

Trapping-induced changes in expression of the *N*-methyl-D-aspartate receptor in the hippocampus of snowshoe hares

Rudy Boonstra, Norio Takagi¹, Nankie Bissoon, Shilpa Vij, James W. Gurd^{2,*}

Centre for the Neurobiology of Stress, Division of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Toronto, Ontario, M1C 1A4 Canada

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Abstract

Live-trapping of animals in natural populations is one of the main ways to determine population processes. We examined the effects of live-trapping on the expression of *N*-methyl-D-aspartate (NMDA) receptor subunits in the hippocampus of snowshoe hares. Snowshoe hares were obtained either with or without the stress of live-trapping. The CA1, CA3 and dentate gyrus were dissected and analyzed for the presence of NMDA receptor subunits. Trapping resulted in a significant reduction of NMDA receptor 1 (NR1) in each of the regions examined but did not affect the levels of either NMDA receptor 2A or B (NR2A or NR2B). Co-immunoprecipitation analysis showed that the association between NR1 and NR2A was decreased in the trapped animals. These results suggest that stress associated with the trapping experience may adversely affect the structure and/or function of the NMDA receptor. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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The hippocampus plays a central role in regulating the stress response to maintain homeostasis in mammals [18]. However, both short-term and chronic stress have direct effects on the function and morphology of hippocampal neurons [14]. *N*-methyl-D-aspartate (NMDA) receptors, the main excitatory receptors in the brain, consist of heteromeric assemblies of NR1 and NR2A–D subunits and are present at a high density in the cerebral cortex and hippocampus. Acute and chronic stressors have direct effects on NMDA receptors [1,13].

In studies of natural populations, live-trapping is often used for the capture of animals to obtain information on population processes. The implicit assumption is that

these methods do not compromise survival and reproduction and hence do not ultimately result in a biased picture of population processes [5,6,9]. However, even though live-trapping is known to be stressful to the animal, the physiological impacts of the trapping procedure, particularly at the neurological level, remain to be determined.

Snowshoe hares are the dominant herbivore throughout the boreal forests of North America [12]. Hares are sensitive to a variety of stressors, including the threat of being killed by predators during periods of population decline [4] and the stress of live-trapping [3]. To begin to identify possible short-term effects of trapping-induced stress on the hippocampus, we investigated the expression of the NMDA receptor in snowshoe hares that had been captured with or without the stress of live-trapping.

All procedures were approved by the Animal Care Committee of the University of Toronto and were in accordance with the Canadian Council on Animal Care. Animals were collected in late February in the boreal forest near the Arctic Institute Base at Kluane Lake in the southern Yukon, Canada. Two groups of male snowshoe hares were collected near the Alaska Highway. (i) Baseline hares, collected without the stress of capture and handling which is involved in

* Corresponding author. Tel.: +1-416-287-7410; fax: +1-416-287-7649.

E-mail address: gurd@utsc.utoronto.ca (J.W. Gurd).

¹ Current address: School of Pharmacy, Department of Pharmacology, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03 Japan.

² Dr Gurd will be at the Discipline of Medical Biochemistry, Faculty of Medicine, University of Newcastle, Callaghan, NSW 2308, Australia (Tel.: +61-02-4921-7891; fax: +61-02-4921-5444, e-mail address: james.gurd@newcastle.edu) until June of 2002. Correspondence should be addressed to him at this address.

live-trapping, were collected between 18:00 and 22:00 h by a gun shot (22 caliber) to the neck. The head was removed immediately, all fur and the lower jaw were cut off (within 1 min), and the remainder of the skull was immersed in an isopentane/dry ice bath (approximately -40°C). The frozen skulls were stored at -80°C until analysis. (ii) Live-trapped hares were caught by the methods described in [11]. Animals were trapped between 16:00 h in the evening and 07:30 h the next morning. Overnight, the temperature dropped to -22°C . The exact time at which a hare entered a trap was unknown. On removal from the trap, animals were immediately sexed, weighed, and injected intracardially with T61 euthanizing solution (Hoechst Roussel Vet, Regina, Saskatchewan). The heads were removed and treated as above.

