Assessment of Behavioral Disruption in Rats with Abdominal Inflammation Using Visual Cue Titration and the Five-choice Serial-reaction Time Task

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ABSTRACT

Background: Both acute and chronic pain result in a number of behavioral symptoms in patients, including cognitive effects such as decreased attention and working memory. Intraperitoneal administration of dilute lactic acid in rodents has been used to induce abdominal inflammation and produce effects in behavioral assays of both sensory-discriminative and affective pain modalities.

Methods: Intraperitoneal injection of dilute lactic acid was used to study the impact of abdominal inflammation on an operant task requiring sustained visual attention in rats (N = 7 to 15/group) that adapts dynamically to performance ability. The effects of ketoprofen and morphine on lactic acid–induced impairment were compared with those on the disruptive effects of scopolamine.

Results: Lactic acid impaired performance in a concentration-dependent manner, increasing the duration of cue presentation required to maintain optimal performance from 0.5 ± 0.2 s (mean ± SD) to 17.2 ± 11.4 s after the administration of 1.8% (v/v) (N = 13). The latency to emit correct responses and to retrieve the food reward were both increased by lactic acid. All effects of lactic acid injection were reversed by both ketoprofen and morphine in a dose-dependent manner. Scopolamine, however, produced dose-dependent, nonpain-related disruption in sustained attention that was not altered by either ketoprofen or morphine.

Conclusions: These data demonstrate that abdominal inflammation induced by lactic acid produces robust disruption in a visual attention-based operant task and that this disruption is reversed by analgesics. Future studies will focus on pain-related circuitry and its impact on both limbic forebrain and frontal cortical mechanisms. **(Anesthesiology 2017; 127:372-81)**

PAIN is a multisensory experience comprising sensory/discriminative and affective/motivational components, which in turn influence cognitive ability.[1–4] Efforts have been directed in recent years to develop paradigms that complement sensory/discriminative measures of pain and include other behavioral domains, including cognition. To this end, a rodent gambling task has proven to be sensitive to knee pain induced by intraarticular injection of irritants in rats.[5,6] This task is a correlate of the Iowa Gambling Task used to assess executive function, decision-making, and risk-taking behavior in humans. Importantly, in humans performance on the Iowa Gambling Task is disrupted by pain.[7] Other cognitive domains are disrupted by pain in humans as well, including working memory and attention, and chronic pain is associated with loss in frontal cortical gray matter.[8–11] Several behavioral paradigms have been developed in rodents to assess attention, and one commonly used paradigm is the five-choice serial-reaction time task.[12,13] This procedure requires the subject to identify which of five apertures located along one wall is illuminated briefly. If the animal correctly identifies the aperture by poking its nose into the opening, the subject is rewarded with food. In this manner, the animal must sustain attention in discreet trials to obtain food reward. An intact prefrontal cortex is required for efficient performance of this task, and neuronal activity in this region is influenced negatively in both rats and humans by pain.[12,14–17]

What We Already Know about This Topic

- Better nonevoked animal models to study antinociceptive effects of analgesics are needed, which can inform on and translate to human pharmacology
- Intraperitoneal lactic acid in rodents has been used to cause abdominal inflammation and nociception
- Affective models of disrupted behavior have been used to study rodent response to nociception

What This Article Tells Us That Is New

- Performance in an operant task requiring sustained visual attention was developed as a rat model to study responses to nociception from intraperitoneal lactic acid–induced acute abdominal inflammation
- Known analgesics morphine and ketoprofen dose-dependently reversed the effects of abdominal inflammation, but not the effects of the attention disruptor scopolamine, on performance deficits

We recently developed a titration variant of the classical five-choice serial-reaction time task procedure in which the duration of aperture illumination varies dynamically between trials based on performance.[18] With this paradigm, the aperture is illuminated (cue duration) for a relatively

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long duration of 30 s in the first trial. If the animal responds correctly, then the cue duration decreases in the next trial. The cue duration continues to decrease with subsequent correct responses, following a preprogrammed time array. When the animal fails to respond to the visual cue (omission) or responds in the incorrect aperture, the cue duration increases. Advantages of this cue titration variant of the five-choice serial-reaction time task (5CTV) are the wide dynamic range of behavioral measurements and task difficulty, as well as the systematic and automated titration of task difficulty depending on individual performance capability. One other advantage is the relatively short amount of training time required for animals to achieve stable performance criteria compared with the classical method.

In this study, we assessed the ability of acute abdominal inflammation to disrupt performance in the 5CTV assay, as well as the efficacy and potency of the analgesics morphine and ketoprofen. As a negative control for the relevance of pain, we assessed the disruptive effects of scopolamine in the 5CTV alone and in conjunction with these analgesics. These data demonstrate that acute abdominal inflammation disrupts performance in this operant model, that these effects are sensitive to clinically relevant opioid and antiinflammatory analgesics, and that these effects can be distinguished from generalized disruption in performance by scopolamine.

Materials and Methods

Subjects

Male Fisher 344 rats (N = 60; Harlan Industries, USA) were used for all studies. Animals were acclimated to the laboratory for a minimum of 7 days or until they achieved a body weight of at least 275 g. Animals were then singly housed and reduced to approximately 90% of their free-feeding weight and fed sufficient standard rat laboratory chow (Lab Diet, USA) to maintain proper growth based on published growth curves from the vendor. Rats were kept on a reversed light/dark cycle (dark 5:00 AM to 5:00 PM) in a room immediately adjacent to the behavioral laboratory. Water was available *ad libitum* at all times except during experimental sessions. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (Bethesda Maryland) and guidelines adopted by the International Association for the Study of Pain (Washington, DC) and were approved by the Wake Forest School of Medicine Animal Care and Use Committee (Winston-Salem, North Carolina).

