAR's within the spinal cord may act in a distinct manner. For example, it is generally assumed that NMDAR antagonists affect the induction, but not the maintenance, of amygdala-dependent plasticity [27]. However, administration of an NMDAR antagonist disrupts both the induction and maintenance of instrumental learning and C-fiber dependent windup [25,28]. The latter suggests that both effects may depend on slow NMDAR-mediated synaptic potentials and the sensitization of the NMDAR [28]. The presumption AMPA-signaling plays a pivotal role is also called into question by studies linking spinal LTP to C-fiber activity and the neurokinin (substance P) receptor [29,30]. A final complexity stems from the observation that approximately a third of the NMDARs within the spinal cord are presynaptic, which suggests that the receptor may also regulate transmitter release [31].

Thus, while our theorizing has assumed that the AMPAR plays a central role in spinal plasticity, we lack any direct evidence that the AMPAR is involved and current data implicate other neural signals. We address this issue by testing the impact of the AMPAR antagonist CNQX on instrumental learning (Experiments 1 and 2) and the induction of the learning deficit (Experiment 3). We also examine whether pretreatment with the agonist AMPA has an acute (Experiment 4), and/or long-term (Experiment 5), effect on instrumental learning. We hypothesize that the AMPAR signal is a necessary component of spinal instrumental learning.

2. Materials and methods

2.1. Animals

All subjects, male Sprague-Dawley rats (100–120 days old; 300–450 g), were obtained from Harlan Laboratories (Houston, TX). Subjects were individually housed with water and food *ad libitum*, and maintained on a 12-h light–dark cycle. Behavioral testing and surgeries were performed during the light portion of the cycle. All experiments were carried out in accordance with the NIH standards for the care and use of laboratory animals (NIH publications No. 80-23), and were approved by the University Laboratory Animal Care Committee at Texas A&M University. Every effort was made to minimize suffering and limit the number of animals used.

2.2. Surgery

The surgical procedure consisted of a complete transection of the spinal cord at the second thoracic vertebra (T2). Anesthesia was induced using a concentration of 5% isoflurane, which was maintained at a 2% concentration during surgery. The T2 vertebra was located and an incision was made rostral–caudal to the vertebra. A laminectomy was performed to expose the cord rostral of T2. Heat cautery was used to transect the exposed cord and the cavity formed was filled with gelfoam (Harvard Apparatus, Holliston, MA). A 25 cm catheter (PE-10, VWR International Bristol, CT), held rigid with a 0.9 mm stainless steel wire (Small Parts Inc., Miami Lakes, FL), was inserted 9 cm into the subarachnoid space on the dorsal surface of the spinal cord [32]. Following insertion, the wire was gently removed and the exposed tubing adhered to the skin externally using cyanoacrylate. Following testing catheter placement was verified during a post-mortem inspection. The incision was closed using Michel Clips (Fine Science Tools, Foster City, CA). All subjects received injections of 0.9% saline (2.5 ml i.p.) immediately following surgery and the subject's legs were taped using a piece of porous tape (ortholetic 1.3 cm width). The tape maintained the legs in a secure natural position and was removed prior to testing. Subjects were then allowed to recover for 18–24 h before testing in a temperature-controlled room (25–27° C) with free access to food and water. During the recovery period, subjects were able to move about within their home cage (via their forelimbs). Bladders were expressed twice daily and immediately before any behavioral procedures were conducted. When behavioral testing was complete, all animals were euthanized with a lethal dose of pentobarbital (100 mg/kg).

The surgical transections were verified by (1) observing behavior during the recovery period to confirm complete paralysis and a lack of vocalization to leg shock, (2) visual inspection of the transection site during surgery, and (3) post-mortem cord examination in a random sample of subjects.

2.3. Instrumental learning apparatus and procedure

Instrumental training/testing was conducted while rats were loosely restrained in tubes (23.5 cm [length] × 8 cm [internal diameter]). Two slots in the tube (5.6 cm [*l*] × 1.8 cm [*w*]), 4 cm apart, 1.5 cm from the end of the tube, allowed both hind legs to hang freely. To minimize the effects of upper body movement on leg position, a wire belt was used to secure the rat's trunk within the tube. Hind limbs were shaved and marked for electrode placement prior to testing. A wire electrode was then inserted through the skin over the distal portion of the tibialis anterior (1.5 cm from the plantar surface of the foot), and one lead from a BRS/LVE (Laurel, MD, USA) constant current (60 Hz, AC) shock generator (Model SG-903) was attached to this wire. A 7-cm long, 0.46-mm diameter stainless steel contact electrode was secured to the foot between the second and third digits with a piece of porous tape. The last 2.5 cm of the electrode was insulated from the foot with heat shrink tubing. A fine wire (0.01 mm^2 [36 AWG], 20 cm) attached to the end of the contact electrode extended from the rear of the foot and was connected to a digital input monitored by a Macintosh computer. A plastic rectangular dish (11.5 cm [*w*] × 19 cm [*l*] × 5 cm [*d*]) containing an NaCl solution was placed approximately 7.5 cm below the restraining tube. A drop of soap was added to the solution to reduce surface tension. A ground wire was connected to a 1-mm wide stainless steel rod, which was placed in the solution. The shock generator was set to deliver a 0.4-mA shock, and the proximal portion of the tibialis anterior (approximately 1.7 cm proximal to the wire electrode) was probed with a 2.5-cm stainless steel pin attached to a shock lead to find a robust flexion response. The pin was then inserted 0.4 cm into the muscle.

To standardize response parameters across subjects, we adjusted the intensity of electrical shock necessary to generate a 0.4-N flexion force for each subject. Flexion force was measured by attaching a monofilament plastic line ("4 lb test" Stren, DuPont, Wilmington, DE, USA) to the rat's foot immediately behind the plantar protuberance. The 40-cm length of line was threaded through an eyelet attached to the apparatus directly under the paw, 16 cm beneath the base of the tube. The end of the line was attached to a strain gauge (Fort-1000, World Precision Instruments, New Haven, CT, USA) fastened to a ring stand. After the line was connected to the rat's paw, the ring stand was positioned so that the line was taut, just barely registering on the gauge. The strain gauge was calibrated by determining the relationship between voltage and force in Newtons. These data revealed a linear relation, which allowed us to convert voltage to force.

To minimize lateral leg movements, a 20-cm piece of porous tape was wrapped around the leg and attached to a bar extending across the apparatus directly under the front panel of the restraining tube. The tape was adjusted so that it was taut enough to slightly extend the knee. Finally, three short (0.15 s) shock pulses, given at the intensity previously determined to elicit a 0.4-N flexion response, were applied and the level of the salt solution was adjusted so that the tip of the contact electrode (attached to the rat's foot) was submerged 4 mm below the surface. Recognizing that some experimental manipulations could potentially affect the subject's capacity to perform the target (flexion) response, we recorded two measures of baseline behavioral reactivity: the shock intensity required to elicit a flexion force of 0.4 N and the