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BRIEF REPORT

Emergence Neophobia Correlates with Hippocampal and Cortical Glutamate Receptor Binding in Rats

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Previous work from our laboratory indicated that emergence neophobia is highly correlated with perforant path long-term potentiation (LTP) in rats. In the present study, we examined the relationship between hippocampal and cortical α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors and emergence behavior in rats. Emergence neophobia was assessed in an exploratory task that provided a choice between a novel alley and a familiar nest box. Quantitative autoradiography using radiolabeled ligands specific for the AMPA subclass of glutamate receptors was performed on frozen brain sections. Both [^{3}H]AMPA and [^{3}H]CNQX (6-cyano-7-nitro-[^{3}H]quinoxaline-2,3-dione, an AMPA receptor antagonist) binding in the dentate gyrus (*stratum moleculare*), hippocampal area CA1 (*stratum radiatum*), and the parietal cortex overlying the hippocampus were significantly correlated with emergence behavior. The correlations indicated that neophobic rats, which had longer latencies to enter the novel alley, made fewer entries into the alley, and spent less time in the novel alley during a 10-min test than their neophilic counterparts, had higher levels of AMPA receptor binding. These results suggest that individual differences in specific hippocampal AMPA receptors reflect variability in a specific class of hippocampal-dependent behaviors.

Long-term potentiation (LTP) in the mammalian hippocampus is an enduring form of synaptic plasticity thought to be involved in associative learning and memory processes (Bliss & Collingridge, 1993; Teyler & DiScenna, 1984). In particular, a number of studies have suggested that hippocampal LTP is involved in encoding spatial and contextual information (Kim, DeCola, Landeira-Fernandez, & Fanselow, 1991; Maren, DeCola, Swain, Fanselow, & Thompson, 1994; Morris, Anderson, Lynch, & Baudry, 1986). We recently demonstrated that the magnitude of perforant path-dentate gyrus LTP in anesthetized rats is highly correlated with quantitative parameters for emergence neophobia in an exploratory choice task that is sensitive to hippocampal lesions (Maren, Patel, Thompson, & Mitchell, 1993a; Mitchell, Maren, & Hwang, 1993). Specifically, neophobic rats, which were reluctant to enter the novel compartment of an emergence apparatus from a familiar nest box, exhibited both a lower threshold for the induction of perforant path LTP and a greater magnitude of asymptotic LTP than their neophilic counterparts. Thus, individual differences in LTP appear to be involved in the variability of nonassociative processes, such as habituation, which mediate neophobic responses to novel stimuli (Mitchell, Kirschbaum, & Perry, 1975).

The mechanisms of LTP induction in the hippocampus are now well-understood. Specifically, they involve the activation of the *N*-methyl-D-aspartate (NMDA) subclass of glutamate receptors under conditions of strong postsynaptic depolarization and presynaptic neurotransmitter release (Bliss & Collingridge, 1993). Although there is considerable debate concerning the precise nature of the cellular mechanisms of LTP expression, it is becoming increasingly apparent that LTP is maintained by a modification of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors (Davies, Lester, Reymann, & Collingridge, 1989; Manabe, Renner, & Nicoll, 1992; Maren, Baudry,

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& Thompson, 1992; Shahi & Baudry, 1992; Staubli, Kessler, & Lynch, 1990), a subclass of postsynaptic glutamate receptors that mediates fast synaptic transmission at excitatory hippocampal synapses (Collingridge, Kehl, & McLennan, 1983; Petralia & Wenthold, 1992). In support of this view, we have recently shown that perforant path LTP induction in anesthetized rats is associated with an NMDA receptor-dependent increase in the number of postsynaptic hippocampal AMPA receptors (Maren, Tocco, Standley, Baudry, & Thompson, 1993b; Tocco, Maren, Shors, Baudry, & Thompson, 1992). The involvement of AMPA receptors in the expression of hippocampal LTP suggests that the relationship between emergence neophobia and perforant path LTP may be reflected by individual differences in hippocampal AMPA receptor populations. In the present experiment, we addressed this issue by using quantitative autoradiography to examine the binding of [^{3}H]AMPA (an AMPA receptor agonist) and 6-cyano-7-nitro-[^{3}H]quinoxaline-2,3-dione ([^{3}H]CNQX, an AMPA receptor antagonist) to frozen brain sections obtained from rats tested in an emergence apparatus.

