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Effect of Neonatal Clomipramine Treatment on Adult Alcohol Drinking in the AA and ANA Rat Lines

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HILAKIVI, L. AND J. D. SINCLAIR. *Effect of neonatal clomipramine treatment on adult alcohol drinking in the AA and ANA rat lines.* PHARMACOL BIOCHEM BEHAV **24**(5) 1451–1455, 1986.—In order to test further the hypothesis that neonatal active (REM) sleep suppression by means of clomipramine, an inhibitor of monoamine reuptake, is involved in the subsequent increase of voluntary alcohol consumption in rats, the AA (alcohol preferring) and ANA (alcohol avoiding) rat lines were injected daily with 25 mg/kg clomipramine IP from the 7th to the 20th postnatal days. At the age of 3 months the clomipramine AA rats consumed significantly more 10% (v/v) alcohol solution than the control AA rats. Neonatal clomipramine treatment did not, however, affect the drinking patterns of the ANA rats. Secondly, in order to test the alcohol-deprivation effect; i.e., the increase in alcohol consumption after its deprivation, the AA and ANA rats were deprived of alcohol for 17 days. There was a significant difference between the temporal pattern of changes in alcohol drinking produced by alcohol deprivation in the AA rats and the pattern in the ANA rats. Furthermore, the clomipramine treated AA rats tended to show a decrease and the clomipramine ANA rats an increase in their post-deprivation alcohol intake compared to the control AA and ANA rats. The results are interpreted in terms of active sleep being important for later alcohol drinking and other genetically determined differences in behavior.

Selected rat lines Voluntary alcohol consumption Newborn rats Clomipramine
Active (REM) sleep deprivation Development of behavior Alcohol-deprivation effect

THE high amount of active sleep (AS, also called REM sleep after neonatal period) during early maturation of the central nervous system in mammals is suggested to reflect the importance of AS for the development of the brain [16,24]. More specifically, AS might provide genetically programmed endogenous excitation to the brain during early postnatal period, a time of limited sensory stimulation [15]. According to this hypothesis deprivation of AS would diminish hereditarily-determined programming of behavior and increase the role of environmental influences. Thus, genetically determined differences in behavior between two lines of a same species should be reduced by neonatal active sleep suppression.

Recent studies have shown that AS is characterized by intense neuronal bursts in the cerebral cortex [20] and that neonatal suppression of active sleep by pharmacological and instrumental means leads to disturbances in the brain and behavioral development of Wistar rats [22]. Our previous work indicated that neonatal active sleep suppression by clomipramine (CLM), an antidepressant drug, increases voluntary alcohol drinking in mixed strain rats (derived from Wistar, Sprague-Dawley and Long-Evans rats) [11] and decreases the expected deprivation-induced increase in alcohol intake [12]. Clomipramine is a relatively selective inhibitor of serotonin reuptake [5,18], but it also affects noradrenaline and dopamine reuptake [9,19]. Long-term treatment of clomipramine in adult rats is shown to slightly elevate brain 5-HT content [28] and to induce down-regulation of both beta-adrenoceptors [26] and of the adenylate cyclase [23]. Except for the finding that noradrenaline concentration is normal in the adult brain and medulla oblongata after neonatal clomipramine treatment [22], there is no evidence for how neonatally given chronic exposure to clomipramine affects the brain transmitter systems.

