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Test Type Influences the Expression of Lithium Chloride-Induced Hyperalgesia

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MCNALLY, G. P. AND R. F. WESTBROOK. *Test type influences the expression of lithium chloride-induced hyperalgesia.* PHARMACOL BIOCHEM BEHAV **61**(4) 385–394, 1998.—The hyperalgesic properties of the emetic drug lithium chloride (LiCl) were examined in eight experiments. At a dose of 63.6 mg/kg, LiCl produced hyperalgesia in the radiant-heat (Experiment 1a) and immersion (Experiment 1b) tail-flick tests. At doses of 15.9, 31.8, 63.6, and 127.2 mg/kg, LiCl failed to produce hyperalgesia during the delayed behavioral response in the formalin test (Experiments 2a and 2b), but 63.6 mg/kg LiCl did produce hyperalgesia during the normally quiescent, interphase period of formalin responding (Experiment 2c). At the dose of 63.6 mg/kg, LiCl did not produce hyperalgesia in the hotplate test (Experiments 3a and 3b) and did not exert significant motoric effects in a step-down passive-avoidance task (Experiment 4). The results were discussed with reference to the behavioral effects of LiCl and their implications for demonstrations of associatively mediated morphine analgesic tolerance. © 1998 Elsevier Science Inc.

Lithium chloride-induced hyperalgesia Hyperalgesic properties Tail-flick test

SYSTEMIC administration of the emetic drug lithium chloride (LiCl), the bacterial endotoxin lipopolysaccharide (LPS), or the cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α, produces hyperalgesia in rats (15,28,34). Illness-induced hyperalgesia is produced initially via activation of the vagus nerve by cytokines released from hepatic macrophages, because hyperalgesia is abolished by sectioning hepatic vagal afferents, destruction of hepatic macrophages by gandolinium chloride, or intraperitoneal (IP) administration of an IL-1β receptor antagonist (16,26,27,28). Although the forebrain mechanism for illness-induced hyperalgesia remain unclear, lesion and infusion studies have identified the critical hindbrain and spinal components of this neural circuitry. Specifically, these studies have confirmed a role for pathways originating in the nucleus raphe magnus of the rostral ventromedial medulla, descend via the dorsolateral funiculus to the dorsal horn where they modulate nociceptive transmission by recruiting excitatory amino acid and cholecystokinin systems (26,33).

Evidence for hyperalgesic responses to illness-inducing agents has been obtained typically with the radiant-heat tail-flick test, although such responses have also been reported in the rat-paw formalin test following injecting of LPS and IL-1β (28,34). However, there has been no systematic investigation into the effect of type of noxious stimulation on the expression of illness-induced hyperalgesia. There are several reasons for expecting an influence of the type of noxious stimulation on the expression of hyperalgesia. Firstly, the type of noxious stimulus used to assay the nociceptive modulatory effects of three, 0.75 s, 1-mA tail shocks determines whether hypoalgesia or hyperalgesia is observed (13,14). Secondly, the type of noxious stimulus modulates the outcome of activity in antinociceptive systems. For example, testing rats in the presence of learned danger signals produces a naloxone-insensitive hypoalgesic response when assayed by a thermal nociceptive stimulus, such as a hot plate, but a naloxone-sensitive response is observed when assayed by a noxious chemical stimulus, such as dilute formalin (5). Finally, there have been demonstrations that the type of noxious stimulus used on test determines the neural circuitries underlying morphine analgesia, as well as the efficacy of opiates in suppressing nociceptive responding. For example, the tail flick and formalin tests differ critically in the importance of forebrain and cortical circuitries for the expression of morphine analgesia (17,18).

The present experiments investigated whether the expression of LiCl-induced hyperalgesia is influenced by the type of noxious stimulation employed. Rats were injected IP with LiCl and tested in the radiant-heat and immersion tail-flick, formalin, and hot-plate tests. A passive avoidance test sensitive to the motoric/sedative effects of benzodiazepines (9,10,29) was used to examine any LiCl-induced motoric/sedative and

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postural abnormalities (e.g., ataxia, lying on the belly) (3,21,22) that could have interfered with the expression of hyperalgesia in tests where nociceptive responding requires coordinated actions.

EXPERIMENTS 1A AND 1B

Experiments 1a and 1b examined the hyperalgesic effects of LiCl in the radiant-heat and immersion variants of the tail-flick test. Rats were injected IP with 63.6 mg/kg LiCl or saline and tested in either the radiant-heat (Experiment 1a) or immersion (Experiment 1b) tail flick tests. In both measures, tail-flick latencies were assessed once every 5 min for 55 min following injection of LiCl.

METHODS

Subjects

The subjects were experimentally naive male Wistar rats weighing between 300 and 400 g. They were obtained from the colony of Specific Pathogen Free rats maintained by the Combined Universities Laboratory Animal Services (Little Bay, Sydney). Rats were housed in opaque, white plastic boxes (65 cm long × 40 cm wide × 22 cm high) with eight rats per box. The wire mesh roof of each box held food and water bottles that were continuously available. The boxes were kept in a colony room maintained under natural lighting. There were 16 rats in Experiment 1a and 16 rats in Experiment 1b. The experiments were conducted between 0900 and 1700 h. The procedures used in these experiments were approved by the Animal Care and Ethics Committee at the University of New South Wales.

Apparatus

The radiant-heat tail-flick apparatus consisted of a 45-cm long × 40-cm wide × 20-cm high metal box that supported a 15-cm long × 4-cm wide Perspex plate. A shallow groove was cut in this plate into which the rat's tail was placed during a test trial. A General Electric 150-W projector spotlight was mounted above the slot. A condenser lens was located between the light source and the rats tail and focussed the light beam. A lateral deflection of the tail of 5 mm activated a photocell receiver that automatically terminated the test trial and recorded tail-flick latency. The heat was adjusted so that naive control animals showed tail-flick latencies between 5 and 6 s. The tail-immersion apparatus consisted of a 40-cm long × 30-cm wide × 35-cm deep glass water bath the temperature of which was controlled at 51°C (± 0.5°C) by an Open Bath Thermoregulator (Ratek Instruments, Melbourne). The temperature was chosen to produce tail-flick latencies in naive animals comparable to those obtained in the radiant heat tail-flick test. Latency to completely remove the tail from the water bath was recorded by stopwatch. Both the radiant-heat and tail-immersion apparatus were located in a laboratory, the ambient temperature of which was maintained between 21 and 23°C. The laboratory also contained plastic buckets (26 cm diameter × 45 cm height), with air holes drilled in the lid and sides. These buckets served as chambers in which rats were kept in isolation when they were brought into the laboratory.

