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N-Methyl-Aspartic Acid Lesions of the Arcuate Nucleus in Adult C57BL/6J Mice: A New Model for Age-Related Lengthening of the Estrous Cycle

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Abstract. We report a new effect of the excitotoxin N-methyl-aspartic acid (NMA) on adult mice. Besides confirming cell loss in the arcuate nucleus of animals treated as adults, we also observed lengthened estrous cycles. Cycling female C57BL/6J mice were treated with subcutaneous injections of NMA and estrous cycles monitored for 30 days. NMA treatment lengthened average estrous cycle length by 1 day, to 5.6 days. The elongated cycles were due to an increase in the frequency of 5- and 6-day cycles at the expense of 4-day cycles. This effect was observed in mice aged 4 and 7 months; however, 10-month-old mice had irregular cycles and a higher mortality rate following NMA, which precluded cycle analysis. NMA caused a region-specific 30–45% cell loss in the rostral portions of the arcuate nucleus; no cell loss was detected in the adjacent ventromedial nucleus. Possible neuronal substates for NMA lesions, as analyzed by in vitro receptor autoradiography for glutamate receptor subtypes, include a high density of kainate receptors in the arcuate nucleus; there was little specific binding of ligands to NMDA and quisqualate receptors. Consistent with the regional pattern of cell loss, little specific binding of any glutamatergic ligand was observed in the VMN. NMA caused weight gain in all age groups. Serum LH and PRL were unaffected by NMA. The transition from 4-day to 5- and 6-day estrous cycles produced by NMA treatment mimics the early age-related changes in estrous cycle patterns in rodents. This new model will be useful in analyzing the contributions of neuroendocrine changes in the arcuate nucleus to reproductive senescence.

An early stage of reproductive senescence in C57BL/6J mice and many other laboratory rodent genotypes is the lengthening of estrous cycles, characterized by a loss of the number of 4-day cycles and an increase in the number of 5- and 6-day cycles [12, 20, 21]. The transition to longer cycles precedes the cessation of cyclicity and is due in part to neuroendocrine impairments [4]. A model for the neuroendocrine aspects of *late*-onset acyclicity during reproductive senescence is the permanent acyclicity induced by chronic exposure of young rodents to moderate levels of estrogen [3, 10, 15, 30]. However, there are no rodent models for *early* age changes in estrous cycle lengthening. The present approach drew from reports that neonates treated with a neuronal excitotoxin, monosodium glutamate, subsequently exhibit sporadic or elongated estrous cycles [19, 33]. Monosodium glutamate causes the loss of several neurotransmitters intrinsic to the arcuate nucleus (e.g. dopaminergic, gabaergic, opioidergic) [reviewed in 26] and which are implicated in the regulation of LHRH [2]. Monosodium glutamate is typically administered to neonates [2–10] days postnatal) at a time when hypothalamic synapses are not fully developed [13]. During maturation, considerable remodelling may occur in the hypothalamus to compensate for the marked depletion (up to 80% [26]) of intrinsic arcuate neurons.

Mice lesioned as adults would provide a more suitable model for evaluating the contribution of the arcuate nucleus to neuroendocrine aging. Monosodium glutamate is too toxic and minimally effective in adults, but peripheral administration of N-methyl-*D,L*-aspartic acid (NMA), a related neuronal excitotoxin, produces a partial lesion (15–30%) of the arcuate nucleus in 25-day-old mice [26]. The neuroendocrine changes accompanying these partial lesions of the arcuate nucleus have not been characterized in sexually mature mice treated with NMA. Therefore, we treated mature C57BL/6J female mice of different ages with NMA and evaluated estrous cycle parameters as well as other neuroendocrine indices. In addition, we also investigated whether estrogen could potentiate NMA excitotoxicity in the hypothalamus, as observed with corticosterone and kainate toxicity in the hippocampus [29]. The major findings indicate that subcutaneous injections of NMA effectively lesion arcuate neurons in young sexually mature mice. Animals bearing partial lesions of the arcuate nucleus also displayed

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changes in estrous cycles characteristic of older mice and suggest that dysfunction of arcuate neurons susceptible to NMA may also participate in early phases of reproductive senescence.

Materials and Methods

Animals. Female C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, Me.) as virgins (2–3 months) or as retired breeders (7 months) and singly housed in a tempertaure- and humidity-controlled vivarium. Mice were maintained under a 12-hour light: dark cycle with unlimited access to food and water.

Estrous Cycle Determination. Estrous cycles were monitored by changes in vaginal cytology. Daily vaginal lavages were stained with Giemsa stain and cell types determined by light microscopy [see 21 for detailed description of estrous cycle analyses in the C57BL/6J mouse]. Regularly cycling mice were identified before treatment.

Histology. Mice were anesthetized with 2,2,2-tribromoethanol and perfused with 50 ml of saline followed with 50 ml of phosphate-buffered formaldehyde. Fixed brains were removed and stored in formaldehyde for an additonal 3–4 days. Brains were dehydrated by passing through a series of alcohols, embedded in paraffin, and thin sections (7 μm) cut throughout the extent of the arcuate nucleus. Cell counts were obtained using randomized photographic enlargements of coded cresyl-violet-stained sections of the arcuate and ventromedial nucleus of the hypothalamus. The investigator was unaware of the treatment group at the time of analysis.