The present study was designed to examine the effect of neonatal clomipramine treatment on the alcohol drinking patterns of the AA and ANA rat lines, genetically developed for high and low alcohol intake [6]. In an earlier study [17] the alcohol preferring AA rats failed to show any changes in their alcohol intake after neonatal modification of cerebral noradrenergic activity [13] by means of 6-hydroxydopamine (6-OHDA) or alteration of 5-HT activity [25] by means of 5,7-dihydroxytryptamine (5,7-DHT). The alcohol avoiding ANA rats were not tested in that study. In contrast to

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TABLE 1

MEAN FLUID CONSUMPTION ± S.D. DURING THE THIRD MEASUREMENT WEEK

	AA control (n=11)	AA CLM (n=11)	ANA control (n=11)	ANA CLM (n=11)
ml fluid*	212.4 ± 9.0	225.6 ± 7.6	195.8 ± 9.1	196.1 ± 5.1
body weight (g)	290.4 ± 5.5	291.6 ± 6.1	312.3 ± 16.3	300.2 ± 8.9
fluid/body weight	0.73	0.87	0.63	0.65

*F AA/ANA (1,40)=7.80, $p<0.001$.

clomipramine, neonatal 6-OHDA or 5,7-DHT treatments are known not to affect sleep-wake patterns of rat pups [2]. Based on Jouvet's theory [15] outlined above, we tested whether neonatal AS deprivation would diminish the differences in alcohol drinking between the AA and ANA rat lines, by decreasing the intake of AA rats and/or increasing that of ANA rats.

METHOD

A total of 44 (out of 56 animals) six day old male AA and ANA rats of the 46th generation served as subjects. Each litter consisted of a nursing mother, four of her pups, and four to six randomly exchanged pups from other litters. Each pup in one of the four litters received the same treatment, while two of the litters contained both experimental and control animals identified with a colored mark on the tail. After weaning at the age of four weeks, the rats were housed in group cages of 4 rats. At the age of 65 days they were housed individually. All rats were housed continuously in a room with a 12-hr light-dark illumination cycle, a temperature of 22–24°C and a relative humidity of about 55%. Standard food (powdered R3 rat diet, Astra-Ewos, Sweden) was available throughout the experiment.

From the seventh postnatal day until the rats were 20 days old, half of the AA and ANA pups were injected subcutaneously with 25 mg/kg clomipramine (Anafranil, Ciba-Geigy) diluted in 0.9% saline, and the remaining pups with saline only. Clomipramine was demonstrated earlier to reduce AS selectively and chronically without affecting normal weight gain or the day of eye-opening in rat pups [12,21].

Beginning at the age of 71 days, the voluntary alcohol consumption of the rats was measured. The rats were given a choice between 10% (v/v) alcohol solution and tap water in two calibrated drinking tubes fitted onto the front wall of the cage for 3 weeks. The alcohol consumption during the third week was used as an index of individual predisposition toward alcohol. The tubes were interchanged weekly to counteract position preference. Fluid intakes were recorded daily at the same time and body weights were recorded weekly. The above procedure is generally the same as that used for over 20 years in the development of the AA and ANA rat lines, and it has been shown to enhance the differences between animals predisposed to prefer and avoid alcohol [7]. Thereafter, all rats were deprived of alcohol for 17 days, and then the alcohol was returned for measurement of the alcohol-deprivation effect (ADE) [27]. Two- and three-way analyses of variance and Student's t-test were used for statistical analyses of the data.

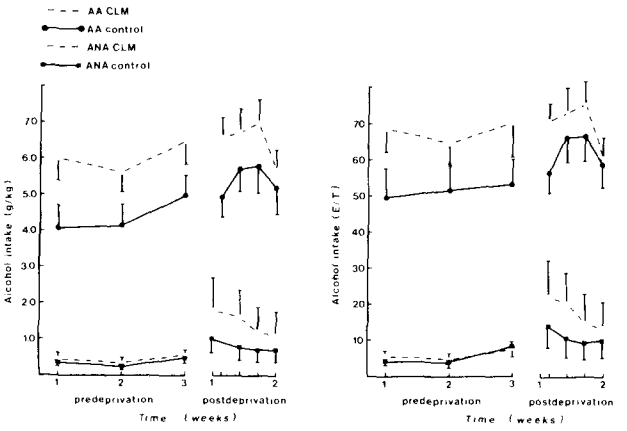

FIG. 1. Effect of neonatal treatment with clomipramine on subsequent alcohol consumption and alcohol deprivation effect in the AA and ANA rats expressed as g/kg and E/T (ethanol solution/total fluid intake, as %). Each point represents the mean±S.E.M. of 11 rats.