For biochemical analysis, heads were thawed to approximately 0°C , brains rapidly removed and hippocampi dissected to obtain the CA1, CA3, and dentate gyrus (DG) subfields. Dissected tissue was rapidly frozen on dry ice. Tissue samples were homogenized in 0.32 M sucrose containing 0.1×10^{-3} M sodium orthovanadate, 0.1×10^{-3} M phenylmethylsulfonyl fluoride (PMSF), 0.02 M *p*-nitrophenyl phosphate, 0.02 M glycerophosphate, and 5 $\mu\text{g/ml}$ each of antipain, aprotinin, and leupeptin. Proteins were solubilized by the addition of an equal volume of 2% sodium dodecyl sulfate (SDS) containing 10% β -mercaptoethanol and heating at 100°C for 5 min. Proteins were separated by SDS–polyacrylamide gel electrophoresis and then either visualized by Coomassie blue staining or transferred to nitrocellulose. Protein blots were incubated with antibodies specific for NR1 (Pharmin-gen-Transduction Laboratories, Lexington, KY), NR2A [20], or NR2B (Clone 13, Pharmin-gen-Transduction Laboratories) subunits of the NMDA receptor and immunoreactive proteins detected by enhanced chemiluminescence (ECL; Pierce, Rockford, IL). Developed blots were scanned and quantified using a BioRad GS700 imaging densitometer. Care was taken to ensure that the ECL signal was within the proportional range. Co-immunoprecipitation experiments were performed as described [20]. Briefly, total homogenates were extracted with 0.05 M Tris–HCl buffer, pH 7.4, containing 1% Nonidet[®] P40, 0.5% sodium deoxycholate (DOC), 0.1% SDS, 0.15 M NaCl, 1 mM EDTA, 0.1 mM PMSF, and 5 $\mu\text{g/ml}$ each of antipain, aprotinin, and leupeptin. Extracts were immunoprecipitated with anti-NR2A antibodies and immunoprecipitates analyzed by immunoblotting with anti-NR1 and anti-NR2A antibodies. Statistical analysis was done using an analysis of variance and the Tukey Honest Significant Difference post-hoc test (Fig. 2) or the Student's *t*-test (Fig. 3).

We initially compared total protein profiles, as detected by staining with Coomassie blue, of hippocampal regions from baseline and live-trapped animals. The results presented in Fig. 1 show that there was no effect of the method of capture on the overall protein profile of the CA1 region. Similar results were obtained for the CA3 and DG subfields (not shown).

Individual NMDA receptor subunits were assessed by immunoblotting tissue homogenates with subunit-specific antibodies. Preliminary experiments confirmed that the antibodies reacted with the expected proteins of M_r 116,000 (NR1) and 180,000 (NR2A and NR2B) in both rat and snowshoe hare hippocampal homogenates (Fig. 2A). The effects of trapping on NR1, NR2A and NR2B in the CA1, CA3, and DG subfields are shown in Fig. 2B–E. NR1 levels in the trapped animals were significantly decreased relative to baseline values in all three regions. In contrast, there were no changes in NR2A or NR2B.

To determine if the reduction in NR1 levels affected the assembly of heteromeric NMDA receptors, membranes were extracted with sodium deoxycholate under conditions that do not disrupt interactions between receptor subunits. Extracts were immunoprecipitated using anti-NR2A antibodies and the immunoprecipitates were analyzed for the presence of NR2A and NR1 subunits. The results show that in immunoprecipitates from trapped hares there was a trend towards a decrease in the ratio of NR1 to NR2A as compared with the baseline group (Fig. 3), although the difference failed to reach statistical significance ($P = 0.06$).

The main finding of the present study is that live-trapping resulted in the preferential loss of NR1 subunits of the NMDA receptor in the hippocampus of male snowshoe hares. The simplest interpretation of these results is that the stress of trapping induces accelerated degradation of NR1 subunits. One possible explanation for these findings may be that stress-induced increases in cortisol in the serum of trapped hares [3] induce an increase in intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$) [10]. Increases in $[\text{Ca}^{2+}]_i$ may in turn activate the calcium-dependent protease calpain which then degrades NR1 [2]. In contrast to the present findings, a single, 24 h immobilization stress resulted in increased expression of NR1 and NR2B mRNA levels in

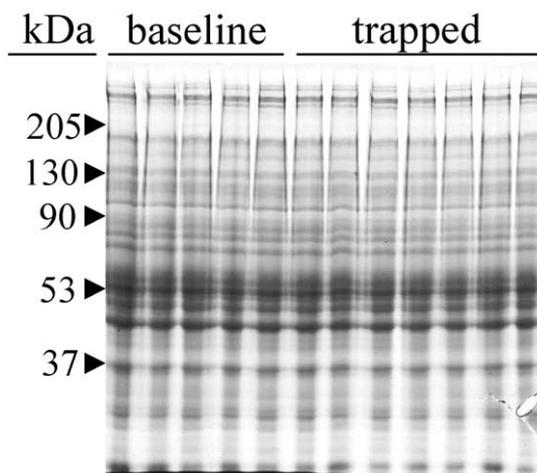


Fig. 1. The effect of trapping on the protein composition of the CA1 region of snowshoe hares. Total homogenates of the CA1 region from baseline or trapped animals were separated on 8% polyacrylamide gels and protein stained with Coomassie blue. Each lane represents a different animal.