Brain Monoamine Analysis. Dopamine and norepinephrine were measured in homogenates prepared from medial basal hypothalamus and anterior pituitary as described previously [27, 34]. Briefly, frozen tissue was homogenized in 0.1 *N* HCl containing 1 m*M* EDTA and 0.57 m*M* ascorbic acid. After centrifugation (15,000 *g*, 10 min), an internal standard (dihydroxybenzylamine) was added to the supernatant, and the pH adjusted to 7.6. Catecholamines were batch-absorbed onto alumina in 0.1 *M* Tris buffer, pH 7.6, washed twice with water and eluted with phosphoric acid. Catecholamines in the eluate were analyzed by reversed-phase HPLC with electrochemical detection.

Protein Assay. Protein was determined by the Commassie blue dye binding method (BioRad Laboratories, Richmond, Calif.) using bovine serum albimin as a standard.

LH and PRL Radioimmunoassay. LH was measured by means of a double antibody RIA adapted for mouse LH [5] using materials obtained from the NIAMDD rat pituitary hormone program (Dr. A. Parlow, Harbor General Hospital, Torrance, Calif.). Rat LH (rLH-I-5) was radiolabelled with (^{125}I)-NA (Amersham, Arlington Heights, Ill.) by the chloramine T procedure and chromatographically purified on Sepharose concanavalin A with alpha-*D*-methyl glucopyranoside as an eluant. The first antibody, rabbit antiovine LH (prep. No. 15) was obtained from Dr. G.D. Niswinder (Colorado State Univ., Ft. Collins, Colo.); the second antibody was purchased from Antibodies, Inc. (Davis, Calif.). Data are expressed as immunoreactive nanograms LH-RP-1 per milliliter serum. All samples were assayed in one run, with an intraassay coefficient of variation of 10% and a sensitivity of 20 ng/ml. PRL was measured using an antibody against purified mouse pituitary PRL [32].

Glutamate Receptor Autoradiography. Three subtypes of glutamate receptors (kainate, NMDA and quisqualate-preferring) were measured by receptor autoradiography using tritium-labeled kainate, glutamate and amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) as ligands [16]. Frozen sections (8 μm) were thawed and preincubated in 50 m*M* Tris-acetate, pH 7.2 (NMDA, AMPA) or 50 m*M* Tris-citrate, pH 7.0 (kainate) for 10 min at 30 °C. For kainate receptor analysis, 100 n*M* ^{3}H-kainate (vinylidene(^{3}H)-kainic acid, 60.0 Ci/mmol, New England Nuclear) in preincubation buffer was applied to tissue sections and incubated for 30 min at 0 °C. Nonspecific binding was assessed in the presence of 100 μ*M* unlabeled kainic acid. For quisqualate receptor analysis, 100 n*M* AMPA (*D,L*-α-(5-methyl-^{3}H)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, 27.6 Ci/mmol, New England Nuclear) in preincubation buffer was applied to sections and incubated for 10 min at 0 °C. Nonspecific binding was assessed in the presence of 500 μ*M* glutamate. For NMDA receptor analysis, sections were exposed to 100 n*M* ^{3}H-glutamate (*L*-3,4-^{3}H-glutamic acid, 51.9 Ci/mmol, New England Nuclear) in 50 m*M* Tris-Acetate, pH 7.2 buffer containing 100 μ*M* 4-acetoamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (to block chloride-dependent glutamate uptake) and 5 μ*M* quisqualate for 10 min at 0 °C. Nonspecific binding was assessed in the presence of 200 μ*M* NMDA. All incubations were terminated by 4 rapid washes at 0 °C in their respective preincubation buffer, quickly dried under a stream of air and apposed to ^{3}H-sensitive film for 4 weeks.

Statistical Analysis. Main effects of treatment upon estrous cycles were evaluated by multivariate analysis (MANOVA) using a SAS statistical package (SAS Institute, Cary, N. C.). Dependent variables used in the analysis were the number of 4-day, 5-day and 6-day estrous cycles in the 30-day pre- and posttreatment observation period. To further characterize changes in cyclicity, the effects of treatment upon each dependent variable were individually assessed by least-squares ANOVA (General Linear Model, SAS). Least-squares ANOVA was also used to evaluate NMA effects upon body weight, arcuate nucleus cell counts and anterior pituitary dopamine content. The effects of treatment upon mortality were assessed by χ^2 analysis.

Experimental Design

To analyze neuroendocrine effects, estrous cycles and body weight were monitored for 30 days prior to treatment (Studies 1 and 3). To minimize weight fluctuations due to diurnal changes in water and food intake, weight measurements were taken weekly between 11.00 and 12.00 h. Cycling mice received three subcutaneous injections of NMA (200 mg/kg) or saline, spaced 24 h apart. This NMA dose is intermediate to a range of doses (50–300 mg/kg) reported to produce 15–30% neuron loss in the arcuate of 25-day-old mice [26]. Estrous cycle characteristics and body weight were followed for an additional 30 days, when brains from NMA and control groups were sampled for histology.