RESULTS

As expected the control AA rats consumed significantly more alcohol than the control ANA rats during the measurement period: the mean (±S.D.) alcohol dose during the third week was 4.85±2.04 g/kg body wt. of alcohol per day in the AA rats, while the ANA rats drank only 0.51±0.57 g/kg, $t(20)=6.77$, $p<0.01$. These values were used as the baselines when studying the alcohol-deprivation effect. Alcohol preferences (i.e., the ratio of alcohol solution to total fluid intake) of the AA and ANA rats were 0.57±0.25 and 0.07±0.09, respectively, $t(20)=6.22$, $p<0.01$.

Figure 1 shows that AA rats treated neonatally with clomipramine had significantly higher alcohol intake, expressed as g/kg, during the third week than the control AA rats, $t(20)=2.74$, $p<0.02$. A similar difference with the alcohol preference measure, however, only approached significance, $t=1.87$, $p<0.1$, because of more variable water intakes. Neonatal clomipramine treatment did not affect voluntary alcohol consumption in the ANA rats. Furthermore, the treatment did not affect adult body weights significantly in either line (Table 1).

On the first day when alcohol was again available after the deprivation period, the clomipramine treated ANA rats and the control ANA rats both tended to show an increase in their alcohol intake compared to the baseline levels (g/kg measure, clomipramine ANA: $t=1.84$, $p<0.1$; control ANA: $t=1.52$, $p<0.2$) but in neither case was the alcohol-

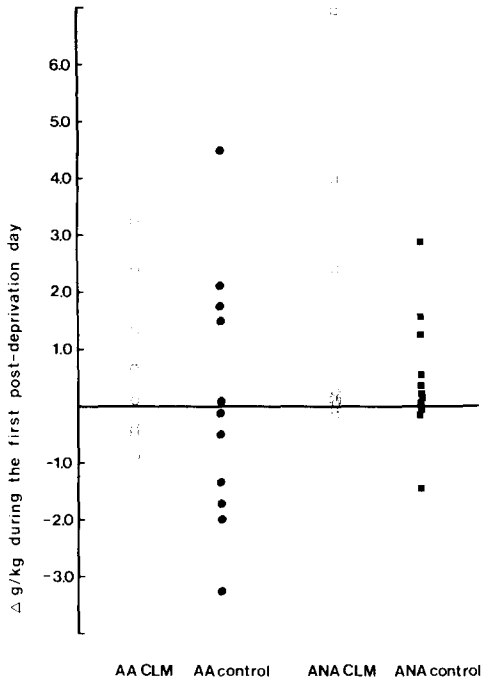

FIG. 2. Distribution of changes in alcohol consumption after 17 days of deprivation in the control and clomipramine treated AA and ANA rats.

deprivation effect significant (Fig. 1). The increases in the mean alcohol intakes were caused almost entirely by only 6 ANA rats (3 in the CLM group, and 3 controls), that showed large alcohol-deprivation effects with increases ranging from 1 to 7 g/kg (Fig. 2). The remaining ANA animals in both groups continued avoiding practically all alcohol as they had done before deprivation.

The temporal pattern of changes in alcohol drinking produced by alcohol deprivation was significantly different in the AA groups relative to the ANA groups, $F(3,120)=4.24$, $p<0.007$. Both ANA groups showed their largest increases on the first day of renewed access, as is usually true with alcohol-deprivation effect, but neither AA group showed any increase on this first post-deprivation day. Instead, the AA rats showed a delayed alcohol-deprivation effect with the largest increase not being reached until the 5th post-deprivation day. The tendency for the deprivation-induced increases among the ANA rats to be larger in the CLM treated group, but among the AA rats to be larger in the controls was not significant, F for interaction $(1,172)=1.99$, $p<0.2$. The failure to reach significance is caused largely by those animals that did not show an alcohol-deprivation effect (see Fig. 2). Among those animals that did increase their alcohol intake after deprivation ($n=5$ in both AA groups, $n=8$ in both ANA groups), there was a significant interaction between rat line and neonatal treatment, $F(1,100)=6.62$, $p<0.02$.

