Neuropathic pain and its treatment with ARA 290 and ketamine : overlapping pathways

Swartjes, M.

Chapter 5

Nonselective and NR2B-selective N-methyl-D-aspartic acid receptor antagonists produce antinociception and long-term relief of allodynia in acute and neuropathic pain

Maarten Swartjes, Aurora Morariu, Marieke Niesters, Leon Aarts, Albert Dahan

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Introduction

There is ample evidence for the importance of the excitatory N-methyl-D-aspartate (NMDA) glutamate receptor in the development and perseverance of chronic neuropathic pain.[1] NMDA receptors consist of multiple subunits: the obligatory NR1 subunit combines with at least one NR2 subunit.[2,3] Multiple NR2 subunits (A-D) have been identified. The NR1/NR2A NMDA receptor is ubiquitously distributed throughout the brain and spinal cord; NR2Bcontaining NMDA receptors are restricted to specific areas with importance for pain signaling, including dorsal root ganglia, lamina I and II of the dorsal spinal horn, thalamus, hippocampus, and cortex.[4] It is, therefore, not surprising that there is increasing experimental evidence that NR2B-containing receptors are involved in chronic pain responses.[2,5] Both nonselective and NR2B-selective NMDA receptor antagonists induce antinociception in experimental chronic pain models.[6-9] However, an important difference between the two subsets of pharmacologic agents is that, whereas nonselective antagonists cause severe side effects, there are suggestions that NR2B selective agents are devoid of such actions.[10] Extrapolation of animal data on the link between the NMDA receptor and chronic pain has led to the increased popularity of ketamine for the treatment of therapy-resistant chronic noncancer pain, as may be concluded from the increase in the number of case studies and clinical studies on ketamine treatment in neuropathic and chronic, noncancer pain.[11] Currently, proof for the efficacy of ketamine – or any NMDA receptor antagonist in the treatment of neuropathic chronic pain – is, however, limited.[11] Ketamine is effective when used in combination with opioids in the treatment of acute postoperative pain and cancer pain management.[11] Ketamine is currently the most potent NMDA receptor available for use in humans and is nonselective (so far no selective NR2B NMDA receptor antagonists have been registered for pain treatment). Akin to animal observations, ketamine produces serious side effects in humans,[11] including nausea, sedation, and psychedelic effects. Ketamine is rapidly metabolized into another NMDA receptor antagonist, norketamine.[12,13] Some contribution of norketamine to ketamine effects has been observed,[8] but the magnitude of this contribution to ketamine analgesia and side effects remains unknown.

We agree that there is a need for an NMDA receptor antagonist with a clear separation of analgesic effects and side effects, most importantly psychedelic side effects.[8] The antinociceptive properties of three NMDA receptor antagonists – ketamine, norketamine, and Traxoprodil (a highly selective NR2B NMDA receptor antagonist) – were characterized in a model of acute antinociception and a well-established rat model of persistent neuropathic pain, the spared nerve injury (SNI) model.[14] In addition, side effects (stereotypical behavior and activity level) and injured paw func-

tionality were quantified. In contrast with previous studies,[6-9] we used a long-term infusion paradigm (3 h for 5 consecutive days) administered 7 days after peripheral nerve injury. The delay in treatment was chosen to mimic established neuropathic pain states in patients by allowing the development of NMDA receptor sensitization and plasticity. A long-term infusion was used to mimic the observation in humans that prolonged infusion schemes are needed to ensure analgesic effects lasting for weeks rather than hours or days.[11,15]

The aims of the study were to compare ketamine, norketamine, and Traxoprodil with respect to: (1) analgesic behavior in acute and chronic pain paradigms, (2) separation in effect versus side effect, and (3) functionality of the injured paw after nerve injury. We hypothesized that all three NMDA receptors would produce analgesia, with Traxoprodil showing an improved utility index (i.e., analgesia with less side effects than either ketamine or norketamine). We further hypothesized that as a result of the reduction in pain, locomotor function would be improved after treatment with all three drugs in the chronic nerve injury model.