Drug

Lithium chloride anhydrous (Becton Dickinson, Sydney) was dissolved in distilled water to yield a concentration of 6.36 g/l (0.15 M) and was injected IP with 26-gauge needles in a volume of 10 ml/kg, producing a dose of 63.6 mg/kg. Isotonic nonpyrogenic saline (0.9% w/v) was used for control injections and injected in the same volume as LiCl.

Procedure

Rats were handled each day for 1 min for 5 days prior to the start of the experiments.

Familiarization. Across days 1 to 4 of the experiments, rats were transported to the laboratory. On arrival rats were placed in the plastic buckets for 20 min, removed, handled, and returned to the buckets. For rats in Experiment 1a this handling consisted of being placed in the radiant-heat tailflick apparatus with the tail placed in the Perspex groove, for 8 s. For rats in Experiment 1b handling consisted of being held by the experimenter for 8 s. Rats were familiarized to prevent fear, novelty-induced hypoalgesia or both on test.

Test. On day 5 rats were randomly allocated to either LiCl ($n = 8$) or saline ($n = 8$) groups and tested using either the radiant-heat (Experiment 1a) or tail-immersion (Experiment 1b) tests. For rats in Experiment 1a, baseline tail-flick latencies were determined by taking the average of the last three out of four tail flick trials spaced 2 min apart. Radiant-heat was focussed on the dorsal surface 4 cm from the tip of the tail. For rats in Experiment 1b, baseline latencies were determined by taking the average of the last three out of four tail-flick trials spaced 5 min apart. The distal 4 cm of the tail was immersed in the water bath. Immediately following each trial, tails were wiped with a flannel cloth to prevent hot water clinging to the tail. In both Experiments 1a and 1b, rats were injected with LiCl or saline 5 min following final baseline trial, tail-flick latencies were assessed 5 min after injection and repeated once every 5 min for 55 min.

Statistical Analyses

Mean tail-flick latencies for tests 5 to 55 min following injection of LiCl or saline were analyzed by means of contrasts assessing differences between groups, and linear trend across minutes of test, and tested using a multivariate model. The Decision-Wise error rate ($\alpha = 0.05$) was controlled for each contrast tested using the procedure described by Hays (11).

RESULTS AND DISCUSSION

The mean and SEM tail-flick latencies for rats in Experiments 1a tested in the radiant-heat tail-flick test following IP injection of 63.6 mg/kg LiCl or equivolume saline are shown in the left panel of Fig. 1. There was no difference between groups in baseline tail-flick latencies ($F < 1$).

Inspection of this panel indicates that rats injected with LiCl responded faster than rats injected with saline, and that this hyperalgesic response persisted for only 25 min following injection of LiCl. The statistical analysis confirmed that injection of LiCl rendered rats hyperalgesic relative to rats injected with saline averaged across the 55 min test period, $F(1, 14) = 28.5$; $F_c = 4.6$. There was also evidence for a significant linear trend in tail-flick latencies over the test period ($F = 22.2$), and a significant interaction between this linear trend and the contrast-assessing differences between groups ($F = 23.2$), confirming that tail-flick latencies for LiCl-treated rats did not differ from those of control rats during the second half of the test period.

The mean and SEM tail-flick latencies for rats in Experiment 1b tested in the immersion tail-flick test after an injec-

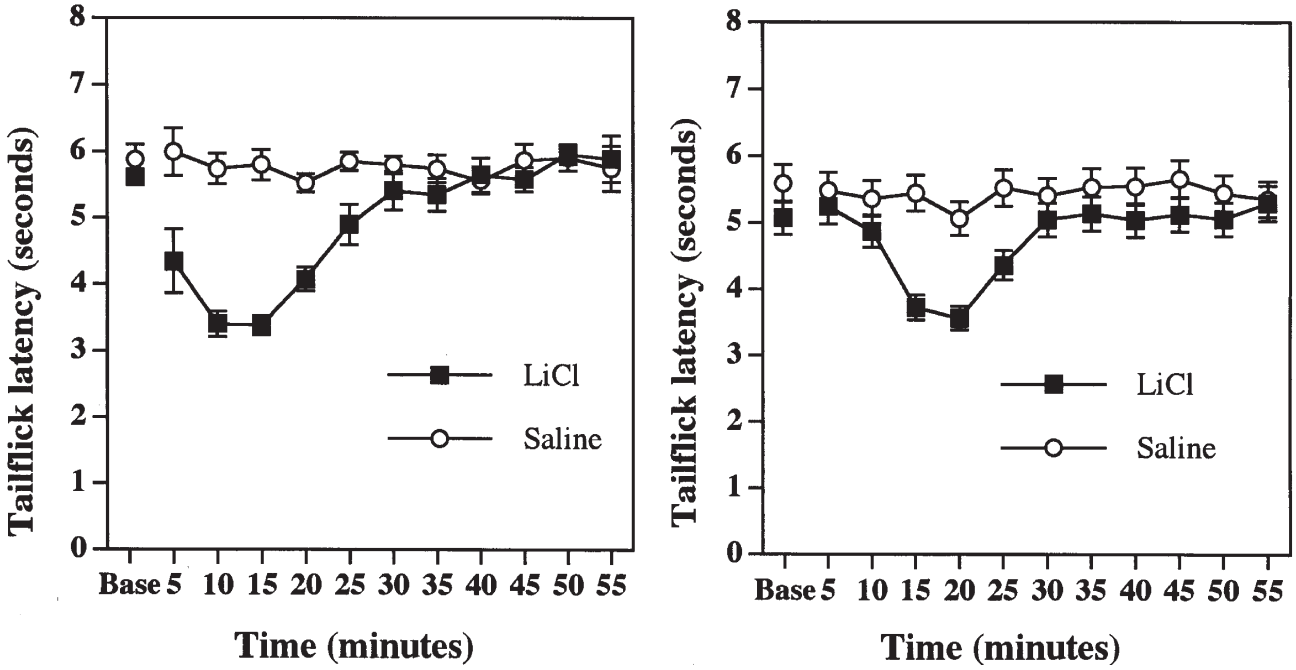

FIG. 1. Mean and SEM tail-flick latencies in the radiant heat tail-flick (Experiment 1a, left panel) and tail-immersion (Experiment 1b, right panel) tests. LiCl (63.6 mg/kg) produced hyperalgesia in both variants of the tail-flick test.

tion of LiCl or saline are shown in the right panel of Fig. 1. There was no difference in baseline tail-flick latencies between groups ($F < 1$). Inspection of this panel indicates that rats injected with LiCl responded faster than rats injected with saline. The time course of this hyperalgesia was similar to that observed using the radiant-heat tail-flick test in that the hyperalgesic response persisted for only 25 min following injection of LiCl. The statistical analysis confirmed that injection of LiCl rendered rats hyperalgesic relative to control rats injected with saline, $F(1, 14) = 18.4$; $F_c = 4.6$, averaged across the 55-min test period. There was also evidence for a significant linear trend in tail-flick latencies over the test period ($F = 18.4$) and a significant interaction between this linear trend and the contrast assessing differences between groups ($F = 9.4$), confirming that tail-flick latencies for LiCl-treated rats did not differ from those of control rats during the second half of the test period.