For biochemical studies (Study 2), 4-month-old mice were ovariectomized (OVX) to control for effects of endocrine status upon brain monoamine content and serum hormone levels. In addition, to determine whether estrogen could potentiate NMA excitotoxicity in the hypothalamus as observed with corticosterone and kainate toxicity in the hippocampus [29], half of the OVX mice received estrogen replacement prior to treatment with NMA. Mice which had been OVX 7 days earlier received daily injections of E_2 in peanut oil (20 μg/kg/day, subcutaneously) or vehicle alone for 3 days followed by 3 injections of NMA (200 mg/kg/day) or saline. Six days after the final NMA injection, mice were sacrificed by decapitation and trunk blood collected for determination of serum LH and PRL levels. The anterior pituitary and medial basal hypothalamus were dissected out and frozen on powdered

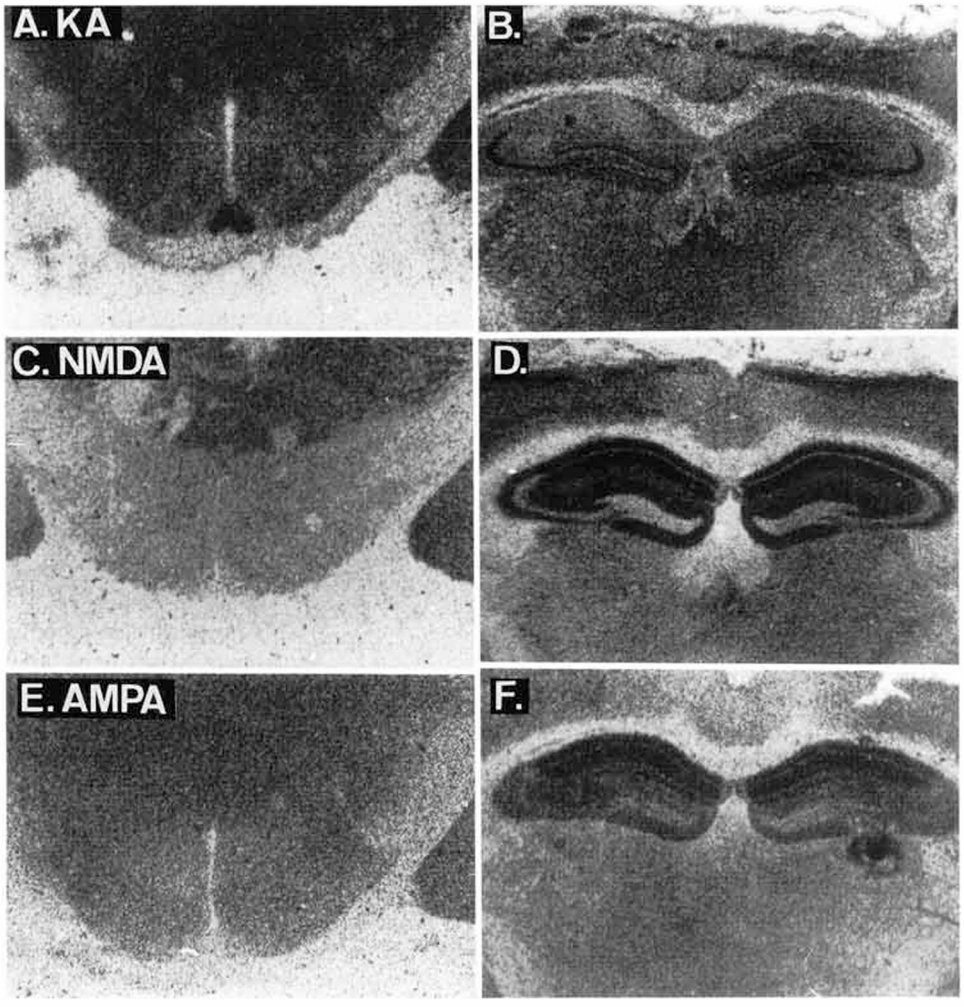

Fig. 1. Glutamate receptor distribution in the female mouse brain. In vitro receptor autoradiographic analysis for kainic acid, NMDA and quisqualate receptors were performed on 8 μm frozen sections through the arcuate nucleus and hippocampus from brains of intact 7-month-old female mice. Representative autoradiographs depict total binding of kainate (**A, B**), NMDA (**C, D**) and AMPA (**E, F**) ligands to selective glutamate receptor subtypes in the arcuate nucleus (**A, B, E**) and hippocampus (**B, D, F**; see Methods).

Table 1. Effect of NMA and E_2 on hypothalamic monoamines and serum hormones

Group	MBH		ANT PIT	Serum	
	DA	NE	DA	LH	PRL
OVX					
Vehicle (n = 8)	5.8 ± 0.4	19.7 ± 0.7	3.2 ± 0.5	314 ± 59	8.7 ± 1
NMA (n = 6)	6.8 ± 0.7	21.5 ± 1.8	3.8 ± 0.7	290 ± 56	8.7 ± 0.6
E_2					
Vehicle (n = 9)	6.4 ± 0.4	22.8 ± 2.2	6.2 ± 0.4*	168 ± 43	7.7 ± 0.6
NMA (n = 8)	7.0 ± 0.6	22.3 ± 2.9	9.1 ± 0.8**	198 ± 38	7.8 ± 0.6

*$p < 0.05$ OVX vs. E_2, ANOVA; **$p < 0.01$ OVX vs. E_2, ANOVA.

Four-month-old female mice were treated 7 days after OVX with 3 daily injections of E_2 (20 μg/kg/day) or vehicle followed by 3 injections of NMA (200 mg/kg/day). Mice were analyzed for medial basal hypothalamic (MBH) and anterior pituitary (ANT PIT) monoamines and serum luteinizing hormone (LH) and prolactin (PRL). Monoamines (ng/mg protein) and serum hormone (ng/ml serum) values represent mean ± SEM (n = 6–9).

