## Light Reversal of a Morphine-Induced Analgesia Susceptibility Rhythm in Mice

The role of the lighting regimen as a dominant synchronizer of physiological rhythms has been well documented for both plants and animals[1,2]. If the regimen is reversed, the temporal placement of the studied rhythm within the 24-h time period can be phase shifted through 180 degrees. Several 24-h rhythms in mice follow this resynchronization phenoma: blood eosinophils[3], liver glycogen content[4], audiogenic convulsions[5], kidney transaminase[6] and epidermal mitoses[7]. Similar studies have not been made with drugs except for the resynchronization of ethanol mortality rhythm[8]. Morphine sulfate, for which the 24-h analgesic pattern in mice has been characterized[9], was selected to illustrate the potential significance of resynchronization to drug therapy.

A colony of adult female albino CF-1 mice were used for 2 successive experiments (October 17 and November 14, 1967). The time between experiments allowed for rest and recycling of the morphine response. In experiment I, 160 mice, $23.4 + 0.2$ g body weight, were programmed on a standard alternating schedule of 06.05 to 18.05 h lights on and 18.05–06.05 h totally dark. The lighting regimen was reversed (i.e. 06.01–18.05 h dark and 18.05–06.05 h lighted) immediately after experiment I and maintained on this schedule throughout experiment II ($n = 144$ mice, body weight $= 25.4 +$ 0.4 g). Thus, the change from experiments I–II was an 180 degree shift in lighting schedule although each mouse was still injected at the same clock hour. As a general rule 3–6 days are required for a 6 h (90 degree) phase shift[2], therefore 14 days of resynchronization stimulus should have sufficed for a 12-h time shift in the morphine response. Presence of pain was determined by a modified Haffner tail pinch clamp test[10]. All statistics were performed by the methods of SNEDECOR[11]. The experimental details have been reported earlier[9].

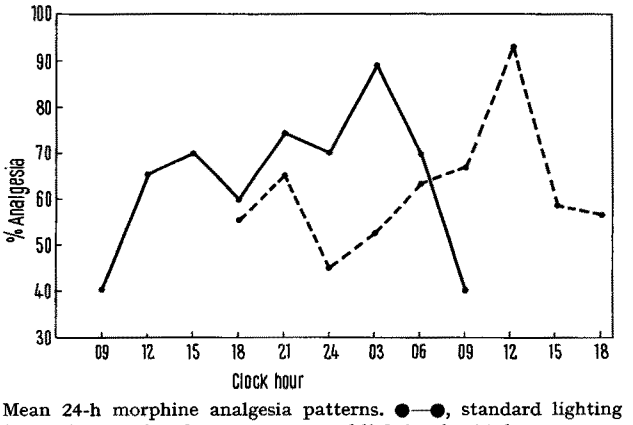
The mean 24-h morphine analgesia patterns for experiments I and II are shown in the Figure with mean percent analgesia of $67.4 \pm 5.0$ and $62.6 \pm 5.1\%$ respectively. By analyses of variance there is no significant difference ($p = 0.1$) between the individual responses within the dark or within the light phases of experiment I. However, there is a significant difference ($p = 0.02$) between the pooled responses of the light and dark phases. Of all the 3 h ratio responses only the 03.00 h crest ($p = 0.02$) and the 09.00 h trough ($p = 0.001$) differ significantly from the mean 24-h response. A highly significant difference exists between the crest and trough ($p = 0.001$) as demonstrated by Chi-square analysis.

Analysis of experiment II showed no statistical difference between the light and dark phase percent responses ($p = 0.2$); however, a Chi-square test of each 3 h ratio response against the mean 24-h morphine ratio response did demonstrate a crest at 12.00 h ($p = 0.01$) and possibly, a trough at 24.00 h ($p = 0.09$). The ratio responses for the crest and trough were highly significantly different ($p = 0.001$). Since no significant difference existed between experimental days ($p = 0.3$), the data from experiments I and II were pooled into dark and light phases for which a significant difference ($p = 0.01$) was demonstrated.