DISCUSSION

The present results with the alcohol preferring AA rats replicate our earlier findings of increased adult alcohol intake after neonatal clomipramine treatment in rats of another strain [11]. However, the alcohol avoiding ANA rats failed to show the same effect. These results are contrary to Jouvet's idea [15] about the role of REM sleep in regulating species-specific neural mechanisms underlying genetically determined behavior, since AS deprivation did not reduce the differences in alcohol intake between the AA and ANA lines, but rather increased them.

Neonatal AA and ANA lines have recently been shown to differ in their amount of REM sleep: the low alcohol drinking ANA rats had significantly more active sleep than the high drinking AA rats [1]. This finding was interpreted as an indication of functional relationship between a low amount of neonatal active sleep and high adult alcohol intake. Furthermore, both sleep-waking cycle and alcohol drinking has been suggested to be dependent upon central monoaminergic regulation [3, 4, 14]. Thus, it is possible that the clomipramine treatment suppressed further the already low amount of active sleep of the AA rats, and consequently increased their subsequent alcohol drinking but the initial level of AS was so high in the ANA rats that clomipramine could not reduce it to a level that would permit more than minimal alcohol intake. Alternatively, the brain of the ANA rat may be more resistant both to internal factors that suppress active sleep—and thus shows more AS than the AA rat—and to external factors, such as the clomipramine injections, that suppress active sleep—and thus showed less effect from the CLM injections on alcohol drinking.

On the basis of previous results (percentages of active sleep of total sleep time in the AA and ANA rats at the age of 10 days: 31.2 and 52.1, and at the age of four months: 7.8 and 16.00) [1] we predict that the amount of active sleep at the end of clomipramine injections was lower in the AA rats than in the ANA rats. Mirmiran *et al.* [22] have demonstrated that there is a threshold for AS suppression producing effects. Behavioral effects were minimal when AS was suppressed only moderately with the pendulum technique and a more complete deprivation of AS with a pharmacological procedure was necessary for inducing the major behavioral changes. Consequently, it is possible that the ANA rats had such high AS levels that even after CLM treatment their AS was not beyond the threshold needed for changing their alcohol drinking behavior.

So far, no studies have been conducted to determine if there is a difference between the AA and ANA rats in the vulnerability of their brains to environmental influences. Over two dozen line differences have, however, been identified in the AA and ANA rats [8,10] including differences in the brain contents of serotonin and dopamine [3,4]. Thus, it is possible that clomipramine treatment affected the balance of brain neurotransmitters differently in the AA and ANA rats, which again might have led to differences in the amount of active sleep suppression between the rat lines.

A third alternative for explaining the lack of increase in alcohol consumption in the clomipramine treated ANA rats is that the ANA rats are incapable of drinking more alcohol, perhaps because of acetaldehyde accumulation. Contrary to this idea, however, after the alcohol deprivation period the CLM rats did increase their alcohol intake, on the average by 1.3 g/kg, thus demonstrating that at least after deprivation they are capable of drinking more alcohol.

The line difference in the temporal pattern of the alcohol-deprivation effect seen here is similar to that reported previously [27]. In that study the ANAs showed only a small increase and returned to baseline in about 4 days.

Also as in the present study, the AA rats showed a delayed alcohol deprivation effect which differentiated them for all other rats studied. In contrast to the present results, the magnitude of the increase was greater in the earlier study and it already was significant on the first post-deprivation day. Whether the reduction in deprivation effect seen in the present AAs is due to procedural differences or to the revitalization [10] of the AA and ANA rat lines remains to be seen.