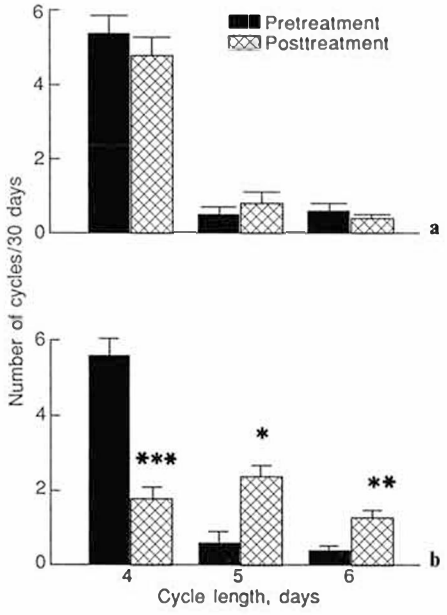

Fig. 2. NMA-induced change in estrous cycle length of young C57BL/6J mice. Cycle distribution of cycling female mice treated at 7 months with vehicle (**a**, n = 22) or NMA (**b**, n = 19). Data are presented as the mean ± SEM of 4-, 5- and 6-day cycles within the observation period. NMA treatment altered cycle distribution ($p < 0.01$; MANOVA). Least square means analysis indicated a shift towards longer cycles due to a loss of 4-day cycles (***$p < 0.001$; ANOVA) with a concomitant increase in 5-day (*$p < 0.05$; ANOVA) and 6-day (**$p < 0.01$; ANOVA) cycles.

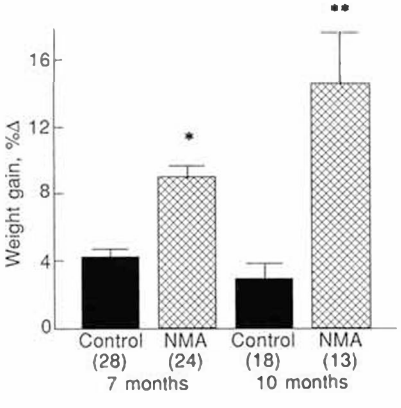

Fig. 3. Effect of NMA upon body weight. Adult cycling female mice (7 and 10 months) were treated with 3 subcutaneous injections of NMA (200 mg/kg) or vehicle. Data represent the percent change in body weight 30 days after treatment compared to pretreatment weight. NMA treatment increased body weight in each age group (*$p < 0.001$; ANOVA). In addition, there was a significant interaction between age and treatment (**$p < 0.001$; ANOVA).

dry ice for subsequent monoamine analysis. Three mice from each group were randomly selected for histological examination of the arcuate nucleus.

Results

Study 1. In a pilot study, intact 4-month-old cycling mice were given 3 subcutaneous injections of NMA (200 mg/kg) or saline on successive days. Estrous cycles during the month following treatment were irregular and lengthened 6.6 ± 0.4, NMA; 5.4 ± 0.4 days, vehicle-treated controls. These results led to more detailed analysis.

Study 2. To identify cellular and biochemical changes associated with elongated estrous cycles, we studied the effects of NMA upon arcuate nucleus cell number, hypothalamic monoamine content, and plasma LH and PRL. Mice (4-month-old) which were OVX and replaced with estrogen or vehicle were treated with NMA (200 mg/kg s.c. for 3 days) and sacrificed 6 days after the final NMA injections (see Experimental Design). Histological analysis of the number of arcuate neurons per unilateral arcuate indicated a 30% loss of cells in the rostral arcuate nucleus of NMA-treated mice (100 ± 6 cells, mean ± SEM, n = 4) compared to saline treated controls (147 ± 6 cells, n = 3). Neither norepinephrine nor dopamine levels in the medial basal hypothalamus were changed by NMA treatment or estradiol (table 1). Transient exposure of OVX mice to estradiol increased dopamine content in the anterior pituitary by twofold ($p < 0.05$, ANOVA; table 1). NMA had little effect upon anterior pituitary dopamine in OVX mice, but potentiated the increase in anterior pituitary dopamine observed in OVX mice treated with estrogen ($p < 0.01$, ANOVA; table 1). Basal serum LH was elevated as expected in control mice 18 days post-OVX, while transient exposure to estrogen for 3 days reduced serum LH by 30–40% (table 1). NMA had no effect upon basal serum LH in either OVX or estrogen-treated mice (table 1). Similarly, basal serum PRL was unaffected by NMA (table 1).

Glutamate receptor subtypes in the arcuate nucleus were mapped by receptor autoradiography in the hypothalamus and hippocampus of intact mice. The arcuate nucleus had a high density of kainate receptors (fig. 1). In contrast, little specific binding of glutamate ligands labeling NMDA and quisqualate receptors was observed in the arcuate nucleus, although a high density of both receptor types was evident in the hippocampus (fig. 1). Negligible labeling of any glutamatergic receptor ligand was observed in the ventromedial nucleus.

Study 3. The increased cycle length of NMA-treated mice was reminiscent of the age-related lengthening of estrous cycle in female C57BL/6J mice after 7–8 months [12, 21]. To further explore this observation, mice at ages which bracket the change in cycle length (7 and 10 months) were treated with NMA as above. As observed with monosodium glutamate treatment of adult rodents [1], NMA elicited transient seizures in some but