These experiments have confirmed that systemic injection of the emetic drug LiCl provokes hyperalgesia in rats when assayed by the radiant-heat tail-flick test. Moreover, these experiments have demonstrated that LiCl-induced hyperalgesia can also be detected in the hot water immersion variant of the tail-flick test.

EXPERIMENTS 2A, 2B, AND 2C

Experiments 2a, 2b, and 2c examined the hyperalgesic effects of LiCl in the rat paw formalin test. Injection of dilute formalin into the paw produces a concentration-dependent, biphasic, stereotypic pattern of responding in rats (2,6,12). Specifically, following injection of formalin rats display a characteristic favoring, lifting, licking, biting, and flinching of the injected paw for approximately 5 min. This immediate, "first phase" of responding is followed by a quiescent period characterized by an absence of responding directed at the formalin-injected paw that has been labeled the "interphase". Ten to 15 min after formalin injection, responding directed at the injected paw returns and persists for a further 30 to 40 min, the so-called "second-phase." As used here, the labels "first-", "inter" and "second" phases refer only to the distinct time course variations in behavioral responses to formalin. Systemic administration of LPS and IL-1β produces hyperalgesia in the formalin test (28,34). Thus, the present experiments examined whether systemic administration of LiCl also produced hyperalgesia in the formalin test.

In Experiment 2a rats were injected with a submaximal concentration of formalin (1%) followed 15 min later by an injection of either LiCl or saline. Rats were tested with doses of 15.9, 31.8, 63.6, or 127.2 mg/kg LiCl. Responding to the formalin-injected paw was recorded 10 min later for 15 min (25–40 min postformalin). In Experiment 2b rats were injected with either 1 or 1.5% formalin followed 15 min later by either 63.6 mg/kg LiCl or saline. Responding to the formalin-injected paw was recorded 10 min later for 15 min (25–40 min post formalin). Previous work from this laboratory has shown that 1% formalin produces submaximal responding in the present strain of rats (2). Accordingly, two concentrations of formalin were employed in Experiment 2b to confirm that the results of Experiment 2a were not affected by a ceiling on behavioral responding. In Experiment 2c, rats were injected with 63.6 mg/kg LiCl followed 10 min later by 1.5% formalin. Rats were tested immediately following formalin injection for a period of 15 min.

METHOD

Subjects

Subjects were experimentally naive adult male Wistar rats weighing between 300 and 400 g. They were obtained from

the same source and maintained under the same conditions described previously. There were 48 rats in Experiment 2a, 32 rats in Experiment 2b, and 16 rats in Experiment 2c. Experiments were conducted between 0900 and 1700 h.

Apparatus

Formalin testing was carried out in Plexiglas chambers (30 cm long × 30 cm wide × 30 cm high) with a mirror placed at 45° beneath the floor to permit an unobstructed view of the paws. The amount of time spent lifting and/or licking/biting the injected paw was recorded by pushing buttons connected to a computer. These chambers were kept in a laboratory the temperature of which was maintained between 21 and 23°C.

Drugs

Lithium chloride was prepared in the manner described for Experiments 1a and 1b, and injected in a volume of 2.5, 5, 10, or 20 ml/kg producing doses of 15.9, 31.8, 63.6, and 127.2 mg/kg LiCl, respectively. Isotonic nonpyrogenic saline (0.9% w/v) was used for control injections and injected in a volume of 20 ml/kg in Experiment 2a and 10 ml/kg in Experiments 2b and 2c. LiCl and saline were injected IP using 26-gauge needles. A saturated formaldehyde solution (37.5–40% w/v) was diluted in sterile nonpyrogenic saline (0.9% w/v) to obtain concentrations of 1.0 or 1.5% formalin, and injected subcutaneously into the plantar surface of the right hind paw in a volume of 50 μl using 29-gauge needles. During formalin injection rats were loosely wrapped in a flannel cloth, the right hind-quarters marked, the needle inserted between the distal tips of the two basal tori, and advanced 2–3 mm proximally.

Procedure

Rats were handled each day for 1 min for 5 days prior to the start of the experiments.

Familiarization. Across days 1 to 4 rats were transported to the laboratory. On arrival rats were placed in the Plexiglas chambers for 20 min. Rats were familiarized to prevent fear-induced, novelty-induced hypoalgesia or both on testing.

Test: Experiment 2a. On day 5 rats were randomly allocated to one of four doses of LiCl (n = 8 per dose) or saline (n = 8) then injected with SC formalin and placed in the Plexiglas chambers. Fifteen minutes later rats were removed from the Plexiglas chambers, injected IP with either LiCl or saline, and returned to the Plexiglas chambers. Responding to the formalin-injected paw was recorded 10 min later for 15 min (25–40 min after formalin injection).

Experiment 2b. On day 5 rats were randomly allocated to either 1 or 1.5% formalin groups (n = 16 per group), injected with SC formalin and placed in the Plexiglas chambers. Fifteen minutes later rats were removed from the Plexiglas chambers, half were injected with 63.6 mg/kg LiCl, whereas the remainder were injected with saline, and then returned to the Plexiglas chambers. The amount of time spent lifting or biting/licking the formalin-injected paw was recorded 10 min later for 15 min (25–40 min after formalin injection).

Experiment 2c. On day 5 rats were randomly allocated to either LiCl or saline groups and injected with either 63.6 mg/kg (n = 8) LiCl or equivolume saline (n = 8) and placed in the Plexiglas chambers. Ten minutes later rats were removed from the Plexiglas chambers, injected SC with 1.5% formalin, then returned to the Plexiglas chambers. The amount of time spent lifting or biting/licking the formalin-injected paw was then recorded for 15 min (0–15 min after formalin injection).