Behavioral Procedures

Apparatus. Commercially available equipment was used for all studies (Med Associates, Inc., USA) and has been described previously.[18] Operant chambers were connected to a PC-compatible computer through a commercially available interface (Med Associates, Inc.) and controlled by a program written in Med PC-IV (Med Associates, Inc.).

Procedure. The 5CTV has been described previously, including the four discreet phases of training.[18] In summary, rats were first trained to nose poke in the receptacle for food delivery to obtain a single reward (45-mg chocolate-flavored purified rat chow pellet, formula F0299; Bio-Serv, Inc., USA). Each successful nose poke was accompanied by a brief tone (0.5 s), and rats were allowed to obtain a maximum of 100 pellets per 30-min session. Once each subject earned the maximum number of pellets for at least 2 consecutive days, the second phase of training was initiated. This phase consisted of training the subject to nose poke in the middle aperture located on the wall opposite of the food receptacle to obtain two pellets. Sessions consisted of 50 trials or 30 min. Each trial began with illumination of the light-emitting diode (LED) stimulus at the rear of the aperture for 30 s or until the animal made a nose poke response. Only nose pokes in the middle aperture were reinforced. If the animal did not respond within 30 s or made a response in one of the other four apertures, the LED was turned off and a 5-s time-out period initiated, after which the next trial began. Once animals responded correctly for a minimum of 45 of the 50 trials for three consecutive sessions, the next phase of the training was initiated. In the third phase of training, one of the five apertures was illuminated at random and the animal was required to respond in the illuminated aperture for food reinforcement. All other aspects of the procedure were the same as the second phase. Once subjects responded correctly for a minimum of 40 of 50 trials for three consecutive sessions, the final phase of training was initiated. In this phase, the duration that the LED cue was illuminated (cue duration) was varied systematically according to the array (in seconds): 30, 25, 20, 15, 10, 8, 6, 4, 2, 1, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1. The structure of each 5CTV session and of each individual trial is the same as that typically used for five-choice serial-reaction time task studies (see fig. 1 from Fizet *et al.*[13] for a visual depiction of typical trial and session structure). To summarize, the session began by illumination of the food receptacle light and delivery of two pellets into the food receptacle. The subject initiated trials by poking its head into the food receptacle, which began with illumination of the house light and turning off the food receptacle light after 2 s. After the house light had been illuminated for 5 s (intertrial interval), one of the aperture LEDs was illuminated at random for the cue duration, until the subject made a response, or within an imposed time limit (limited hold). The limited hold was set to the cue duration or to 5 s if the cue duration was less than 5 s (*i.e.*, the subject always had a minimum of 5 s to respond after illumination of the cue light). Correct responses during the cue duration or within the limited hold resulted in extinguishing the cue light, illumination of the food receptacle light, and delivery of two food pellets. There were no time requirements or limits for the subject to retrieve the food reward, and the next trial was initiated 2 s after detection of head entry into the food receptacle, signaled by extinguishing the food receptacle light. The next trial consisted of illumination of an aperture after the intertrial interval. If the subject made an incorrect response or failed to respond within the limited hold (omission), then both the

cue and house light were turned off and a 2-s time-out period was imposed. If a subject responded in any aperture during the time-out (time-out response), the time-out was reset to 2 s. In addition, if a subject responded in an aperture during the intertrial interval (before illumination of the cue light, premature response), a time-out period was initiated. After the time-out, the next trial was initiated, signaled by illumination of the house light, followed by illumination of the cue light after the intertrial interval of 5 s. The cue duration was decreased according to the array if the subject made correct responses or was increased with incorrect responses or omissions. In this manner, the cue duration is titrated to within the limits of performance capabilities of each subject throughout each individual session.[18] Sessions consisted of 100 trials or 30 min, whichever occurred first. No animals were excluded from this study, and the training period required was consistent with that published previously for the 5CTV procedure for all subjects.[18]

Behavioral Endpoints. The behavioral endpoints collected were cue duration for each trial, latency to each correct or incorrect response from initiation of the visual cue, latency to retrieve food reward after each correct response, number of correct responses, incorrect responses, omissions, premature responses (responses in aperture before illumination of cue), perseverative responses (multiple responses in aperture after a correct response in the same trial), time to completion of trials, and time-out responses. The median cue duration was the primary behavioral endpoint and was calculated in Excel (Microsoft Corporation, USA) with the cue duration values from trials 15 to 100 or from 15 to the final trial completed. The percentage of correct responses (total number of correct responses as a percentage of total number of trials completed), percentage of incorrect responses, and percentage of omissions were calculated as well and are presented as supplementary data (Supplemental Digital Content, http://links.lww.com/ALN/B466).