Materials and Methods

Female Sprague-Dawley rats (Charles River Nederland BV, Maastricht, Netherlands), 9 weeks old and weighing approximately 230 g were housed two per cage in individually ventilated cages under standard laboratory conditions with water and food ad libitum and 12 h light/dark cycles (lights on/off at 7:00 AM/PM). After surgery, animals were housed separately. Body weights were determined on the day of testing. All studies were performed during the light phase of the cycle. At the end of the studies, animals were euthanized by exsanguination under 6% sevoflurane anesthesia. The experiments were performed after approval of the protocol by the animal ethics committee of Leiden University (Dier Ethische Commissie, Leiden University Medical Center).

Acute Pain Model

Fifteen rats each received an intravenous cannula in the external jugular vein for drug administration under general anesthesia (6% sevoflurane induction, 3.5% maintenance). The cannula was subcutaneously directed toward the scalp and fixed to the skull. After surgery, animals were allowed to recover for 7 days, after which they were randomly allocated to one of three test groups (5 per group). Group 1 received ketamine (Eurovet Nederland, Bladel, Netherlands) at the following doses: 0, 1.25, 2.5, 5, 7.5 and 10 mg/kg. Group 2 received norketamine (Tocris Bioscience, Bristol, United Kingdom) at the following doses: 0, 2.5, 5, 7.5, 10 and 12.5 mg/kg.

Group 3 received Traxoprodil (Pfizer Inc., New York, NY) at the following doses: 0, 10, 20, 30, 40 mg/kg. Higher doses were not tested because they induced loss of consciousness. Each dose was tested on a separate day. The order of doses was random. Acute antinociception was assessed by applying an infrared thermal stimulus to the plantar surface of the hind paws (Plantar Test, Ugo Basile, Comerio VA, Italy). After a period of adaptation, the test drug was infused (dissolved in 200 µl normal saline) and paw withdrawal times (PWT) were obtained at regular intervals (5, 10, 15, 25, 40, and 55 min after infusion). The intensity of the heat stimulus was set to obtain baseline PWT at approximately 5 s and a cut-off value of 20 s was used to prevent burning of the skin. Each animal received one heat stimulus to each of the hind paws per time point. Measurements were averaged for further analysis.

Stereotypical behaviors and activity levels were scored as an indication of the drug's side effects. Stereotypical behaviors were scored on a scale from 0 to 3, with 0 = normal behavior, 1 = increased explorative (sniffing) behavior, 2 = increased urge to move around the cage, and 3 = inability to hold still with weaving/shaking/twitching of the head and body. Activity level was scored on a scale from 0 to 3, with 0 = normal activity, 1 = mildly impaired activity (i.e., disturbance in paw support), 2 = moderately impaired activity (i.e., a tendency to fall over but able to regain an upright position after falling), and 3 = severely impaired activity (i.e., inability to maintain paw support, falling with an inability to regain the upright position). These scores were adapted from Holtman et al.[8] and possibly relate to psychedelic side effects observed in humans.

Chronic Pain Model

The SNI model was designed according to the model of persistent neuropathic pain by Decosterd and Woolf.[14] In 32 animals, the skin on lateral surface of the thigh was incised under sevoflurane anesthesia (induction 6%, maintenance 3.5%). The sciatic nerve, with its three terminal branches (sural, common peroneal, and tibial nerves), was exposed by blunt preparation. The common peroneal and the tibial nerves were tightly ligated with 5.0 silk sutures and sectioned distal to the ligation, removing 2–4 mm of distal nerve stump. Great care was taken to avoid any contact with or stretching of the intact sural nerve. The nerve stumps were dislocated (more than 1 cm) to prevent regeneration. Muscle and skin were closed in two layers. The SNI model results in early (within 24 h), prolonged (more than 6 months), and robust (all animals are responders) behavioral changes. Next, a venous catheter was placed in the jugular vein for intravenous drug infusion. The catheter was placed subcutaneously at the back of the neck and fixed on the surface of the skull.

After a 1-week rest period, animals were randomly allocated to receive intravenous ketamine, norketamine, Traxoprodil, or vehicle. Eight animals were assigned to each

study group. Randomization was performed in blocks of four; four successively operated animals were randomized into one of the four treatment groups. Intravenous infusions were given continuously for 3 h for 5 consecutive days: 3 mg/kg/h ketamine (9 mg/kg per day for 5 days), 9 mg/kg/h norketamine (27 mg/kg per day for 5 days), or 10 mg/kg/h Traxoprodil (30 mg/kg per day for 5 days). Vehicle was normal saline (0.9% NaCl) given at a rate of 1 ml/h. Ketamine, norketamine, and Traxoprodil doses were based on a pilot study aimed at producing equiefficacy with respect to mechanical antiallodynic effect combined with minimal side effects.