The deprivation-induced increases in alcohol consumption by the control ANA rats were also somewhat smaller in the present study than in the previous work; this also may be an indirect result of the revitalization. Just prior to revitalization the ANA line actually drank rather much alcohol: the baseline level in the previous study was 1.06 g/kg [27]. Revitalization has apparently reintroduced genetic factors that can further reduce alcohol intake, so by the time the present study was done, the ANA baseline was down to 0.51 g/kg, and since then with further selection, the ANA intake in most recent generation has decreased about 0.30 g/kg. Since animals which completely avoid alcohol are often unaffected by alcohol deprivation, the lower baseline, with more complete abstainers, may have been responsible for the mean increase after deprivation.

Previously neonatal clomipramine treatment has been found to decrease the magnitude of the alcohol-deprivation effect in mixed strain rats [11]. A similar tendency was found in the AA rats now, but the opposite tendency, an increase in the deprivation effect was seen in the ANA rats. Unlike the findings concerning the baseline level of alcohol drinking, this result is in agreement with Jouvet's hypothesis about the role of active sleep in providing genetically programmed endogenous excitation to the brain during the early postnatal period. In the AA and ANA rat lines the alcohol-deprivation effect may reflect a more general pattern of behavior than the alcohol drinking itself: the alcohol-deprivation effect is not related to prior level of alcohol intake during continuous access.