Drug and Lactic Acid Administration. Once the median cue duration did not vary by more than 15% from the mean for a minimum of five consecutive sessions for each subject, the effects of intraperitoneal administration of lactic acid or 0.9% (w/v) saline were assessed in 22 animals. Lactic acid (0.9, 1.8, 5.4% v/v, 1 ml/kg) was administered on Tuesdays or Fridays immediately before 5CTV sessions, and only one injection of lactic acid was given per week, consistent with other studies using this nociceptive stimulus.[19] In a separate group of 24 animals, ketoprofen (0.01, 0.03, 0.1, or 0.3 mg/kg) was injected subcutaneously 30 min before injection of either saline or 1.8% lactic acid intraperitoneally, and 5CTV sessions were conducted immediately after the intraperitoneal injection. These animals also were administered morphine (0.3, 1.0, or 3.0 mg/kg) subcutaneously 30 min before injection of saline or 1.8% lactic acid intraperitoneally, and 5CTV sessions were conducted in a similar manner. Animals received only one injection of lactic acid per week. In a separate group of 14 animals, scopolamine (0.03, 0.1, or 0.3 mg/kg) was administered subcutaneously 30 min before 5CTV sessions. These animals also received ketoprofen (0.3 mg/kg subcutaneously) or morphine (3.0 mg/kg subcutaneously) at the same time as injection of scopolamine (0.1 mg/kg subcutaneously) 30 min before separate 5CTV sessions. None of the animals received all injection combinations from each group; however, all animals received saline injections. All animals were randomized individually to treatment and treatment order, with the exception that each individual animal was limited to only one injection of lactic acid per week and received intraperitoneal saline injection on the other test day for that week, such that each dose of either ketoprofen or morphine was given in the same week for each animal in combination with either saline or lactic acid, consistent with previous studies using lactic acid.[19] The randomization for dose and drug order for each animal was obtained with the random number generator in Microsoft Excel and numerical coding for ketoprofen, morphine, or scopolamine dose or lactic acid concentration. Only animals that displayed stable performance in the 5CTV on Monday, Wednesday, or Thursday as defined previously were administered drugs or lactic acid on the following Tuesday or Friday. The experimenter was not blinded to treatment.

Data Analysis

All data analyses were performed with Prism 6.0 (GraphPad Software, Inc., USA) for Macintosh. A power analysis was performed with G*Power 3.1.9.2 for Macintosh (http://www.gpower.hhu.de/en.html) with F test and two-tailed one-way ANOVA parameters set to effect size of 0.7, alpha level at 0.05, power at 0.9, and number of groups set to five. The estimated effect size was based on median cue duration estimates of 0.5 ± 0.2 s (mean ± SD) at baseline and 20 ± 10 s after 1.8% lactic acid administration. The primary outcome measure for all pharmacologic manipulations was median cue duration, which was analyzed with two-tailed, one-way ANOVA. ANOVAs were performed separately with data from animals that received intraperitoneal lactic acid or intraperitoneal saline in combination with morphine or ketoprofen; however, the data from animals that received two saline injections were included in both analyses. *Post hoc* analyses were performed with the Bonferroni *t* test with correction for multiple comparisons. Secondary outcome measures analyzed were latency to correct responses and latency to retrieve food reward after correct responses, and these endpoints were analyzed similarly as median cue duration. The Robust regression and Outlier removal method to identify outliers was applied to the primary and secondary outcome measures for all groups, with false error rate Q set to 1%.[20] A two-tailed *P* value of 0.05 or less was considered statistically significant. All other behavioral endpoints collected are summarized in the supplementary data section (Supplemental Digital Content, http://links.lww.com/ALN/B466) but were not analyzed statistically.

Results

Effect of Lactic Acid Concentration on Performance in the 5CTV

Lactic acid increased the median cue duration in a concentration-dependent manner after intraperitoneal injection

(F[4,50] = 57.2, $P < 0.00001$, fig. 1). Neither intraperitoneal administration of saline nor 0.9% lactic acid significantly altered the median cue duration compared with baseline; however, both 1.8% and 5.4% lactic acid disrupted performance, increasing the median cue duration 34-fold and 60-fold compared with baseline, respectively. Injection of 5.4% lactic acid produced a significantly greater disruption in attention performance than 1.8% lactic acid ($P < 0.05$), and none of the rats were able to complete all 100 trials within 30 min after the administration of 5.4% lactic acid (data not shown). Representative visual cue duration titration curves are shown in the lower panel of figure 1.

Lactic acid affected both the latency to emit a correct response from the initiation of the visual cue (F[4,44] = 20.9, $P < 0.0001$) and the latency to retrieve the food reward after a correct response (F[4,440] = 6.1, $P < 0.001$) in a concentration-dependent manner (table 1). Both latency values were increased significantly after administration of 1.8% or 5.4% lactic acid compared with baseline values ($P < 0.05$) (table 1). The latency to respond correctly was increased 11.0 ± 3.8-fold after administration of 5.4% lactic acid compared with an increase of 1.5 ± 0.4-fold in the latency to retrieve the food reward.

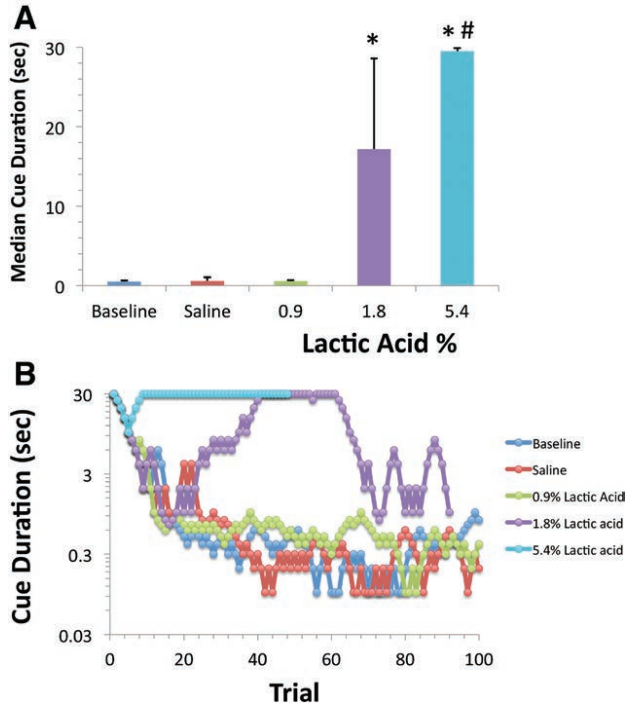

Fig. 1. Effect of lactic acid concentration on visual attention threshold in the five-choice serial-reaction time task. (*A*) Lactic acid increased the median cue duration in a concentration-dependent manner after intraperitoneal injection (F[4,50] = 57.2, $P < 0.00001$). Saline and 0.9% lactic acid did not alter the median cue duration from baseline. *$P < 0.05$ compared with baseline, saline, and 0.9% lactic acid; #$P < 0.05$ compared with 1.8% lactic acid. Mean and SD are shown, N = 7 to 13/group. (*B*) Representative visual cue duration titration curves are shown. Each curve represents an individual session of 100 trials on a given day and treatment.