The data indicate that the crests and probably the troughs of morphine induced analgesia can be resynchronized by altering the light-dark regimen. Consequently the analgesia peak, which 14 days earlier had been at 03.00 h, now after light reversal occurs at 12.00 h. In like manner, the trough shifted from 09.00 to 24.00 h. The crest occurs 6–9 h after the lights go off and the troughs 3–6 h after the lights turn on, regardless of the actual clock hour. 2 weeks subjection of mice to a reversed light-dark cycle appeared to be adequate time for resynchronization of the morphine induced analgesia pattern. Thus, for morphine analgesia, the inversion of the light-dark regimen can be employed to shift the temporal placement of the peak and trough hours within a 24-h time scale.

This investigation, as well as previous morphine studies[9], infers a probable relationship between motor activity and morphine susceptibility pattern in mice. The nocturnal mouse is more susceptible to morphine analgesia during its active dark than inactive (light) phase. In man, however, where social routine is the dominant synchronizer, one would expect man to be more susceptible to morphine analgesia during the daylight hours at which time greater activity is exhibited. Subjective clinical observations tend to support this impression. Some of the unexplicable clinical responses following morphine therapy could very well be related to time of administration, sleep habits, occupation, environmental conditions, etc. of the patient.

The results of our studies suggest that either, the quantity of administered morphine interfering with cortico-thalamic transmission (i.e. a site of morphine analgesic action) remains constant throughout the 24-h time study and the pain threshold varies during the circadian cycle of the mice or the quantity of morphine arriving at and reacting with its receptor site displays a rhythmic variation along a 24-h time scale. This latter effect could




Mean 24-h morphine analgesia patterns. ●—●, standard lighting from 06.00 to 18.00 h; ○—○, reversed lighting for 14 days.


[1] E. BUNNING, *The Physiological Clock* (Springer, Heidelberg 1964).
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[3] F. HALBERG, M. B. VISSCHER and J. J. BITTNER, Am. J. Physiol. *179*, 229 (1954).
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[10] C. BIANCHI and J. FRANCESCHINI, Br. J. Pharmac. *9*, 280 (1954).
[11] G. W. SNEDECOR, in *Statistical Methods* (Iowa State University Press, Ames 1956).

be the result of a circadian variation in the detoxification and/or distribution of morphine, or variable permeability of the blood brain barrier with time of day, or other 24-h changes in physiological systems. However, the rate of morphine destruction would appear to play but a minor role in the morphine rhythms demonstrated in this paper since analgesic activity was determined only 20 min after the morphine administration. Studies have been initiated to explore the relationship between pain threshold and morphine analgesia rhythms.

*Zusammenfassung.* Durch Tag-Nacht-Umkehr konnte bei Mäusen eine entsprechende Phasenverschiebung beim Muster der Sensibilität für die schmerzstillende Wirkung des Morphins erzielt werden.

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## Kompensation der teratogenen Wirkung eines Thalidomid-Metaboliten durch L-Glutaminsäure

Der Thalidomid-Metabolit N-Phthalyl-DL-glutaminsäure[1] weist bei der SWS-Maus nach i.p. Applikation eine starke embryotoxische Aktivität auf[2], die ausschliesslich auf das L-Isomere zurückgeht[3]. In der vorliegenden Arbeit soll untersucht werden, ob die embryotoxische Wirkung der N-Phthalyl-L-glutaminsäure (N-P-L-Glu) durch zusätzliche Verabreichung von L- bzw. D-Glutaminsäure (L-Glu bzw. D-Glu) reduziert oder vollständig kompensiert werden kann.

Die Versuche erfolgten wieder an der Maus (SWS); Angaben über Tierhaltung, künstliche Besamung sowie die Methode der Schnittentbindung und Untersuchung auf embryotoxische Wirkung finden sich unter l.c.[2]. Synthese und physikalische Daten der verabreichten N-P-L-Glu wurden ebenfalls früher mitgeteilt[3]. Zur Herstellung der Injektionslösung versetzte man in einem 25-ml-Erlenmeyerkolben 400 mg fein gepulverte N-P-L-Glu mit 5 ml Tween 20[4], rührte 30 min mit einem Magnetrührer und tropfte dann langsam unter fortwährendem Rühren 15 ml physiologische Kochsalzlösung zu. Die Lösung war nach ca. 5 min klar und kam innerhalb 1 h zur Anwendung. Alle Verabreichungen erfolgten durch i.p. Injektion als einmalige Dosis.