Statistical Analyses

The amount of time spent lifting or biting/licking the formalin injected paw was converted to a percentage of the total test period and analyzed by means of planned orthogonal contrasts. The Decision-Wise error rate (α = 0.05) was controlled

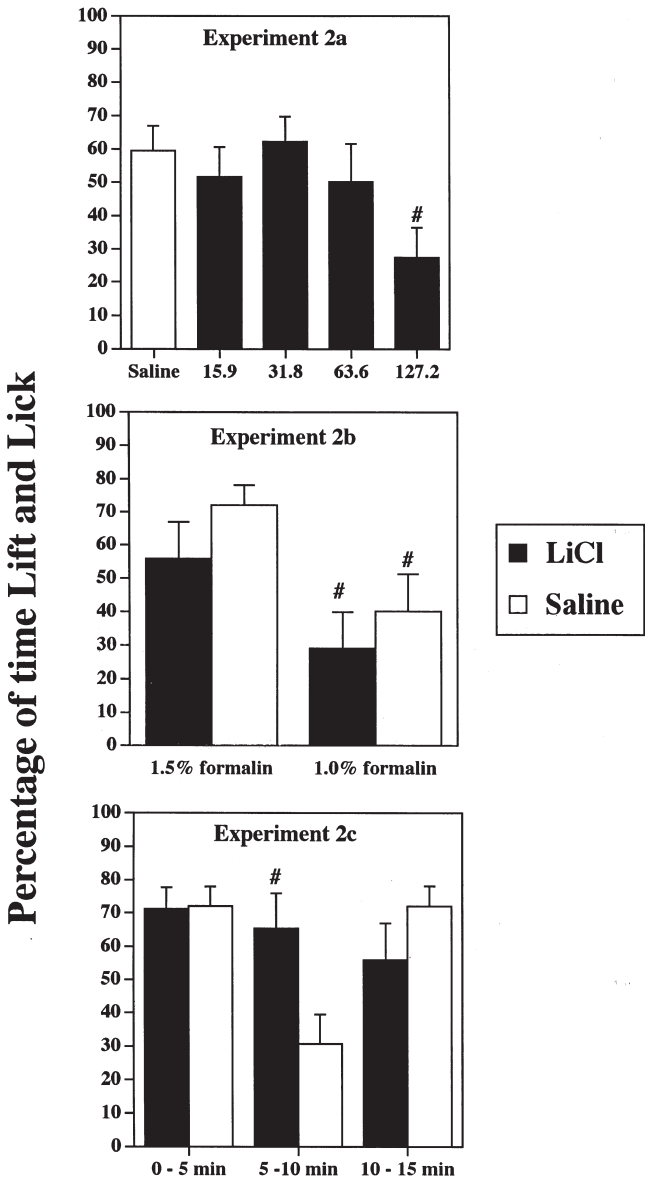

FIG. 2. Mean and SEM percentage of time spent lifting or licking/biting the formalin-injected paw in Experiment 2a (top panel), 2b (middle panel), and 2c (bottom panel). Rats tested under 127.2 mg/kg LiCl responded significantly less than rats tested under saline (#) (top panel). Rat tested with 1% formalin responded significantly less than rats tested with 1.5% formalin (#) (middle panel). LiCl produced hyperalgesia 5 to 10 min after injection of 1.5% formalin (#) (bottom panel).

for each contrast tested using the procedure described by Hays (11).

RESULTS

The mean and SEM percentage of time spent lifting or biting/licking the formalin-injected paw during the 15-min test period, 25–40 min after formalin, for rats injected with LiCl or saline in Experiment 2a is shown in the top panel of Fig. 2. Inspection of the panel indicates that there was no evidence for a hyperalgesic effect of LiCl at any of the doses tested. However, those rats tested with 127.2 mg/kg LiCl displayed less pain responding that rats in the saline control group, and rats tested with lower doses of the drug. There was no significant difference in the percentage of time spent responding to the formalin-injected paw between rats tested under LiCl and rats tested under saline, averaged across doses of LiCl, $F(1, 28) = 1.2$; $F_c = 4.2$. There was, however, a significant difference in the percentage of time spent responding to the formalin-injected paw between rats tested under 127.2 mg/kg LiCl and rats tested under lower doses of the drug ($F = 6.1$). There was neither a significant difference in percentage of time spent responding to the formalin-injected paw between rats tested under 63.6 mg/kg LiCl and rats tested under 15.9 and 31.8 mg/kg LiCl ($F < 1$), nor between rats tested under 31.8 mg/kg LiCl and rats tested under 15.9 mg/kg LiCl ($F < 1$).

The mean and SEM percentage of time spent lifting or licking/biting the formalin-injected paw during the 15 min test period, 25–40 min after formalin, for rats injected with LiCl or saline combined with either 1 or 1.5% formalin, in Experiment 2b is shown in the middle panel of Fig. 2. There was no overall difference in percentage of time spent responding to the formalin-injected paw between rats tested under 63.6 mg/kg LiCl vs. those tested under saline, averaged across formalin concentration, $F(1, 28) = 1.9$; $F_c = 4.2$. There was, however, a significant difference in the percentage of time spent responding to the formalin-injected paw between rats injected with 1 and 1.5% formalin, averaged across LiCl and saline ($F = 8.6$), such that rats injected with 1.5% formalin spent a greater percentage of time responding to the injected paw than rats injected with 1% formalin. This result confirms that injection of 1% formalin does not produce maximal responding under present conditions (2). However, there was no interaction between the contrast-assessing differences in percentage of time spent responding to the formalin-injected paw between rats injected with LiCl and saline, and the contrast-assessing differences between 1 and 1.5% formalin ($F < 1$).

The mean and SEM percentage of time spent lifting or licking/biting the formalin-injected paw during the 15-min test period, 0–15 min after formalin, for rats injected with LiCl or saline in Experiment 2c is shown in the bottom panel of Fig. 2. Inspection of the panel reveals that rats injected with LiCl and saline responded to the formalin injected paw for approximately 70% of the time during the first 5 min following formalin injection. This immediate, first-phase, of responding was followed by a 5-min period of reduced responding among rats injected with saline but not LiCl. This reduction in formalin responding is characteristic of the interphase of the behavioral response to formalin. Finally, 10–15 min after formalin injection, responding to the injected paw increased in both LiCl and saline-treated rats. During the first 5 min of formalin responding, the formalin first phase, there was no difference between LiCl and saline-treated rats in percentage of time spent responding to the formalin-injected paw, $F(1, 14) < 1$; $F_c = 4.6$. However, during the second 5 min of formalin responding, the formalin interphase, there was an overall difference between LiCl and saline-treated rats in percentage of time spent responding to the formalin-injected paw ($F = 10.8$), such that LiCl-treated rats responded to the injected paw for a greater percentage of time than rats injected with saline. There was no overall difference between LiCl and saline-treated rats in percentage of time spent responding to the injected paw during the third 5-min period of formalin responding, the start of the formalin second phase ($F < 1$).