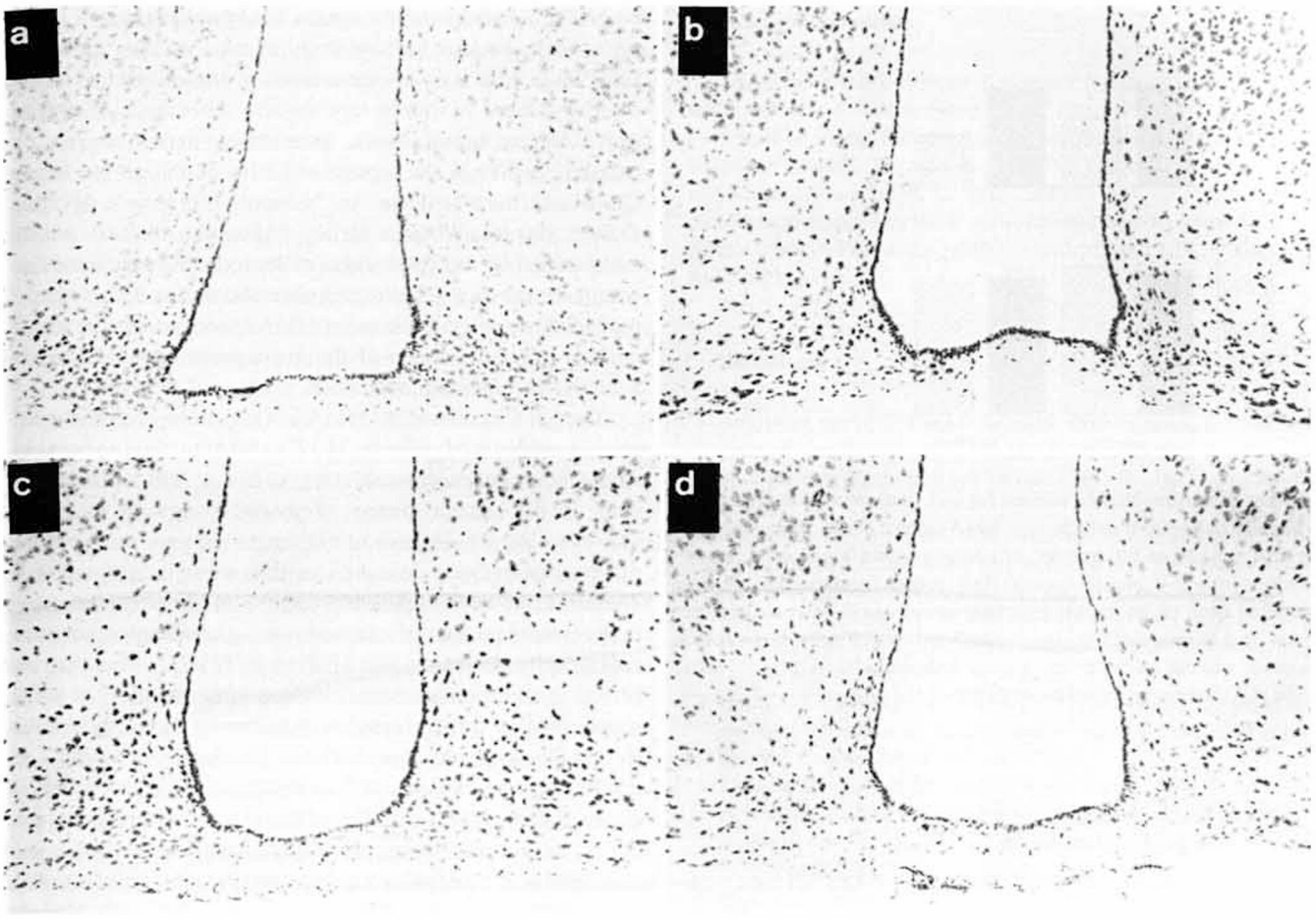

Fig. 4. Effect of NMA upon arcuate nucleus histology. Representative cresyl violet stained coronal sections (7 μm) through the rostal arcuate nucleus of vehicle (**a, c**) and NMA-treated (**b, d**) mice. NMA treatment resulted in a net loss of neurons in the arcuate nucleus of 7- (**b**) and 10-month-old (**d**) mice. × 200.

not all mice. Death ensued within 24 h of initial treatment for 14% (4/28) of 7-month-old mice and 40% (8/21) of 10-month-old mice ($p \leq 0.05$, 10 vs. 7 months; χ^2). The estrous cycle stage at treatment did not predict mortality nor did the presence or absence of seizures correlate with subsequent elongated estrous cycles.

In 7 month old mice, average estrous cycle length was determined from vaginal smear patterns of cycling mice prior to treatment (cycle length = 4.4 ± 0.2 days, mean ± SEM). NMA treatment lengthened estrous cycles by approximately 1 day (5.6 ± 0.3 days, mean ± SEM, n = 19), while saline-injected controls were unaffected (4.5 ± 0.2 days, n = 22). Multivariate analysis indicated a main effect of NMA treatment upon cycle length distribution ($p < 0.01$, MANOVA). Least-square means analysis of the number of 4-day, and 5-day and 6-day or longer cycles which occured in the observation period indicated NMA-lesioned mice had fewer-4-day cycles ($p < 0.001$, ANOVA) and more 5-day ($p < 0.05$, ANOVA) and 6-day cycles ($p < 0.01$, ANOVA) than controls (fig. 2). In a separate group of 10-month-old mice, irregular cycling characteristics plus the high mortality rate following NMA treatment (see above) precluded accurate cycle analysis in this age group. NMA-treated mice of both age groups had increased body weight the month following treatment ($p < 0.001$; ANOVA; fig. 3). Older mice showed greater weight gains ($p < 0.001$, age by treatment interaction; ANOVA).