Ten adult male Sprague–Dawley rats (65 days of age, Simonsen Laboratories) were individually housed in an air-conditioned room on a 12:12-h light:dark cycle (lights on at 0600 h) for 10 days before the beginning of the experiment. Each animal occupied a standard stainless steel isolation cage suspended over a stainless steel tray containing a layer of wood chips. Food (Purina Lab Chow) and tap water were continuously available. The rats were transported to and from the emergence apparatus, which was located in an adjacent room, in their home cages. The emergence apparatus is described in detail in Maren et al. (1993a). Briefly, it consisted of a set of four parallel, independent wooden alleys painted grey and covered with hardware cloth. Four identical nest boxes with hinged hardware cloth tops were separated from the alleys by guillotine doors; each nest box permitted access to one of the adjacent alleys. The nest boxes and doors were painted black and a black wooden shelf mounted above the nest boxes partially shaded the back portion of each box and supported a digital clock and animal identification cards. Each nest box was ventilated through a common manifold by an exhaust fan mounted outside the apparatus; the fan also provided masking noise. Food and wood-chip bedding were supplied on the floor of each nest box and water was available from an externally mounted glass bottle with a stainless steel spout that projected through the back wall of each nest box. The entire apparatus was positioned on the floor of a small enclosure, which had a rectangular opening in the center of the top and a sliding door along one side. Indirect lighting was provided by four fluorescent tubes (20 W) mounted vertically on the walls of the enclosure; two behind and two in front of the apparatus. A monitor and videocassette recorder were positioned on a cart outside the enclosure and a video camera mounted on scaffolding above the apparatus permitted a clear view of the entire apparatus.

Twenty-four hours before each emergence test (1800 h) a set of four rats was placed in the nest boxes adjacent to the alleys. Food and water were freely available in the nest boxes and the lighting cycle was the same as that in the vivarium where the rats were housed. At the beginning of each test the enclosure lights were reset to remain on during the test, the camera was turned on, and the guillotine doors were removed. The rats were permitted to enter and explore the novel alley adjacent to their nest box for 10 min. Following the test, the rats were returned to their home cages. After each set of rats had been tested, the soiled litter and uneaten food were discarded and the entire apparatus thoroughly cleaned and washed with a mild detergent solution. The water bottles were cleaned and refilled and each nest box was resupplied with fresh food and wood-chip bedding. Subsequent sets of rats were housed in the nest boxes on the following day and the procedure repeated until all sets had been run. The videotaped emergence tests were scored for each animal's latency to enter the alley, number of alley entries, and duration of time spent in the alley during the 10-min test.

One week following emergence testing, the rats were decapitated and their brains were rapidly removed, frozen in -20°C isopentane, and stored at -70°C until sectioning. Frozen coronal sections (10 μm thick) were used for the ligand binding experiments. [^{3}H]AMPA binding (sp act = 55.6 Ci/mmol; NEN) was performed as described in Maren et al. (1993b). After equilibration to room temperature, slides were preincubated in Tris–acetate buffer (100 m*M*, pH 7.2–7.4) containing 100 m*M* potassium thiocyanate and 100 μM EGTA for 30 min at 35°C. Sections were then incubated at 0–4°C for 45 min in the same buffer containing 120 n*M* [^{3}H]AMPA with or without 1 m*M* quisqualate (to determine nonspecific binding). Slides were rinsed at 0–4°C twice (10 s per rinse) in 100% buffer and once (5 s) in 50% buffer, dipped three times in distilled water, and rapidly dried under a stream of warm air. [^{3}H]CNQX (29.2 Ci/mmol; NEN) binding was

TABLE 1
Pearson Correlation Coefficients for Glutamate Receptor Binding and Emergence Neophobia