Although providing evidence for LiCl-induced hyperalgesia in the formalin test, the present experiments suggest that the expression of this hyperalgesia is influenced by the time course of the behavioral response to formalin. Thus, evidence for LiCl-induced hyperalgesia was restricted to the 5–10-min period following formalin injection normally characterized by a diminution of the behavioral response. Indeed, rats tested with the highest dose of LiCl in Experiment 2a spent significantly less time responding to the formalin-injected paw, 25–40 min after formalin, than rats injected with lower doses of the drug. This apparent hypoalgesic response may have arisen from the irritating effects of the drug in the gut (35).

EXPERIMENTS 3A AND 3B

Experiments 3a and 3b investigated the hyperalgesic effects of LiCl in the hot-plate test. Rats were injected with either 63.6 mg/kg LiCl or saline and exposed for 30 s to the heated floor of a hot-plate apparatus, and latency to first paw lick was recorded. In both experiments the heated floor was maintained at 50, 52, or 54°C. Three floor temperatures were employed to avoid the influence of floor or ceiling effects upon the expression of hyperalgesia. Confined to the heated floor of a hot-plate apparatus for 30 s and tested the next day in that apparatus, rats display a pronounced conditioned hypoalgesia, reflecting a learned fear of the hotplate apparatus (8). This conditioned hypoalgesia is sensitive to variations in the temperature of the heated floor during initial exposure such that as floor temperature increases; so, too, does the level of conditioned hypoalgesia displayed on retest (8,29,30). It was reasoned that if LiCl had increased the functional intensity of the heated floor (i.e., produced hyperalgesia), this would be reflected not only by decreased paw-lick latencies during initial exposure but also by enhanced conditioning of hypoalgesic responses. Therefore, rats in Experiments 3a and 3b were retested the following day on a 52°C floor to examine any effects of LiCl on the acquisition of conditioned hypoalgesia. In Experiment 3a rats were simply reexposed to the 52°C floor, whereas in Experiment 3b rats were reexposed to the 52°C floor under the influence of the same drug (LiCl or saline) as test. This manipulation was employed to control for potential state-dependent learning effects of the expression of enhanced levels of conditioned hypoalgesia among LiCl-treated rats.

METHOD

Subjects

Subjects were experimentally naive adult male Wistar rats weighing between 300 and 400 g. They were obtained from the same source and maintained under the same conditions described previously. There were 48 rats in Experiment 3a and 46 rats in Experiment 3b. Experiments were conducted between 0900 and 1300 h.

Apparatus

The hot-plate apparatus consisted of a Plexiglas chamber (24 cm diameter × 48 cm height) that stood in a bath of water, the temperature of which was maintained by the thermoregulator described in Experiment 1b. A copper floor 1-mm thick was fixed 12 cm above the base of the chamber and the portion of the chamber below the copper floor was perforated with holes 3 cm in diameter to permit circulation of water beneath the floor. The latency with which rats licked their paws was recorded by pushing buttons connected to a computer. The temperature of the copper floor was measured using a digital thermal probe (Anritsu, Tokyo). The hot-plate apparatus was kept in a laboratory, the temperature of which was maintained between 21 and 23°C. The laboratory also contained the plastic buckets described in Experiments 1a and 1b, which were used to keep rats in isolation during familiarization, test, and retest.

Drugs

Lithium chloride was prepared in the manner described for Experiments 1a and 1b, and injected in a volume of 10 ml/kg producing a dose of 63.6 mg/kg. Isotonic nonpyrogenic saline (0.9% w/v) was used for control injections and injected in a volume of 10 ml/kg. LiCl and saline were injected IP using 26-gauge needles.

Procedure

Rats were handled each day for 1 min for 5 days prior to the start of the experiments.

Familiarization. Across days 1 to 4 rats in Experiments 3a and 3b were transported to the laboratory. On arrival rats were placed in the plastic buckets for 30 min and then placed in the hot-plate apparatus for 60 s. The water surrounding the floor of the hot-plate apparatus was maintained at 23°C. Rats were familiarized to eliminate the effects of novelty-induced hypoalgesia.

Test. On day 5 in Experiments 3a and 3b rats were placed in the plastic buckets for 15 min, removed, injected IP with either 63.6 mg/kg LiCl or saline, and returned to the plastic buckets for 15 min before being exposed for 30 s to the heated floor of the hotplate apparatus. The temperature of the water surrounding the floor was either 50, 52, or 54°C. In Experiment 3a there were eight rats tested with LiCl and eight rats tested with saline at each floor temperature. In Experiment 3b there were eight rats tested with LiCl at each floor temperature, six rats tested with saline at 50°C, and eight rats tested with saline at 52 and 54°C. The latencies with which rats licked their paws were recorded.

Retest: Experiment 3a. On day 6 rats were placed in the plastic buckets for 30 min before being exposed to the heated floor of the hot-plate apparatus. The temperature of the water surrounding the floor was 52°C. The latencies with which rats licked their paws were recorded. Rats were removed from the hot-plate apparatus immediately following first paw lick.

Experiment 3b. On day 6 rats were placed in the plastic buckets. Fifteen minutes later rats were removed from the buckets, injected IP with the same drug as day 5 (63.6 mg/kg LiCl or saline), and returned to the plastic buckets for a further 15 min before being exposed to the heated floor of the hot-plate apparatus. The temperature of the water surrounding the floor was 52°C. The latencies with which rats licked their paws were recorded. Rats were removed from the hot-plate apparatus immediately following first paw lick.

Statistical Analyses

The data were analyzed, and the Decision-Wise error rate was controlled by the same techniques described previously.