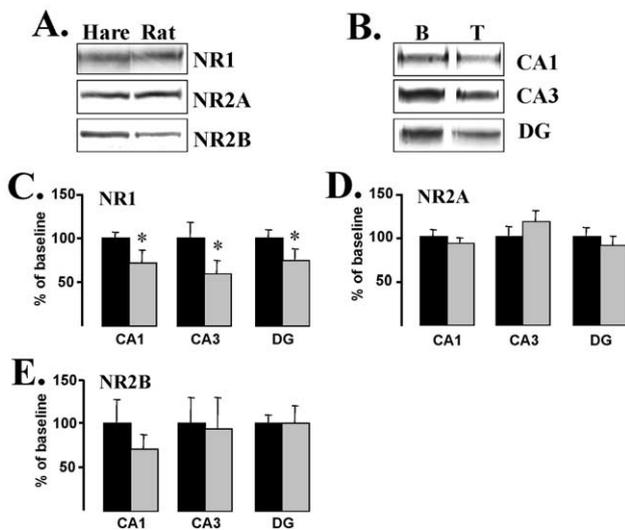


Fig. 2. The effect of live-trapping of snowshoe hares on the expression of NMDA receptor subunits. (A) Hippocampal homogenates from hare and rat were analyzed by immunoblotting with antibodies against NR1, NR2A and NR2B. (B) Representative immunoblots of NR1 in CA1, CA3 and DG subfields of the hippocampus from baseline (B) and trapped (T) hares. (C–E) Immunoblots for NR1, NR2A and NR2B were scanned and values for trapped hares expressed as a percentage of baseline values. Total homogenates from the CA1, CA3 and DG regions of the hippocampus from baseline and live-trapped hares were analyzed by immunoblotting with antibodies against individual NMDA receptor subunits and immunoreactive proteins detected by ECL. Results presented in (C–E) represent the means \pm SD ($n = 5$ for baseline and $n = 7$ for trapped). Significantly different from baseline, $*P < 0.01$. Black bars, baseline; gray bars, live-trapped.

hippocampal CA1 and CA3 pyramidal cells of the rat [1]. The difference between these findings and the current results may reflect differences in the nature of the stress, species variation or failure of an increase in mRNA expression to result in increased protein levels, either because of reduced translation rates or increased proteolysis in the stressed animals.

NR2 subunits exist predominantly (>90%) as assembled heteromeric NMDA receptor complexes expressed at the cell surface. NR1, in contrast, is present both as a component of assembled receptors on the cell surface and in an intracellular pool that represents between 40 and 60% of the total, and is not co-assembled with NR2 [7]. Turnover studies have indicated that non-assembled and assembled pools of NR1 are degraded with half-lives of approximately 2 and 34 h, respectively [8]. Under the present conditions, most of the decrease in NR1 is likely associated with the more rapidly turning over non-assembled, intracellular pool. The small decrease in association between NR1 and NR2A in the trapped animals indicates, however, that trapping may also result in the degradation or dissociation of assembled, heteromeric receptors.

New, heteromeric NMDA receptors may be delivered to the cell surface in response to experience-dependent synap-

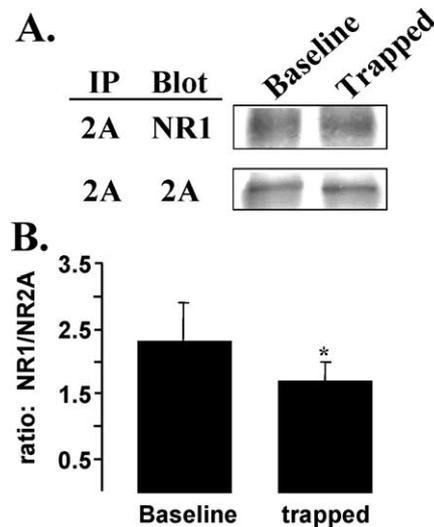


Fig. 3. Co-immunoprecipitation of NR1 and NR2A from baseline and live-trapped snowshoe hares. DOC extracts of the CA3 region were immunoprecipitated using anti-NR2A antibodies and immunoprecipitates analyzed for the presence of NR1 and NR2A. (A) Representative immunoblot of NR1 and NR2A present in the immunoprecipitates obtained from baseline and trapped animals. (B) Immunoblots were scanned and the ratio of immunoreactivity for NR1 to immunoreactivity for NR2A in the immunoprecipitate was determined. Results are expressed as the average \pm SD ($n = 5$ or $n = 7$ different animals for the baseline and trapped conditions, respectively). $*P = 0.06$.

tic activation or long-term decreases in synaptic activity [16,17]. This process requires, and may be regulated by the availability of NR1 subunits [15,19]. Stress-induced decreases in the intracellular pool of NR1 might affect the neuron's ability to insert new NMDA receptors into the synaptic membrane, thereby compromising adaptability to a subsequent stressful challenge, at least in the short-term, before the depleted pool of NR1 has had time to be replenished.

In summary, the present study shows that live-trapping can result in rapid changes in the components of the NMDA receptor that may alter receptor functionality and/or the ability to modulate receptor density in response to subsequent challenges. These changes may have behavioral consequences and introduce unwanted biases in studies of natural populations.

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