NMA treatment caused a net loss of neurons in the rostral arcuate nucleus (fig. 4). Cell counts of cresly-violet-stained sections of the rostral arcuate nucleus indicated a 30% decrease in cell number in the rostral arcuate nucleus of 7-month old mice and a greater decrease (48%) in the 10-month cohort ($p < 0.01$; ANOVA; fig. 5). In contrast, the mid-arcuate nucleus

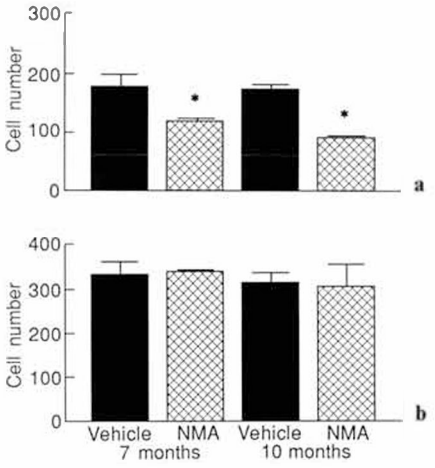

Fig. 5. Rostral arcuate nucleus **(a)** and ventromedial hypothalamic **(b)** cell counts from vehicle and NMA-treated mice. Data represent mean ± SEM of the number of neurons within a unilateral arcuate nucleus (n = 2–4 animals/group). Two sections from each animal were counted from photographic enlargements of cresyl-violet-stained sections and averaged. There was a significant loss of cells in the rostral arcuate nucleus of both age groups following NMA (*$p < 0.01$; ANOVA) but not in the adjacent ventromedial nucleus.

(above the median eminence) showed much less cell loss, significant only in the 10-month-old mice [data not shown]. The ventromedial nucleus of NMA- and saline-treated mice in these same sections showed no cell loss (fig. 5).

Discussion

Systemic administration of NMA to mature C57BL/6J female mice caused subtle impairments in reproductive function as evidenced by an increase in estrous cycle length. This shift in cycle length results from a loss of 4-day cycles and an increase of 5- and 6-day cycles, and mimics the early age-related changes observed in C57BL6J mice and some other genotypes [12, 21]. Further studies of pituitary and ovarian hormones are needed to establish the relationship of the NMA-related cycle lengthening to hormonal differences as observed between 4- vs. 5-day or longer cycles in young rats [23] and in older rodents [4, 5, 20].

Cell loss in the rostral portions of the arcuate nucleus due to NMA treatment ranged from moderate (30%, 7-month-old mice) to severe (48%, 10 months). Comparable arcuate nucleus cell loss (30%) occurred in 25-day-old mice treated with 300 mg/kg NMA and may represent a maximal effect for this age; higher doses increased mortality without increasing arcuate cell loss [26]. More caudal regions of the arcuate were less affected by NMA; neonates show a similar topographic gradient within the arcuate nucleus for sensitivity to monosodium glutamate [31]. While there is no documentation of arcuate cell loss during the early stages of rodent reproductive senescence, several reports indicate neuron loss at later stages; neuron density decreased 30–50% in the arcuate of 18- to 20-month-old female C57BL/6J mice [14] and in 24-month-old female Sprague-Dawley rats [6]. Whether earlier dysfunction of these arcuate neurons lost by the latter stages of reproductive senescence accounts for the cycle lengthening observed during early stages of reproductive aging or following NMA lesions awaits the identification and comparison of the arcuate cell types affected by acute NMA treatment and aging.

The cell loss caused by NMA is likely to be receptor-mediated, as evidenced by the ability of an NMDA receptor antagonist, *D*-aminophosphonovalerate, to block NMA-induced lesions in the arcuate nucleus of young 25-day-old mice [25]. However, the distribution of glutamate receptor subtypes has not previously been described in the mouse arcuate nucleus. Therefore, we made a preliminary map of glutamate receptors in the arcuate nucleus of intact mice using in vitro receptor autoradiography. Little specific binding to NMDA sites was observed in the mouse arcuate nucleus under conditions which clearly labeled these glutamate receptors in the hippocampus (fig. 1). These results agree with the low density of NMDA receptors observed in the rat hypothalamus [18]. Low levels of quisqualate receptors were found in the arcuate nucleus of mice (fig. 1), as reported for rats [28]. Perhaps the very dense, laminar distribution of NMDA and quisqualate receptors in the rodent hippocampus prejudices our appreciation of low levels of these receptor types in the more diffuse hypothalamic nuclei. In contrast to NMDA and quisqualate sites, kainate receptors were present in the arcuate nucleus at high density, the region of marked neuron loss. Little specific binding of kainic acid was observed in the VMN, an area unaffected by the NMA injection. These results are consistent with the moderate levels of kainic acid receptors observed in the rat hypothalamus, although differences in kainate receptor levels between different hypothalamic nuclei were not described [17]. It seems likely that activation of potent excitatory kainate receptors (either directly by administered NMA or indirectly by NMA-induced release of an endogenous excitatory transmitter) have a role in the selective loss of arcuate nucleus neurons.

The neuronal specificity of the NMA lesion in adult mice appears to differ from monosodium glutamate-lesioned neonates. Neurons intrinsic to the arcuate nucleus (e.g., dopaminergic, gabaergic and opioidergic) degenerate after neonatal monosodium glutamate treatment, while neurons projecting into the arcuate (e.g., noradrenergic, serotonergic) are spared [8, 9, 22]. Consistent with this selectivity, noradrenaline content in the medial basal hypothalamus did not change following NMA treatment. Unexpectedly, however, dopamine levels in the MBH also were unaffected by NMA treatment. Perhaps analysis of dopamine content in tissue punches taken from the ar-

cuate nucleus would reveal a dopaminergic deficit. The identification of the cell types affected by NMA may help elucidate neurochemical changes which accompany shifts in cycle length distribution.