RESULTS

The mean and SEM latencies with which rats licked their paws following exposure on test to the heated floor in Experiment 3a are shown in the top left panel of Fig. 3. Inspection of the panel indicates that, whereas increases in the temperature of the heated floor decreased the latencies with which rats licked their paws, LiCl had no effect on paw-lick latencies. There was no evidence for an overall significant difference between paw-lick latencies of rats injected with LiCl and rats injected with saline, averaged across temperature of the heated floor, $F(1, 42) = 1.3$; $F_c = 4.1$. There was a significant linear trend in paw-lick latencies across floor temperatures, averaged across drug injected ($F = 41.7$), such that paw-lick latencies decreased linearly as a function of the increase in floor temperature. There was, however, no significant interaction between LiCl vs. saline and this linear decrease in paw-lick latencies ($F < 1$). Thus, LiCl failed to produce hyperalgesia under the present conditions in the hot-plate test.

The mean and SEM paw-lick latencies from retest when all rats were tested on the 52°C floor in Experiment 3a are shown in the top right panel of Fig. 3. As can be seen from the panel, paw-lick latencies on the 52°C floor were a positive function

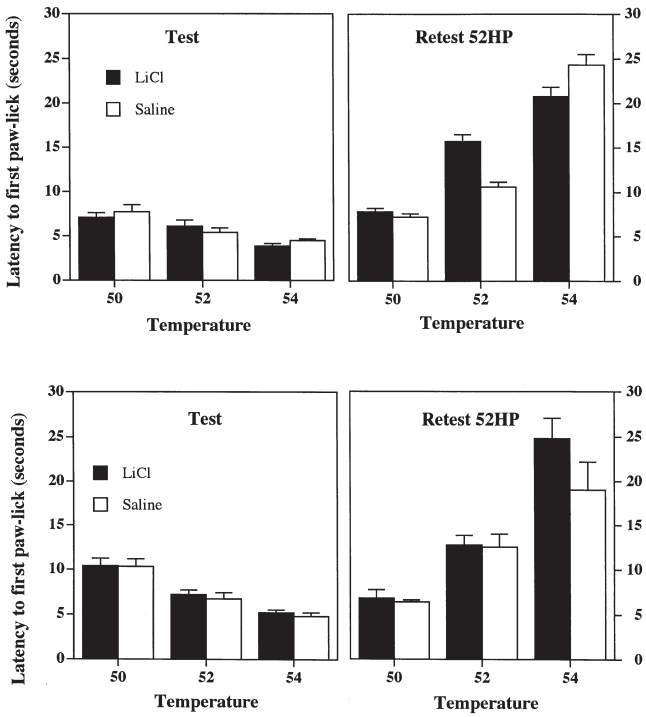

FIG. 3. Top panels: mean and SEM paw-lick latencies in Experiment 3a. Rats tested under 63.6 mg/kg LiCl failed to demonstrate hyperalgesia (left panel) or enhance conditioning of hypoalgesic responses (right panel) in the hotplate test. Bottom panels. Mean and SEM paw-lick latencies in Experiment 3b. Rats tested under 63.6 mg/kg LiCl failed to demonstrate hyperalgesia (left panel) or enhanced conditioning of hypoalgesic responses (right panel) in the hot-plate test.

of the temperature of the heated floor during testing. There was a significant trend in paw-lick latencies, averaged across drug injected, such that latency to first paw-lick on reexposure increased linearly as a function of floor temperature during exposure the previous day ($F = 55.1$). However, there was no overall difference in paw-lick latencies during reexposure to the 52°C floor between rats injected the previous day with LiCl and rats injected with saline ($F < 1$). There was also no evidence for an interaction in paw-lick latencies on the 52°C floor between drug injected the previous day and the temperature of the floor during initial exposure the previous day ($F < 1$). Thus, there was no evidence for enhanced conditioning of hypoalgesic responses when rats were retested in the absence of LiCl on a 52°C floor. Experiment 3b examined whether this absence of increased levels of conditioned hypoalgesia was due to state-dependent learning effects by testing rats under the influence of the drug used during conditioning.

The mean and SEM paw-lick latencies from Experiment 3b are shown in the bottom panels of Fig. 3. On test (bottom left panel), there was no evidence for an overall significant difference between paw-lick latencies of rats injected with LiCl and rats injected with saline, averaged across temperature of the heated floor, $F(1, 42) < 1$; $F_c = 4.1$). There was a significant linear trend in paw-lick latencies across floor temperatures, averaged across drug injected ($F = 68.5$), indicating that paw-lick latencies decreased linearly as a function of the increase in floor temperature. There was, however, no significant interaction between drug injected and this linear decrease in paw-lick latencies ($F < 1$). When reexposed to the 52°C floor the next day under the influence of the training drug (bottom right panel), there was a significant linear increase in paw-lick latencies as a positive function of floor temperature during initial exposure (i.e., conditioned hypoalgesia) ($F = 56.7$), but no effect of LiCl vs. saline on paw-lick latencies, averaged across floor temperature during initial exposure ($F = 1.7$), nor an interaction between LiCl versus saline and floor temperature during initial exposure ($F = 1.8$). Thus, the failure to observe enhanced conditioning of hypoalgesic responses among rats tested initially under the influence of LiCl, and later reexposed to the 52°C floor of the hotplate apparatus during retest in Experiment 3a, cannot be attributed to state-dependent learning processes.

These experiments have failed to provide evidence for LiCl-induced hyperalgesia in the hot-plate test, despite testing rats with a dose of the drug that produced a clear and potent hyperalgesia in the radiant-heat and immersion tail-flick tests. Moreover, there was no evidence that exposure to the heated floor of the hot-plate apparatus under the influence of LiCl affected the level of conditioned hypoalgesia displayed when rats were reexposed to the heated floor of that apparatus the following day. The failure to observe a hyperalgesic effect of LiCl during initial exposure to the heated floor in Experiments 3a and 3b cannot be attributed to a reduction in the aversive quality of the heated floor by LiCl-induced malaise, because there was no evidence for a reduction in the level of aversive conditioning resulting from that exposure as indexed by levels of conditioned hypoalgesia compared to saline controls.