Effects of Ketoprofen and Morphine on Lactic Acid–induced Impairment of 5CTV Performance

Median cue duration values at baseline or after pretreatment with saline, 0.03, 0.1, or 0.3 mg/kg of ketoprofen 30 min before administration of 1.8% lactic acid were compared. Ketoprofen reduced the effect of 1.8% lactic acid on median cue duration in a dose-dependent manner (F[4,67] = 20.1, $P < 0.0001$, fig. 2). The effect of 1.8% lactic acid on median cue duration was reduced significantly by administration of either 0.1 or 0.3 mg/kg of ketoprofen, and the median cue duration was not significantly different from baseline values after pretreatment with either dose of ketoprofen in combination with 1.8% lactic acid (fig. 2). Administration of saline intraperitoneally in combination with saline or any of the doses of ketoprofen subcutaneously did not result in any significant changes in median cue duration (F[4,61] = 2.1, $P = 0.09$, fig. 2). Representative visual cue duration titration curves are presented for a single animal in the lower panel in figure 2.

Ketoprofen produced a dose-dependent inhibition of the effects of 1.8% lactic acid on both the latency to emit a correct response (F[5,78] = 17.0, $P < 0.0001$) and the latency to retrieve the food reward after a correct response (F[5,73] = 13.2, $P < 0.0001$). Using the Robust regression and Outlier removal method in Prism 6.0, we identified three outliers within the latency to correct data set (one from the 0.03-mg/kg ketoprofen/lactic acid group and two from the 0.1-mg/kg ketoprofen/lactic acid group), and we identified eight outliers in the latency to reward data set (three from the saline/lactic acid group, three from the 0.03-mg/kg ketoprofen/lactic acid group, and two from the 0.1-mg/kg ketoprofen/lactic acid group). In all circumstances, the values of outliers were significantly greater than the group mean. Administration of 1.8% lactic acid increased both the latency to correct and the latency to reward compared with baseline, and these effects were inhibited by pretreatment with either 0.1 or 0.3 mg/kg of ketoprofen (table 2). Administration of saline or any of the doses of ketoprofen in combination with intraperitoneal saline (in the absence of lactic acid) did not alter either of the latency measures (data not shown).

Table 1. Effect of Lactic Acid on Response Latencies in 5CTV

% Lactic Acid	Latency to Correct	Latency to Reward
Baseline	1.15 (0.51)	1.49 (0.15)
Saline	0.96 (0.20)	1.65 (0.37)
0.9%	1.14 (0.40)	1.42 (0.24)
1.8%	3.96 (2.82)*†	2.12 (0.50)‡
5.4%	12.69 (7.56)‡§	2.21 (1.08)

Latencies (seconds, mean and SD) from presentation of the visual cue until a correct response is emitted (Latency to Correct) or from correct responses to retrieval of the food pellets (Latency to Reward) are shown.

*$P < 0.05$, ‡$P < 0.01$ compared with baseline. †$P < 0.05$, §$P < 0.01$ compared with saline using Bonferroni correction for multiple pairwise comparisons.

5CTV = cue titration variant of the five-choice serial-reaction time task.

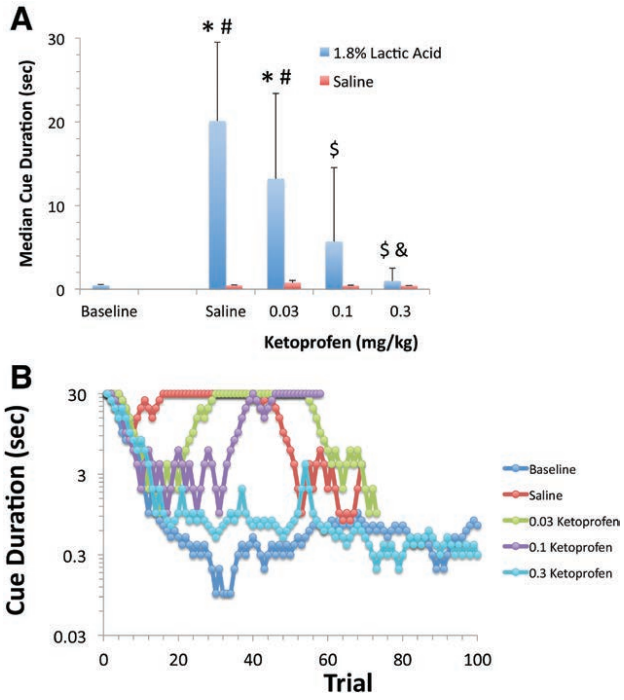

Fig. 2. Effects of ketoprofen on lactic acid–induced impairment of visual attention performance. (*A*) Ketoprofen reduced the effect of 1.8% lactic acid on median cue duration in a dose-dependent manner ($F[4,67] = 20.1$, $P < 0.0001$). Saline and ketoprofen at any dose had no effect on median cue duration in the absence of lactic acid administration. *$P < 0.05$ compared with baseline; #$P < 0.05$ compared with saline/saline; \$$P < 0.05$ compared with saline/1.8% lactic acid; &$P < 0.05$ compared with 0.03 ketoprofen/1.8% lactic acid. Mean and SD are shown, N = 10 to 15/group. (*B*) Representative visual cue duration titration curves are shown following treatment with 1.8% lactic acid in combination with indicated doses of ketoprofen or no treatment (baseline). Each curve represents an individual session of 100 trials on a given day and treatment.