Region	Latency	Duration	Entries
	AMPA		
CA1rad	0.61	−0.41	−0.74*
CA3rad	0.52	−0.25	−0.62
DG	0.70*	−0.46	−0.73*
CTX	0.46	−0.23	−0.45
THAL	0.40	−0.16	−0.59
	CNQX		
CA1rad	0.60	−0.50	−0.76*
CA3rad	0.61	−0.58	−0.45
DG	0.79*	−0.71*	−0.76*
CTX	0.85**	−0.83**	−0.82**
THAL	0.74*	−0.60	−0.84**

Note. The critical two-tailed t values for these levels of significance were $t(8) = 1.9$ and 2.9, respectively.
* $p < .05$.
** $p < .01$.

performed using the same procedures as for [^{3}H]AMPA binding except that 100 μM glycine was included in the incubation to inhibit [^{3}H]CNQX binding to the glycine site associated with the NMDA receptor and the buffer did not contain potassium thiocyanate Each ligand-binding experiment was performed on at least four frontal sections from each animal. Nonspecific binding for both ligands represented <10% of the total binding.

Following ligand binding, autoradiographic film (Hyperfilm, Amersham) was pressed against the tissue sections on the slides and against radioactive standards (ARC). After exposure for 10–15 days, the films were developed for 3–5 min at room temperature in Kodak GBX developer and fixer. Autoradiographs were analyzed with an image analysis system (BRAIN, Drexel University) to determine the amount of ligand binding in *stratum radiatum* of hippocampal areas CA1 and CA3 (CA1rad, CA3rad), *stratum moleculare* of the dentate gyrus (DG), cerebral cortex overlying the dorsal hippocampus (CTX, layers I–VI, corresponding to parietal cortex), and thalamus (THAL). Optical density measurements made in each hemisphere were averaged across the sections from each animal and converted to specifically bound ligand per milligram of protein using autoradiographic standards.

Pearson correlation coefficients, shown in Table 1, were calculated for specific [^{3}H]AMPA and [^{3}H]CNQX binding in each brain region and the three measures of emergence behavior: emergence latency, duration, and entries. In general, [^{3}H]CNQX binding was more highly correlated with emergence behavior than was [^{3}H]AMPA binding. For example, the highest correlation between emergence behavior and AMPA receptor binding was found between [^{3}H]CNQX binding in the cerebral cortex and emergence latency ($r = 0.85$, $p < .01$; Fig. 1). Moreover, [^{3}H]CNQX binding in the dentate gyrus and cerebral cortex was significantly correlated with all three measures of emergence behavior. In contrast, only [^{3}H]AMPA binding in area CA1 and the dentate gyrus was significantly correlated with emergence behavior. In the dentate gyrus, [^{3}H]AMPA binding was significantly correlated with both emergence latency and entries, and in area CA1, [^{3}H]AMPA binding was correlated with emergence entries. For both ligands, the correlations indicated that nonemerging, neophobic rats exhibited more [^{3}H]AMPA and [^{3}H]CNQX binding than their emerging, neophilic counterparts. It is noteworthy that there were no significant correlations between AMPA receptor binding in hippocampal area CA3 and neophobic behavior.

As is apparent from Table 1, there were marked differences in the regional specificity of the correlations between [^{3}H]AMPA and [^{3}H]CNQX binding and emergence behavior. For [^{3}H]AMPA binding, significant correlations were restricted to hippocampal subfields, whereas [^{3}H]CNQX binding in both hippocampal and extra-hippocampal regions correlated significantly with emergence behavior. The basis for the different regional specificity of these correlations is not known as there was a high correlation between [^{3}H]AMPA and [^{3}H]CNQX in all of the brain regions examined (data not shown). However, it is of interest that a number of

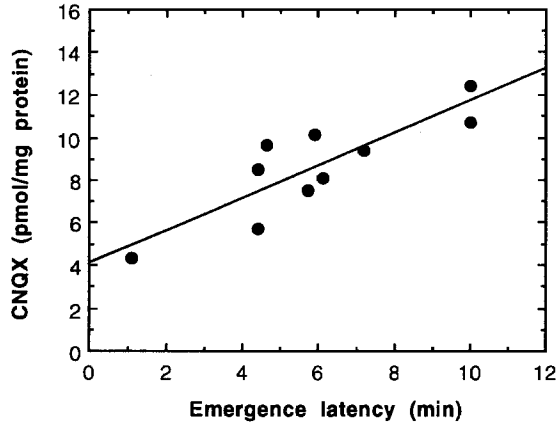

FIG. 1. Linear correlation between [^{3}H]CNQX binding (pmol/mg protein) in the parietal cortex overlying the dorsal hippocampus and emergence latency (min). The Pearson correlation coefficient ($r = 0.85$) for the regression line was highly significant ($p < .01$).