EXPERIMENT 4

The failure to detect LiCl-induced hyperalgesia in the formalin and hot-plate tests may be attributable to motoric incapacitation or postural abnormalities produced by the drug (3,21,22). The present experiment examined this interpretation by testing LiCl-treated rats in a passive avoidance task. Rats in an experimental group (conditioned) were rendered fearful of the floor of the hot-plate apparatus by exposing them to a 54°C floor for 30 s, whereas rats in a control group were exposed to a nonheated floor for 30 s. The following day rats were tested for latency to step down off a platform onto the nonheated floor of the hot-plate apparatus under the influence of either LiCl or saline. This passive avoidance task is sensitive to the motoric/sedative effects of benzodiazepines at doses that impair responding in the hot-plate and formalin tests (9,10,29). A dose of 63.6 mg/kg LiCl was used in this experiment, as it produced hyperalgesia in the radiant-heat and immersion variants of the tail-flick test but not hot-plate test, and only during restricted portions of the time course of the behavioral response to formalin. If this dose of LiCl produces significant motoric/sedative or postural effects that prevented the expression of hyperalgesia in the formalin or hot-plate tests then it would be expected, like systemic administration of a benzodiazepine, to increase step-down latencies among conditioned and not conditioned rats, compared to control rats injected with saline (29).

METHOD

Subjects

Subjects were 20 experimentally naive adult male Wistar rats weighing between 300 and 400 g. They were obtained from the same source and maintained under the same conditions described previously. The experiment was conducted between 0900 and 1300 h.

Apparatus

The hot-plate apparatus was that described in Experiments 3a and 3b. A solid wooden platform that occupied a quadrant of the Plexiglas cylinder and whose top stood 9 cm from the floor was used for the step-down task. All other apparatus was that used in Experiments 3a and 3b.

Drugs

Lithium chloride was prepared in the manner described for Experiments 1a and 1b, and injected in a volume of 10 ml/kg producing a dose of 63.6 mg/kg. Isotonic nonpyrogenic saline (0.9% w/v) was used for control injections and injected in a volume of 10 ml/kg. LiCl and saline were injected IP using 26-gauge needles.

Procedure

Rats were handled each day for 1 min for 5 days prior to the start of the experiment.

Familiarization. Across days 1 to 4 rats were transported to the laboratory. On arrival rats were placed in the plastic buckets for 30 min and then placed in the hot-plate apparatus for 60 s. The water surrounding the floor of the hotplate apparatus was maintained at 23°C. Rats were familiarized to prevent novelty-induced hypoalgesia on conditioning and test.

Conditioning. On day 5 rats were placed in the plastic buckets for 30 min, before being exposed for 30 s to the hot-plate apparatus. The temperature of the water surrounding the floor was 54°C for rats in groups conditioned, and 23°C for rats in groups not conditioned. Rats exposed to the 54°C floor of the hot-plate apparatus paw-licked with latencies between 3.1 and 5.3 s, whereas rats exposed to the 23°C floor of the hot-plate apparatus did not paw-lick.

Test. On day 6 rats were placed in the plastic buckets for 15 min, after which time they were removed, injected with LiCl or saline, then returned to the plastic buckets for 15 min. Rats were tested by being placed on the wooden platform that stood on the 23°C floor of the hot-plate apparatus. There were five rats each in the four groups LiCl-conditioned, LiCl-not conditioned, saline-conditioned and saline-not conditioned. An observer pushed buttons connected to a computer to record the latencies with which rats stepped off the platform and placed all four paws on the floor.

Statistical Analyses

The data was analyzed, and the Decision-Wise error rate was controlled by the same techniques described previously.

RESULTS

The mean and SEM latencies to step-down the platform onto the 23°C floor for rats in each of the groups in Experiment 4 are shown in Fig. 4. Inspection of the figure indicates that rats preexposed to the 54°C floor (groups conditioned) stepped down with longer latencies than rats preexposed to the 23°C floor (groups not conditioned), however, 63.6 mg/kg LiCl did not appear to affect step-down latencies in either groups conditioned or not conditioned. The analysis confirmed that rats in groups conditioned were frightened because they took significantly longer to step down than rats in groups not conditioned, $F(1, 16) = 10.1$; $F_c = 4.5$. Conditioned rats that received an IP injection of LiCl did not differ in step-down latencies from those injected with saline ($F < 1$), nor did not conditioned rats injected with LiCl differ from those injected saline ($F < 1$). Thus, the results of the present experiment demonstrate that a dose of LiCl that was effective in producing hyperalgesia in the radiant-heat and immersion variants of the tail-flick test, but failed to produce hyperalgesia in the hot-plate test and produced hyperalgesia only during a restricted period of the behavioral response to formalin, did not exert significant motoric/sedative or postural effects in a step-down passive avoidance test. Specifically, there was no evidence that when tested with 63.6 mg/kg LiCl rats, which were either fearful of the floor of the hot-plate apparatus (group LiCl-conditioned) or not fearful of that floor (group LiCl-not conditioned), took longer to step down from the platform onto the nonheated floor than rats tested with saline. This failure to detect an increase in step-down latencies among rats injected with LiCl contrasts with the present sensitivity of step-down latencies to the effects of fear, as indexed by the overall difference between groups conditioned and not conditioned.

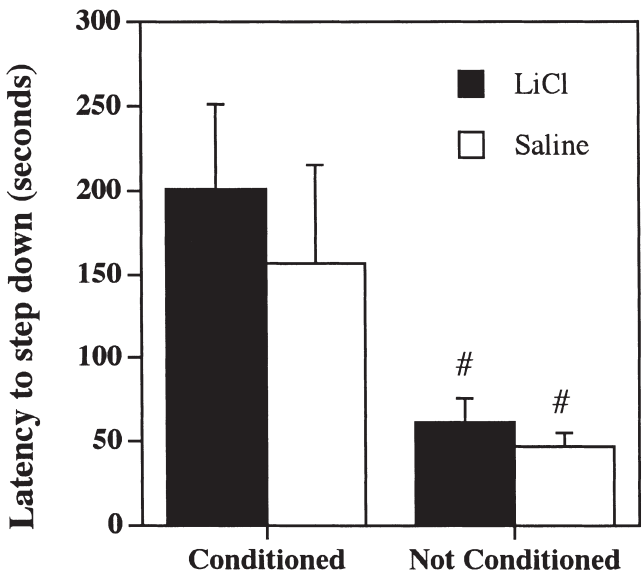

FIG. 4. Mean and SEM latency to step down onto the nonheated floor of the hotplate apparatus by rats previously exposed to a heated (groups conditioned) or nonheated (groups not conditioned) floor (Experiment 4). Rats in groups conditioned stepped down with longer latencies that group not conditioned (#). LiCl had no effect on step-down latencies.