The effects of morphine on 5CTV performance were assessed and analyzed statistically as described for ketoprofen. Morphine reduced the effect of 1.8% lactic acid on performance as measured with the median cue duration in a dose-dependent manner ($F[4,47] = 19.4$, $P < 0.0001$, fig. 3). The effect of 1.8% lactic acid on median cue duration was reduced significantly after pretreatment with 1.0 or 3.0 mg/kg of morphine, and the median cue duration was not significantly different from baseline values after pretreatment with 3.0 mg/kg of morphine in combination with intraperitoneal treatment with 1.8% lactic acid (fig. 3). Administration of saline intraperitoneally (in the absence of lactic acid) in combination with saline or any dose of morphine subcutaneously did not alter median cue duration values ($F[4,45] = 0.95$, $P = 0.4$, fig. 3). Representative visual cue duration titration curves are presented for a single animal in the lower panel of figure 3.

Morphine significantly reduced the effect of 1.8% lactic acid on both the latency to emit a correct response ($F[5,57] = 11.8$, $P < 0.0001$) and the latency to retrieve the food reward

Table 2. Effect of Ketoprofen and 1.8% Lactic Acid on Response Latencies in 5CTV

Ketoprofen, mg/kg	Latency to Correct	Latency to Reward
Baseline	1.03 (0.22)	1.67 (0.33)
Saline/saline	0.99 (0.13)	1.57 (0.15)
Saline/1.8% lactic acid	5.06 (2.93) *†	2.39 (0.44)*†
0.03/1.8% lactic acid	4.03 (2.29)*†	2.56 (0.66)*†
0.1/1.8% lactic acid	1.73 (0.96)‡§	1.78 (0.37)‡§
0.3/1.8% lactic acid	1.66 (0.65)‡§	1.78 (0.34)‡§

Latencies (seconds, mean and SD) from presentation of the visual cue until a correct response is emitted (Latency to Correct) or from correct responses to retrieval of the food pellets (Latency to Reward) are shown. Baseline indicates baseline sessions, and saline indicates sessions with saline (intraperitoneal) administration. Saline and ketoprofen were administered subcutaneously 30 min before administration of 1.8% lactic acid (intraperitoneally, 1 ml/kg).

*$P < 0.0001$ compared with baseline. †$P < 0.0001$ compared with saline/saline. ‡$P < 0.01$ compared with saline/1.8% lactic acid. §$P < 0.01$ compared with 0.03/1.8% lactic acid using Bonferroni correction for multiple comparisons.

5CTV = cue titration variant of the five-choice serial-reaction time task.

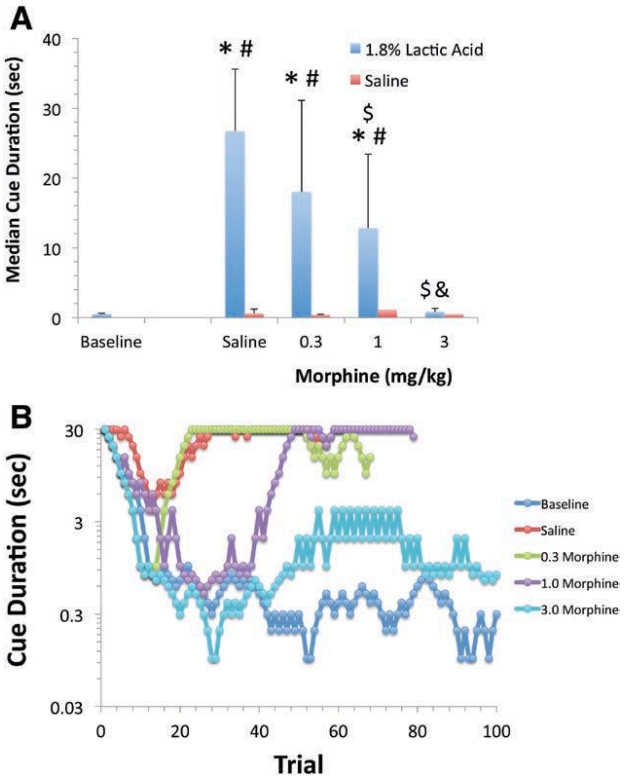

Fig. 3. The effects of morphine on lactic acid–induced impairment of visual attention performance. (*A*) Morphine reduced the effect of 1.8% lactic acid on performance as measured with median cue duration in a dose-dependent manner ($F[4,47] = 19.4$, $P < 0.0001$). Saline and morphine at any dose had no effect on median cue duration in the absence of lactic acid administration. *$P < 0.05$ compared with baseline; #$P < 0.05$ compared with saline/saline; \$$P < 0.05$ compared with saline/1.8% lactic acid; &$P > 0.05$ compared with 0.3 morphine/1.8% lactic acid. Mean and SD are shown, N = 8 to 14/group. (*B*) Representative visual cue duration titration curves are shown following treatment with 1.8% lactic acid in combination with indicated doses of morphine or no treatment (baseline). Each curve represents an individual session of 100 trials on a given day and treatment.

(F[5,51] = 3.2, P = 0.014) at all doses tested compared with baseline (table 3). Both the latency to emit a correct response and the latency to retrieve the food reward were increased significantly after administration of 1.8% lactic acid intraperitoneally compared with baseline values. However, neither measure was significantly different from baseline when animals were pretreated with 0.3, 1.0, or 3.0 mg/kg of morphine subcutaneously before lactic acid injection (table 3). Administration of saline or any of the doses of morphine in combination with intraperitoneal saline (in the absence of lactic acid) did not alter either of the latency measures (data not shown).