[^{3}H]AMPA binding sites are not associated with postsynaptic receptors (i.e., some are localized to subcellular sites), whereas [^{3}H]CNQX binding sites are localized primarily to synapses (Standley et al., submitted for publication). This may account for the higher and more numerous correlations of [^{3}H]CNQX with emergence behavior. Nonetheless, the fact that both [^{3}H]AMPA and [^{3}H]CNQX binding in the dentate gyrus and hippocampal area CA1 correlated highly with emergence behavior suggests the existence of a close relationship between hippocampal AMPA receptors and emergence behavior, although cortical receptors may also play an important role in regulating this behavior.

Taken together, these data raise some interesting questions concerning the relationship of AMPA receptors, LTP, and emergence neophobia. Namely, are individual differences in AMPA receptors the cause of individual differences in LTP and, in turn, neophobia, or are they an effect of differential LTP induced in neophobic and neophilic rats by the behavioral procedures used to assess neophobia? This is a difficult issue to resolve with the present data; nonetheless we tend to favor the second possibility because of the role of AMPA receptors in the expression of LTP. In this view, individual differences in AMPA receptor populations reflect, but do not cause, individual variability in already-induced or "endogenous" LTP. The source of this endogenous LTP may be the contextual cues of the emergence apparatus, but could also be various stimuli in the home cage environment, for instance. In either case, LTP is more readily induced in neophobic compared to neophilic rats (Maren et al., 1993a), and this differential induction of LTP is reflected by greater AMPA binding in neophobic animals. Interestingly, the correlations between AMPA binding and emergence behavior were restricted to brain regions where LTP is thought to be expressed as a postsynaptic change; AMPA binding in in hippocampal area CA3, where LTP is expressed by an increase in presynaptic neurotransmitter release (Staubli & Lynch, 1990; Zalutsky & Nicoll, 1991), did not correlate with emergence behavior.

If the individual differences in AMPA receptor binding observed in the present study are not the cause of the variability in LTP and emergence and neophobia reported in Maren et al. (1993a), then what is? One possibility is that the differential induction of perforant path LTP in neophobic and neophilic rats is related in some way to NMDA receptors. It may be the case, for instance, that neophobic rats possess a greater number of NMDA receptors and therefore exhibit a greater potential for LTP induction. In support of this hypothesis, a recent report indicates that the differential thresholds of inducing LTP in slow versus fast shuttlebox learners (i.e., rats that would also be expected to show differences in neophobia) is related to the number of hippocampal NMDA receptors (Keller, Borghese, Carrer, & Ramirez, 1992). In addition, the binding of glutamate to NMDA receptors has been shown to be correlated with learning ability in the radial arm maze (Wenk, Grey, Ingram, Spangler, & Olton, 1989).

In combination with our earlier reports, the present experiments suggest that LTP and the AMPA receptors which express it are significantly involved in the mediation of neophobic behavior. In the hippocampus, LTP may serve as a mechanism to represent novel contextual stimuli (e.g., Maren et al., 1994), and this may be critical for organizing behavioral responses, such as neophilic approach and neophobic avoidance, to novel environments. Moreover, because neophobia is regulated by habituation (e.g., Mitchell et al., 1975), we would also suggest that hippocampal LTP is involved in the nonassociative processes that underlie neophobic behavior. These hypotheses are consistent with the growing body of literature implicating NMDA receptor-dependent processes such as LTP in both associative *and* nonassociative learning tasks (e.g., Izquierdo, da Cunha, Rosat, Jerusalinsky, Ferreira, & Medina, 1992). Finally, the relationship between neocortical AMPA receptors and emergence neophobia suggests that cortical LTP mechanisms may be important for this behavior, possibly mediating the permanent storage of contextual information acquired in the hippocampus.

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