Another neuroendocrine function affected by NMA treatment was regulation of body weight, which increased in both 7- and 10-month-old mice treated with NMA. Interestingly, NMA caused more weight gain in the 10-month-old mice. In addition, mortality and cell loss in the arcuate was more extensive in the 10-month-old cohort than in the 7-month-old group. These results suggest that postmaturational aging may increase susceptibility to neuronal excitotoxins, and are similar to observations reported for another class of neurotoxins, MPTP [7, 11]. Whether the apparent enhanced toxicity reflects an age-related change in intrinsic neuronal susceptibility (e.g., altered energy metabolism, altered receptors) or is secondary to a change in clearance requires further study.

Besides neuroendocrine changes in cycle length, most other endocrine parameters examined were not affected by NMA treatment. The basal levels of LH and PRL in NMA-lesioned mice resembled saline-treated controls, as did anterior pituitary dopamine levels of OVX mice treated with NMA. Estrogen increased the dopamine content of the anterior pituitary of OVX mice, but NMA treatment increased anterior pituitary dopamine content above that seen with estrogen alone. Aging also increases dopamine content in the anterior pituitary of intact female mice but again, this change primarily is dependent upon the presence of estrogen; OVX of aged mice reduces anterior pituitary dopamine content toward levels seen in young intact females [34]. The present data suggest that NMA influenced dopamine clearance from an estrogen-sensitive site in the anterior pituitary and that such a paradigm may be useful in elucidating a function for anterior pituitary dopamine.

Apart from the effect upon clearance of anterior pituitary dopamine, no other clear interactions were observed between estradiol and NMA. Whether this reflects a true lack of interaction or a ceiling effect (see above) at this dose of NMA is unknown. Given the potentiation of kainate excitotoxicity in the hippocampus by corticosterone [29] and the potential for neuroendocrine damage caused by chronic estradiol alone [10, 12, 30], further investigation into this possible interaction is warranted.

In conclusion, NMA treatment creates partial lesions of the arcuate nucleus in young female mice which are accompanied by changes in estrous cycles typically observed in older mice. The degree of arcuate damage following NMA treatment is undoubtedly greater than that occurring during the early phases of reproductive senescence, although similar neuroendocrine loci may be involved. Identification and characterization of arcuate neurons affected by acute NMA treatment may point to neuronal populations undergoing milder age-related degenerative changes. Thus, we believe NMA-lesioned mice will be useful in assessing the contributions of neuroendocrine changes in the arcuate nucleus to reproductive senescence.

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References

1 Arauz-Contreras, J.; Feria-Velasco, A.: Monosodium-*L*-glutamate-induced convulsions. I. Differences in seizure pattern and duration of effect as a function of age in rats. Gen. Pharmac. *15:* 391–395 (1984).

2 Barraclough, C.A.; Wise, P.M.: The role of catecholamine in the regulation of pituitary luteinizing hormone and follicle stimulating hormone. Endocr. Rev. *3:* 1–119 (1982).

3 Brawer, J.R.; Schipper, H. and Naftolin, F.: Ovary-dependent degeneration in the hypothalamic arcuate nucleus. Endocrinology. *107:* 274–279 (1980).

4 Finch, C.E.; Felicio, L.S.; Mobbs, C.V.; Nelson, J.F.: Ovarian and steroidal influences on neuroendocrine aging processes in female rodents. Endocr. Rev. *5:* 467–497 (1984).

5 Flurkey, K.; Gee, D.M.; Sinha, Y.N.; Wisner, J.R.; Finch, C.E.: Age effects on luteinizing hormone, progesterone and prolactin in proestrous and acyclic C57BL/6J mice during aging. Biol. Reprod. *26:* 835–846 (1982).

6 Hsu, H.K.; Peng, M.T.: Hypothalamic neuron number of old female rats. Gerontology *24:* 434–440 (1978).

7 Jarvis, M.F.; Wagner, G.C.: Age-dependent effects of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). Neuropharmacology *24:* 581–583 (1985).

8 Jennes, L.; Stumpf, W.E.; Bissette, G.; Nemeroff, C.B.: Monosodium glutamate lesions in rat hypothalamus studied by immunohistochemistry for gonadotropin releasing hormone, neurotensin, tyrosine hydroxylase, and glutamic acid decarboxylase and by autoradiography for (^{3}H)estradiol. Brain Res. *308:* 245–253 (1984).

9 Kawakami, F.; Terubayashi, H.; Okamura, H.; Fukui, K.; Ibata, Y.; Yanaihara, N.; Zimmermann, E.A.; Shiota, K.; Nakayama, R.: Influence of monosodium-*L*-glutamate upon peptide neurons of the hypothalamus of the rat. Biomed. Res. *4:* suppl., pp. 75–81 (1983).

10 Kohama, S.; Anderson, C.P.; Osterburg, H.H.; May, P.C.; Finch, C.E.: Oral administration of estradiol to young C57BL/6J mice induces age-like dysfunctions in the capacity for estrous cycles. Biol. Reprod. (In press)

11 Langston, W.J.; Irwin, I.; DeLanney, L.E.: The biotransformation of MPTP and disposition of MPTP: The effects of aging. Life Sci. *40:* 749–754 (1987).

12 Lerner, S.P.; Anderson, C.P.; Walford, R.L.; Finch, C.E.: Genotypic influences on reproductive aging of inbred female mice: effects of H-2 and non-H-2 alleles. Biol. Reprod. *38:* 1035–1043 (1988).