Den Mäusen in Gruppe I–IV applizierten wir am Tag 9 p.c. je 400 mg/kg N-P-L-Glu in Form der oben beschriebenen Lösung (Tabelle I). Zusätzlich erhielten die Tiere in Gruppe II–IV nach 1–3 min eine zweite Injektion, und zwar je 50, 100, 200 bzw. 400 mg/kg L-Glu[5] in Gruppe II, je 400 mg/kg D-Glu[6] in Gruppe III sowie 40 ml/kg physiologische Kochsalzlösung in Gruppe IV; die Lösungen der L- und D-Glu waren 2%ig in physiologischer Kochsalzlösung und unmittelbar vor der Injektion frisch zubereitet.

Zur Kontrolle bekamen am Tag 9 p.c. die Tiere in Gruppe V je 40 ml/kg physiologische Kochsalzlösung und in Gruppe VI je 40 ml/kg der Mischung physiologische Kochsalzlösung/Tween 20 = 3:1.

Allen in Tabelle II aufgeführten Tieren wurden 9 Tage 0 h p.c. je 400 mg/kg N-P-L-Glu appliziert. Jeweils 4 Mäuse hatten bereits 3, 6, 12 bzw. 18 h vor diesem Zeitpunkt je 400 mg/kg L-Glu erhalten; jeweils 4 anderen

[1] J. W. Faigle, H. Keberle, W. Riess und K. Schmid, Experientia *18*, 389 (1962).
[2] F. Köhler und H. Ockenfels, Experientia *26*, 1157 (1970).
[3] H. Ockenfels und F. Köhler, Experientia *26*, 1236 (1970).
[4] Tween 20 rein, M∼1200; Serva GmbH & Co., Heidelberg.
[5] L(+)-Glutaminsäure für biochemische Zwecke (LAB); Merck, Darmstadt.
[6] D-Glutaminsäure puriss.; Fluka AG, Buchs, Schweiz.

Tabelle I. Versuche zur Kompensation der embryotoxischen Wirkung von N-Phthalyl-L-glutaminsäure durch zusätzliche Verabreichung von L- bzw. D-Glutaminsäure (SWS-Maus)

| Gruppe Nr. | Verabreichte Substanzen | Dosis[c] | Muttertiere | Implantationen | Resorptionen | | Feten Gesamt | Missgebildet | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | I | R | R/I (%) | F | M | M/F(%) |
| I | N-Phthalyl-L-glutaminsäure[a] | 400 mg/kg | 10 | 134 | 75 | 56,0 | 59 | 56 | 94,9 |
| II | N-Phthalyl-L-glutaminsäure[a] + L-Glutaminsäure[b] | 400 mg/kg + 50 mg/kg | 5 | 68 | 29 | 42,6 | 39 | 34 | 87,2 |
| | | 100 mg/kg | 5 | 64 | 18 | 28,1 | 46 | 26 | 56,5 |
| | | 200 mg/kg | 5 | 61 | 13 | 21,3 | 48 | 11 | 22,9 |
| | | 400 mg/kg | 5 | 72 | 6 | 8,3 | 66 | 0 | 0 |
| III | N-Phthalyl-L-glutaminsäure[a] + D-Glutaminsäure[b] | 400 mg/kg + 400 mg/kg | 5 | 74 | 25 | 33,8 | 49 | 49 | 100 |
| IV | N-Phthalyl-L-glutaminsäure[a] + physiol. NaCl-Lösung | 400 mg/kg + 40 ml/kg | 5 | 67 | 52 | 77,6 | 15 | 15 | 100 |
| V | physiol. NaCl-Lösung | 40 ml/kg | 10 | 138 | 2 | 1,4 | 136 | 0 | 0 |
| VI | physiol. NaCl-Lösung/Tween 20 (3:1) | 40 ml/kg | 20 | 287 | 11 | 3,8 | 276 | 0 | 0 |
| VII | Normaltiere | – | 30 | 399 | 12 | 3,0 | 387 | 0 | 0 |

[a] 2%ige Lösung in einer Mischung aus physiologischer Kochsalzlösung und Tween 20 im Verhältnis 3:1. [b] 2%ig in physiologischer Kochsalzlösung; 1–3 min nach der ersten Injektion appliziert. [c] Alle Verabreichungen erfolgten am 9. Tag p.c. als einmalige i.p. Injektion.