Effects of Scopolamine on 5CTV Performance and Lack of Reversal by Ketoprofen and Morphine

Scopolamine disrupted performance in the 5CTV, as evidenced by a dose-dependent increase in the median cue duration (F[4,66] = 22.6, P < 0.0001, fig. 4). Administration of either saline or 0.03 mg/kg of scopolamine did not produce an increase in the median cue duration relative to baseline values; however, both 0.1 and 0.3 mg/kg increased the median cue duration by 27- and 41-fold, respectively. Comparison of the effects of scopolamine in the presence or absence of 0.3 mg/kg of ketoprofen or 3 mg/kg of morphine found a significant effect of group (F[2,35] = 3.5, P = 0.04); however, neither 0.3 mg/kg of ketoprofen nor 3 mg/kg of morphine altered the effect of 0.1 mg/kg of scopolamine on the median cue duration (P > 0.05, fig. 4). There was a difference between the median cue duration after scopolamine treatment after administration of the two analgesics, however, with the median cue duration after morphine treatment being less than after treatment with ketoprofen (fig. 4). Representative visual cue duration titration curves are shown in the lower panels of figure 4.

Scopolamine increased both the latency to emit a correct response (F[4,64] = 39.5, P < 0.0001) and the latency to retrieve the food reward (F[4,63] = 9.6, P < 0.0001) in a dose-dependent manner (table 4). Ketoprofen (0.3 mg/kg) did not inhibit the effects of scopolamine on latency to correct responses or latency to retrieve the food reward. Morphine (3.0 mg/kg) reduced the effect of scopolamine on latency to retrieve the food reward but not on the latency to emit correct responses.

Table 3. Effect of Morphine and 1.8% Lactic Acid on Response Latencies in 5CTV

Morphine, mg/kg	Latency to Correct	Latency to Reward
Baseline	1.08 (0.22)	1.55 (0.21)
Saline/saline	1.11 (0.32)	1.52 (0.24)
Saline/1.8% lactic acid	6.77 (4.60)*†	2.07 (0.44)‡§
0.3/1.8% lactic acid	2.39 (1.24)‖	1.72 (0.64)
1.0/1.8% lactic acid	2.71 (1.82)‖	1.48 (0.26)#
3.0/1.8% lactic acid	1.54 (0.57)‖	1.76 (0.51)

Latencies (seconds, mean and SD) from presentation of the visual cue until a correct response is emitted (Latency to Correct) or from correct responses to retrieval of the food pellets (Latency to Reward) are shown. Saline and morphine were administered subcutaneously 30 min before administration of 1.8% lactic acid (intraperitoneally, 1 ml/kg).

*P < 0.0001 compared with baseline. †P < 0.0001 compared with saline/saline. ‡P < 0.05 compared with baseline. §P < 0.05 compared with saline/saline. ‖P < 0.01 compared with saline/1.8% lactic acid. #P < 0.05 compared with saline/1.8% lactic acid using Bonferroni correction for multiple comparisons.

5CTV = cue titration variant of the five-choice serial-reaction time task.

Discussion

Pain is a multidimensional experience, with sensory-discriminative and affective-motivational components. The sensory-discriminative effects of a multitude of experimental pain states have been explored in rodents for several decades, and significant progress has been achieved in defining the neuronal pathways and correlates for this dimension of pain. Recently, several models have been explored that investigate affective-motivational aspects of experimental pain in rodents, including alteration of behavior in both reinforcement paradigms including drug self-administration, conditioned place preference, and intracranial self-stimulation, as well as in fear/avoidance paradigms such as conditioned place avoidance.[21–29] These behavioral models have likewise been used to explore how pain-related circuitry interacts with reinforcement and fear-related pathways.[30–34] Similar progress in exploring the influence of experimental pain stimuli in rodents on cognitive functioning is needed, because cognitive impairment resulting from pain is an important consequence of pain, and treatment modalities for cognitive impairment may not coincide with those useful for other dimensions depending on the mechanisms involved. The present data demonstrate that acute abdominal inflammation in rats disrupts performance in an operant task requiring sustained visual attention and that clinically useful analgesics are capable of restoring performance to baseline levels. Furthermore, the effects of these analgesics on performance disruption induced by abdominal inflammation are distinct from those induced by scopolamine, a compound frequently used as a positive control for disruption in attention-based operant assays in rodents. It should be noted that 3 mg/kg of morphine did attenuate the effects of scopolamine on latency to retrieve the food reward, and this may indicate a general excitatory effect at this high dose.

Abdominal inflammation induced by lactic acid administration in rodents produces a number of behavioral effects thought to be associated with pain, including abdominal writhing (sensory/discriminative) and suppression of intracranial self-stimulation (affective/motivational).[29] A recent study failed to find a disruptive effect of lactic acid administration on attention using a signal-detection task, however, except at relatively large concentrations (3.2 and 5.6%), which increased the latency to correct responses and the number of omitted trials with no change in accuracy, similar to the effects found at a lower concentration in the present study.[35] The same range of doses of scopolamine used in the present study produced disruption in this signal-detection