13 Matsumoto, A.; Arai, Y.: Developmental changes in synaptic formation in the hypothalamic arcuate nucleus of female rats. Cell Tissue Res. *169:* 143–156 (1976).

14 Miller, M.M.; Gould, B.E.; Nelson, J.F.: Aging and long-term ovariectomy alter the cytoarchitecture of the hypothalamic-preoptic area of the C57BL/6J mouse. Neurobiol. Aging (in press; 1989).
15 Mobbs, C.V.; Flurkey, K.; Gee, D.M.; Yamamoto, K.; Sinha, Y.; Finch, C.E.: Estradiol-induced anovulatory syndrome in female C57BL/6J mice: age like neuroendocrine, but not ovarian impairments. Biol. Reprod. *30:* 556 (1984).
16 Monaghan, D.T.; Holets, V.R.; Toy, D.W.; Cotman, C.W.; Anatomical distributions of four pharmacologically distinct ^{3}H-*L*-glutamate binding sites. Nature *306:* 176–179 (1983).
17 Monaghan, D.T.; Cotman, C.W.: The distribution of (^{3}H)kainic acid binding sites in rat CNS as determined by autoradiography. Brain Res. *252:* 91–100 (1982).
18 Monaghan, D.T.; Cotman, C.W.: Distribution of N-methyl-*D*-aspartate sensitive *L*-(^{3}H)glutamate binding sites in rat brain. J. Neurosci. *5:* 2909–2919 (1985).
19 Nagasawa, M.; Yanai, R.; Kikuyama, S.: Irreversible inhibition of pituitary prolactin and growth hormone secretion and of mammary gland development in mice by monosodium glutamate administered neonatally. Acta endocr., Copenh. *75:* 249–259 (1974).
20 Nelson, J.F.; Felicio, L.S.: Reproductive aging in the female: an etiological perspective. Rev. Biol. Res. Aging *2:* 251–314 (1985).
21 Nelson, J.F.; Felicio, L.S.; Randall, P.K.; Sims, C.; Finch, C.E.: A longitudinal study of estrous cyclicity in aging C57BL/6J mice. I. Cycle frequency, length and vaginal cytology. Biol. Reprod. *27:* 327–339 (1982).
22 Nemeroff, C.B.; Konkol, R.J.; Bissette, G.; Youngblood, W.; Martin, J.P.; Brazeau, P.; Rone, M.S.; Prange, A.J.; Breese, G.R.; Kizer, J.S.: Analysis of the disruption in hypothalamic-pituitary regulation in rats treated neonatally with monosodium glutamate (MSG): evidence for the involvement of tuberoinfundibular cholinergic and dopaminergic systems in neuroendocrine regulation. Endocrinology *101:* 613–622 (1977).
23 Nequin, L.G.; Alvarez, J.; Schwartz, N.B.: Measurement of serum steroid and gonadotropin levels and uterine and ovarian variables throughout 4 day and 5 day estrous cycles in the rat. Biol. Reprod. *20:* 659–670 (1979).
24 Olney, J.W.: Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science *164:* 719–721 (1969).
25 Olney, J.W.; Labruyere, J.; Collins, J.F. and Curry, K.: *D*-Aminophosphonovalerate is 100-fold more powerful than *D*-alpha-aminoadipate in blocking N-methylaspartate neurotoxicity. Brain Res. *221:* 207–210 (1981).
26 Olney, J.W.; Price, M.T.: Excitotoxic amino acids as neuroendocrine research tools. Meth. Enzym. *103:* 379–393 (1983).
27 Osterburg, H.H.; Telford, N.A.; Cohen-Becker, I.; Wise, P.M.; Finch, C.E.: Hypothalamic monoamines and their catabolites in relation to the estradiol-induced luteinizing surge. Brain Res. *409:* 31–40 (1987).
28 Rainbow, T.C.; Wieczorek, C.M.; Halpain, S.: Quantitative autoradiography of binding sites for (^{3}H)AMPA, a structural analogue of glutamic acid. Brain Res. *309:* 173–177 (1984).
29 Sapolsky, R.M.: A mechanism of glucocorticoid toxicity in the hippocampus: increased neuronal vulnerability to metabolic insults. J. Neurosci. *5:* 1228–1232 (1985).
30 Schipper, H.; Brawer, J.R.; Nelson, J.F.; Felicio, L.S.; Finch, C.E.: Role of the gonads in the histologic aging of the hypothalamic arcuate nucleus. Biol. Reprod. *25:* 413–419 (1981).
31 Seress, L.: Divergent effects of acute and chronic monosodium-*L*-glutamate treatment on the anterior and posterior parts of the arcuate nucleus. Neuroscience *7:* 2207–2216 (1982).
32 Sinha, Y.N.; Selby, F.W.; Lewis, V.J.; Vanderlaan, W.P.: Studies of prolactin secretion in mice by a homologous radioimmunoassay. Endocrinology *91:* 1045–1053 (1973).
33 Takahashi, S.: Kawashima, S.; Wakabayashi, K.: Serum and pituitary levels of prolactin and the responsiveness to pimozide on prolactin release in monosodium glutamate-treated rats. J. Sci. Hiroshima Univ. *30:* 221–228 (1982).
34 Telford, N.A.; Mobbs, C.V.; Sinha, Y.N.; Finch, C.E.: The increase of anterior pituitary dopamine in aging C57BL/6J female mice is caused by ovarian steroids, not intrinsic pituitary aging. Neuroendocrinology *43:* 135–142 (1986).

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