GENERAL DISCUSSION

Injected with the emetic drug LiCl and tested in the radiant-heat and immersion variants of the tail-flick test, rats displayed a clear hyperalgesic response compared to control rats injected with saline (Experiments 1a and 1b). There was also evidence for LiCl-induced hyperalgesia during the interphase diminution, but not other periods, of the behavioral response to formalin (Experiments 2a, 2b, 2c), and tested with 127.2 mg/kg LiCl rats spent significantly less time responding to the formalin-injected paw than rats tested with lower doses of the drug (Experiment 2a). There was no evidence for LiCl-induced hyperalgesia in the hot-plate test (Experiments 3a and 3b). Finally, there was no evidence from a passive avoidance task that the differential effects of a moderate dose of LiCl in the tail-flick, hot-plate, and formalin tests could be attributed to motoric/sedative or postural effects of the drug (Experiment 4).

The step-down task used in the present experiments is sensitive to the motoric effects of benzodiazepines at doses that also impair responding in the formalin and hot-plate tests (9,10,29). Thus, the failure of 63.6 mg/kg LiCl to increase step-down latencies among rats that were previously exposed to either the heated or nonheated floor of the hotplate apparatus suggests that the differential effect of this dose across the variants of the tail-flick, hot-plate, and formalin tests cannot be attributed to motoric/sedative or postural effects of the drug. In addition, the results from retest in the hot-plate test in Experiment 3a directly address this issue because the test for enhanced acquisition of conditioned hypoalgesia was separated from exposure to the effects of LiCl. Thus, the failure on retest of rats tested initially under LiCl to display evidence for enhanced acquisition of hypoalgesic responses strongly suggests that the failure to observe hyperalgesia was not due to motoric/sedative or postural effects of the drug preventing the expression of this hyperalgesia. These findings, combined with the observation of LiCl-induced hyperalgesia during the interphase diminution of the behavioral response to formalin that employed the same behavioral end point, argue strongly against an interpretation that accords a causal role to motoric/sedative effects or postural abnormalities produced by LiCl in the failure to observe hyperalgesia in the hot-plate test and latter periods of the behavioral response to formalin. Finally, it is worth noting the differential effects of LiCl across the four assays in the present experiments are preserved when rats are tested in the presence of contextual stimuli previously paired with LiCl, which elicit topographically distinct behavioral conditioned responses compared to the drug (20).

The present results confirm previous reports of LiCl-induced hyperalgesia in the radiant-heat tail-flick test and extend these observations to the tail-immersion variant of this measure. How-

ever, despite providing evidence for hyperalgesia, the effect of LiCl in the radiant heat tail-flick test differs from previous reports of LiCl-induced hyperalgesia. Although the maximal extent of hyperalgesia in the radiant-heat tail-flick test was similar to that previously reported (15,34), the present hyperalgesia was of shorter duration than previously reported, such that tail-flick latencies had returned to baseline levels within 30 min of LiCl administration in the present experiments, but have previously been found to be decreased for in excess of 55 min following LiCl administration. This discrepancy in the time course of LiCl-induced hyperalgesia may be due to differences in the dose of LiCl. In previous studies rats were teste for hyperalgesia following injection of 127.2 mg/kg LiCl (15,34), whereas in the present experiments rats were tested in the tail-flick tests following 63.6 mg/kg LiCl. Accordingly, these findings may be interpreted to mean that dose is an important determinant of the duration, but not maximal extent, of LiCl-induced hyperalgesia.

There was no evidence from the present experiments for a hyperalgesic effect of LiCl in the hotplate test at a dose of the drug effective in the tail-flick tests. Specifically, in the hotplate test 63.6 mg/kg LiCl failed to produce hyperalgesia across a range of floor temperatures or to enhance the acquisition of conditioned hypoalgesia. The first of these results does not appear attributable to either floor or ceiling effects masking LiCl-induced hyperalgesia because paw-lick latencies were sensitive to variations in temperature of the heated floor (50–54°C) The second of these results is more interesting because paw-lick latencies upon reexposure to the heated floor are sensitive to the effects of morphine and naloxone during initial exposure (29), and were sensitive to variations in the temperature of the floor during initial exposure. Thus, the failure to detect either hyperalgesia during initial exposure or enhanced conditioned hypoalgesia upon reexposure suggests that testing rats under 63.6 mg/kg LiCl did not alter the functional intensity of the heated floor. Finally, this failure to detect LiCl-induced hyperalgesia in the hot-plate test cannot be attributed to use of an ineffective dose as higher doses (127.2 mg/kg) produce hypoalgesia not hyperalgesia in the hot-plate test (35).

The results from the formalin test provide evidence for LiCl-induced hyperalgesia during the interphase diminution, but not later periods of the behavioral response to formalin. The presence of LiCl-induced hyperalgesia in the formalin test is consistent with the effects of IL-1β and TNF-α in this assay (28,34). However, the influence of timecourse on the expression of LiCl-induced hyperalgesia is difficult to interpret. Although responding to the injected paw is lower during the formalin interphase than any other period of the behavioral response, Experiment 2b directly examined and excluded the possibility of a potential ceiling on the behavioral response obscuring LiCl-induced hyperalgesia during the later periods of the behavioral response to formalin. It has been suggested that the diminution of responding during the formalin interphase represents an auto-analgesic response elicited by the initial tissue damage produced by injection of formalin (7). Accordingly, the presence of an auto-analgesic state may facilitate or be necessary for the expression of a hyperalgesic response to LiCl in the formalin test (32). Alternatively, the diminution of responding during the interphase may reflect competition between exploratory and pain-indicant behaviors (2). Thus, LiCl may have increased responding to the injected paw during this period by antagonizing the otherwise expected increase in exploratory activity shown by rats during the formalin interphase (2). Finally, LiCl may have acted like a benzodiazepine, $GABA_A$ receptor agonist (7), or decerebration (17) to selectively increase responding to the injected paw during the interphase.

The pattern of responding observed across the four measures in the present experiments suggests that there may be important differences in the sensitivity of the tail-flick, hotplate, and formalin tests to LiCl-induced hyperalgesia. Interestingly, these results may have some explanatory power in accounting for the test specific manifestations of associatively mediated morphine analgesic tolerance. Several authors have reported that associatively mediated morphine analgesic tolerance is detected using the tail flick (19,25), but not formalin (1) or hot-plate tests (4,31). The activation of endogenous hyperalgesic circuitries has been accorded a central role in mediating associatively mediated morphine analgesic tolerance (23,24). The present experiments suggest that the two tests insensitive to associative morphine analgesic tolerance are also relatively insensitive to activity in endogenous hyperalgesic circuitries produced by the emetic drug LiCl.

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