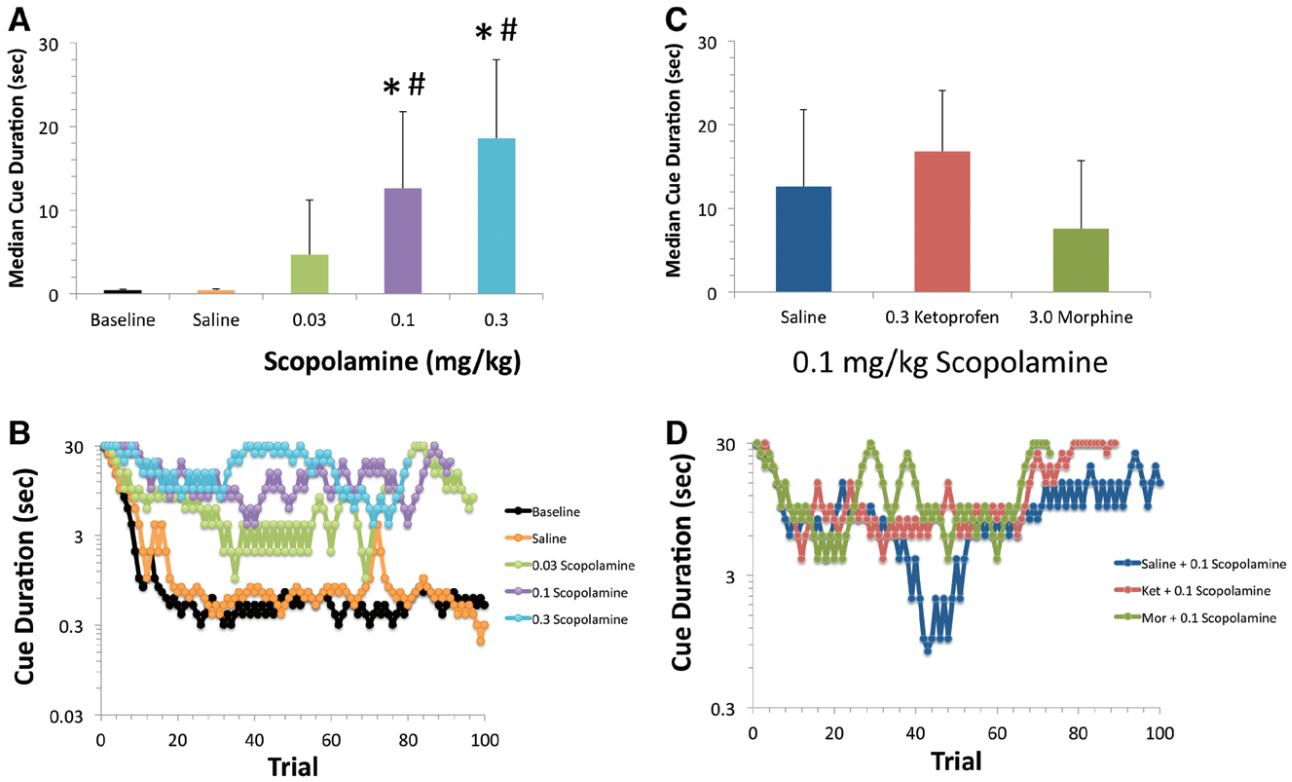

Fig. 4. Effects of scopolamine on visual attention performance and lack of reversal by ketoprofen and morphine. (*A*) Scopolamine disrupted performance in the cue titration variant of the five-choice serial-reaction time task as evidenced by a dose-dependent increase in the median cue duration ($F[4,66] = 22.6$, $P < 0.0001$). Saline or 0.03 mg/kg of scopolamine did not produce a significant increase in median cue duration relative to baseline values. *$P < 0.05$ compared with baseline; #$P < 0.05$ compared with saline. Mean and SD are shown, N = 13 to 15/group. (*B*) Representative visual cue duration titration curves are shown. Each curve represents an individual session of 100 trials on a given day and treatment. (*C*) Neither 0.3 mg/kg ketoprofen nor 3 mg/kg morphine altered the effect of 0.1 mg/kg scopolamine on the median cue duration ($P > 0.05$). Mean and SD are shown, N = 11 to 13/group. (*D*) Representative visual cue duration titration curves are shown for scopolamine and either ketoprofen or morphine.

Table 4. Effect of Scopolamine Alone or with Morphine or Ketoprofen on Response Latencies in 5CTV

Scopolamine, mg/kg	Latency to Correct	Latency to Reward
Baseline	1.03 (0.22)	1.67 (0.33)
Saline	0.99 (0.13)	1.57 (0.15)
0.03	2.23 (0.89)	3.68 (2.16)
0.10	5.80 (2.97)*†	6.27 (4.21)
0.30	9.16 (3.67)*†	4.84 (3.04)
0.3 ketoprofen + 0.10	7.10 (1.90)*†	4.97 (2.86)*†
3.0 morphine + 0.10	3.75 (1.56)*†	2.51 (1.41)

Latencies (seconds, mean and SD) from presentation of the visual cue until a correct response is emitted (Latency to Correct) or from correct responses to retrieval of the food pellets (Latency to Reward) are shown.

*$P < 0.01$ compared with baseline. †$P < 0.01$ compared with saline using Bonferroni correction for multiple comparisons.

5CTV = cue titration variant of the five-choice serial-reaction time task.

task.[35] Induction of colitis with 2,4,6-trinitrobenzenesulfonic acid, a more chronic form of abdominal nociception, disrupts performance in a novel object-recognition task in rats; however, the effects of morphine in this assay were mixed, with only a single dose producing a reversal of these effects.[36] Ligation of the L5 and L6 spinal nerves also impairs performance in the novel object-recognition task in rats.[37] This task has been described as possessing nonsustained attentional components, as well as working memory components and, as such, the role of attention *per se* with these manipulations is not known. Previous studies examining the effects of abdominal nociception and analgesics in attention-based assays have been mixed; however, the present study appears to be relatively more sensitive to both abdominal inflammation and reversal of these effects by analgesics.