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REFERENCES

1. Aalto, J. and L. Hilakivi. Differences in the sleep-wake patterns of the AA and ANA rat lines developed for high and low alcohol intake. *Alcohol* **3:** 77–79, 1986.
2. Adrien, J. Monoaminergic regulation of sleep: development and plasticity. In: *Physiological and Biochemical Basis for Perinatal Medicine*, edited by M. Mouset-Couchard and A. Minkowski. Basel: Karger, 1981, pp. 296–307.
3. Ahtee, L. and K. Eriksson. 5-Hydroxytryptamine and 5-hydroxyindolacetic acid content in brain of rat strains selected for their alcohol intake. *Physiol Behav* **8:** 123–126, 1972.
4. Ahtee, L. and K. Eriksson. Dopamine and noradrenaline content in the brain of rat strain selected for their alcohol intake. *Acta Physiol Scand* **93:** 156–165, 1975.
5. Carlsson, A., J. Jonsson, M. Lindqvist and K. Fuxe. Demonstration of extraneuronal 5-hydroxytryptamine accumulation in brain following membrane-pump blockade by clomipramine. *Brain Res* **5:** 75–79, 1969.
6. Eriksson, K. Genetic selection for voluntary alcohol consumption in the albino rat. *Science* **159:** 739–741, 1968.
7. Eriksson, K. Factors affecting voluntary alcohol consumption in the albino rat. *Ann Zool Fennici* **6:** 229–265, 1969.
8. Eriksson, K. and M. Rusi. Finnish selection studies on alcohol-related behaviors: General outline. In: *Development of Animal Models as Pharmacogenetic Tools*, edited by G. E. McClearn, R. A. Deitrich and V. G. Erwin. Rockville: NIAAA Res Monograph-6, 1981, pp. 87–117.
9. Halaris, A., K. Belendiuk and D. Freedman. Antidepressant drugs affect dopamine uptake. *Biochem Pharmacol* **24:** 1896–1898, 1975.
10. Hilakivi, L., C. J. P. Eriksson, M. Sarviharju and J. D. Sinclair. Revitalization of the AA and ANA rat lines: effects on some line characteristics. *Alcohol* **1:** 71–75, 1984.
11. Hilakivi, L. A., J. D. Sinclair and I. Hilakivi. Effects of neonatal treatment with clomipramine on adult ethanol related behavior in the rat. *Dev Brain Res* **15:** 129–132, 1984.
12. Hilakivi, L. A. and J. D. Sinclair. Neonatal active sleep suppression by clomipramine and later alcohol related behavior in the rat. In: *Sleep 1984*, edited by W. P. Koella, E. Ruther and H. Schulz. Stuttgart: Gustav Fisher Verlag, 1985, pp. 307–309.
13. Jonsson, G. and C. Sachs. Regional changes in 3H-noradrenaline uptake, catecholamine synthetic and catabolic enzymes in rat brain following neonatal 6-hydroxydopamine treatment. *Med Biol* **54:** 286–297, 1975.
14. Jouvet, M. The role of monoamines and acetylcholine containing neurons in the regulation of the sleep-waking cycle. *Ergeb Physiol* **64:** 166–307, 1972.
15. Jouvet, M. Paradoxical sleep and the nature-nurture controversy. In: *Adaptive Capabilities of the Brain, Vol 53, Progress in Brain Research*, edited by P. S. Connell *et al.* Amsterdam: Elsevier, 1980, pp. 331–346.
16. Jouvet-Mounier, D., L. Astic and D. Laconte. Ontogenesis of the states of sleep in rat, cat and guinea pig during the first postnatal month. *Dev Psychobiol* **2:** 216–239, 1970.
17. Kiianmaa, K. and L. M. J. Attila. Alcohol intake, ethanol-induced narcosis and intoxication in rats following neonatal 6-hydroxydopamine or 5,7-dihydroxytryptamine treatment. *Naunyn Schmiedebergs Arch Pharmacol* **308:** 165–170, 1979.
18. Lidbrink, P., G. Jonsson and K. Fuxe. The effect of imipramine-like drugs and antihistamine drugs on uptake mechanisms in the central noradrenaline and 5-hydroxytryptamine neurons. *Neuropharmacology* **10:** 521–536, 1971.
19. Maj, J., L. Stala, Z. Gorka and A. Adamus. Comparison of the pharmacological actions of desmethylimipramine and clomipramine. *Psychopharmacology (Berlin)* **78:** 165–169, 1982.
20. Mirmiran, M. and M. A. Corner. Neuronal discharge patterns in the occipital cortex of developing rats during active and quiet sleep. *Dev Brain Res* **3:** 37–48, 1982.
21. Mirmiran, M., N. E. Van de Poll, M. A. Corner, H. G. Oyen and H. L. Bour. Suppression of active sleep by chronic treatment with clomipramine during early postnatal development: effect upon adult sleep and behavior in the rat. *Brain Res* **204:** 129–146, 1981.
22. Mirmiran, M., J. Scholtens, N. E. Van de Poll, H. B. M. Uylings, J. Van der Gugten and G. J. Boer. Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. *Dev Brain Res* **7:** 277–286, 1983.
23. Mishra, R. and F. Sulser. Role of serotonin reuptake inhibition in the development of subsensitivity of the norepinephrine (NE) receptor coupled adenylate cyclase system. *Commun Psychopharmacol* **2:** 365–370, 1978.
24. Roffwarg, H. F., J. Muzio and W. C. Dement. Ontogenetic development of the human sleep-waking cycle. *Science* **152:** 604–619, 1966.

25. Sachs, C. and G. Jonsson. 5,6-Dihydroxytryptamine included changes in the postnatal development of central 5-hydroxytryptamine neurons. *Med Biol* **53:** 156–164, 1975.
26. Sellinger-Barnette, M. M., J. Mendels and A. Franzer. The effect of psychoactive drugs on beta-adrenergic receptor binding sites in rat brain. *Neuropharmacology* **19:** 447–454, 1980.
27. Sinclair, J. D. Alcohol-deprivation effect in rats genetically selected for their ethanol preference. *Pharmacol Biochem Behav* **10:** 597–602, 1979.
28. Sugrue, M. F. Changes in rat brain monoamine turnover following chronic antidepressant administration. *Life Sci* **26:** 423–429, 1980.