Other pain manipulations have been found to decrease performance in behavioral assays thought to be related to attention processes in rodents. Spared nerve injury decreases performance in the novel object-recognition task and in the classical five-choice serial-reaction time task.[38,39] The effects of analgesics on disruption of attention were not determined in these studies; however, it was demonstrated that the deficits persisted for several weeks or months beyond the time of surgical injury. Induction of paw inflammation with Complete Freund's adjuvant or formalin, respectively, diminishes attention performance in both the novel object-recognition task and the five-choice serial-reaction time task, and morphine reverses these effects in both assays at

relatively high doses.[6,40] In the present study, application of an up-down method to determine the threshold of cue duration at which rats could maintain optimum sustained performance in a visual attention task provided a sensitive assay for inflammatory abdominal pain, as well as dose-dependent reversal by relevant analgesics. Application of this method to assess cognitive effects of chronic pain after nerve injury could provide a means for evaluating mechanisms of disruption of attention and efficacy of analgesics in a longitudinal manner, as the 5CTV procedure provides daily measures of ability within a wide range of performance capabilities.

The role of motivation for food reward in performance within the five-choice serial-reaction time task is thought to be dissociable from that of attention processes. Prefeeding food-restricted rats before five-choice serial-reaction time task sessions produces a similar increase in latency to correct responses and latency to retrieve a food reward, an effect mimicked by the anorectic D-fenfluramine.[41] The dopamine antagonist haloperidol increases latency to correct responses without affecting latency to food reward.[41] Ketamine, an *N*-methyl-D-aspartate antagonist with dissociable anesthetic properties, increases the latency to correct responses and number of omissions without altering the latency to retrieve food reward.[42] In the aforementioned study in which the authors examined the effects of formalin on performance in the five-choice serial-reaction time task, latency to retrieve food rewards was reported as not affected statistically; however, data were not presented.[40] The aforementioned study examining the effect of spared nerve injury on performance in the five-choice serial-reaction time task demonstrated an increase in latency to retrieve the food reward with nerve injury, even in the absence of an effect on motivation as measured with a progressive-ratio schedule of food-maintained behavior. This suggests that the effect may be related to motor impairment rather than motivation for food reward *per se*.[39] However, pain with movement in this task is likely not easily dissociable from motor impairment *per se* resulting from nerve damage. Analgesics would, however, be expected to provide beneficial effects in treating pain with movement but not improve motor impairment resulting from nonsensory nerve damage. Abdominal lactic acid is not likely to produce direct motor impairment in the manner that would be expected from nerve ligation but rather to produce interference of movement due to inflammatory nociception. The effects of lactic acid on response and reward latencies may therefore reflect either decreased motivation to obtain the food reward or interference with movement due to nociception. In either case, both morphine and ketoprofen reversed the effects of intraperitoneal lactic acid, and this is likely due to inhibition of the nociceptive state.

It is important to note that performance in the classical five-choice serial-reaction time task is dependent on a number of behavioral processes, including attention, impulse control, and motivation for reward, as well as intact motor function. In this regard, percent accuracy, defined as the ratio of correct trials to the sum of correct and incorrect trials, generally is accepted as the measure most impacted by attentional processes in this paradigm.[12,13] However, others have noted that determining the role of attention in performance capability is more complex than simply measuring accuracy. For example, it has been suggested that subjects may sacrifice speed to maintain accuracy in this operant paradigm when attentional demand is high or if attention is impaired.[12] In the present study, accuracy was not affected by manipulations that elevated the median cue duration, including both lactic acid and scopolamine. The median cue duration is influenced by correct, incorrect, and omitted trials, however, and thus is likely a measure of overall performance capability, affected by both attention and other processes, and indicates the level of difficulty at which animals are capable of performing efficiently. It has been suggested that examining the overall pattern of behavioral effects is the most robust means to determine behavioral mechanisms related to alteration of performance within the five-choice serial-reaction time task, and this may prove to be the case with the 5CTV titration variant as well. The overall pattern of increased latency to correct responses, retrieval of the food reward, and number of omitted trials has been described as "loss of response vigour" by Robbins,[12] and such effects typically have been associated with diminished dopaminergic drive in the ventral and dorsal striatum. These regions typically are thought to be involved in rewarding effects of food and motor responses associated with reward. It is noteworthy that intraperitoneal lactic acid is thought to suppress intracranial self-stimulation in rats by suppressing dopaminergic neurons within limbic forebrain, and this same mechanism may be involved in the effects of lactic acid seen in the present study.[28] As such, the effects of lactic acid on performance in this visual attention–based operant task may be related indirectly to the affective/motivational component necessary for behavior in this paradigm. Examination of specific brain regions and neurochemistry will be necessary to elucidate this role.

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Competing Interests

The authors declare no competing interests.

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From Coenzyme R to "Arfonad" and from Vitamin H to Hypotension

Using his employer-facilitated Swiss passport to pass through Nazi-occupied France, Croatian-born Leo Henryk Sternbach, Ph.D. (1908 to 2005), escaped anti-Semitism and sailed from Portugal to America in 1940. At the Nutley, New Jersey, laboratories of Hoffman-La Roche, he perfected the world's first commercially successful synthesis of biotin, otherwise known as vitamin H, B_7, or even "coenzyme R." A related synthesis resulted in Dr. Sternbach's discovery of trimethaphan camsylate (trimetaphan camsilate), which was trademarked as "Arfonad" in 1952. Roche advertised Arfonad as a ganglionic blocker for "controlled hypotension" and nearly "bloodless surgery," especially for neurosurgery. Boxed as an "Experimental Preparation," the 10cc ampoule (*right*) of Arfonad is labeled as from "Lot 001." Lowering blood pressure was followed by lowering anxiety, as Dr. Sternbach then discovered benzodiazepines. Before he passed away in North Carolina in 2005, this unassuming "Father of Valium," Sternbach, had been granted 241 U.S. patents. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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