In three other animals, a sham operation was performed. Under sevoflurane anesthesia, the skin on the lateral surface of the thigh and underlying muscle was incised. The sciatic nerve and its three terminal branches were exposed by blunt preparation. No nerve ligation was performed. Subsequently, muscle and skin were closed in two layers. No treatment was given to these animals.

Postoperatively, the animals were given 0.1 mg/kg buprenorphine and monitored for 1 h with body temperature maintained at 38 °C. Thereafter, animals were monitored/tested weekly for motor functions, behavior changes (autotomy), body weight, and allodynia of the injured paw. The study ended 10 weeks post surgery.

In order to measure mechanical allodynia, the animals were placed in a see-through box on a wire mesh floor. With the use of different von Frey hairs (Semmes-Weinstein monofilaments, North Coast Medical Inc., San Jose, CA) with increasing stiffness (0.004–300 g), incremental forces were exerted on the plantar surface of the affected hind paw. The hairs were applied 10 times at intervals of 2–3 s to slightly different loci within the test area.[7] The force necessary to induce a withdrawal reflex was recorded. Peak allodynia was defined as the minimum force that would trigger a withdrawal response in the first 10 postoperative weeks.

Next, animals were tested for cold allodynia with an acetone spray test. The stimulus was applied by spraying 20 µl acetone on the plantar surface of the affected hind paw. Animal response was scored according to the following classification: 0 = no withdrawal, 1 = startle response lasting less than 1 s, 2 = withdrawal lasting 1–5 s, 3 = withdrawal lasting 5–30 s (with or without paw licking), and 4 = withdrawal lasting longer than 30 s (with or without licking and repeated shaking).

Using a video-based, illuminated footprint analysis system (CatWalk, Noldus Information Technology, Wageningen, Netherlands),[16,17] we measured the following gait and footprint parameters during a short walk of the animals on a glass plate: area of the foot print (surface of paw used during step cycle), stand duration (time spent on paw during step cycle), and maximum intensity (maximum exerted pressure on paw during step cycle). All data were collected for further analysis, which was performed on a blinded data set.

Data and Statistical Analysis

Acute Pain Model

To obtain an estimate of the acute anti-nociceptive potency of the test drugs, PWT data (latencies) were fitted to the following model (adapted from Romberg et al.,[18] and Lötsch et al.[19]):

$$PWT(d) + PWT_B + PWT_0 + 5 \cdot [d/X_5]^{\gamma}$$

PWT(d) is the peak latency observed after dose d; PWTB, the latency before drug infusion; PWT_0, peak latency observed after saline; X_5, drug dose causing an increase in latency of 5 s (i.e., approximate ED_{50}); and γ, a shape parameter. The model was fitted to the data with the statistical package NONMEM (Nonlinear Mixed Effects Modeling, version VII; ICON Development Solutions, Ellicott City, MD).[20]

For the two measured side effects, typical behavior and activity level, data were analyzed using the following model (adapted from Romberg et al.,[18] and Lötsch et al.[19]):

$$S(d) = S_0 + 1.5 \cdot [d/Y_{1.5}]^{\gamma}$$

S(d) is the side effect score at dose d; S_0, the score after saline; and $Y_{1.5}$, the drug dose causing an increase in score by 1.5 points. Estimations were performed with NONMEM.[20]

Chronic Pain Model

Power analysis was based on a pilot study of ketamine versus saline treatment effect on mechanical allodynia in the SNI model. Four animals were treated with ketamine, three others with saline. At t=2 weeks (1 week after end of treatment), the mean ± SD force that caused a withdrawal response in ketamine-treated animals was 0.2 ± 0.17 versus 0.02 ± 0.01 g in saline-treated animals. We calculated group sizes of at least eight animals to detect a difference between treatments of at least 1 SD between groups, with a reliability of 5% and a power higher than 80%.

Areas under the curve were calculated from postoperative weeks 1–10 using the trapezoidal rule (AUC1–10). Among treatments, a comparison on AUC, peak effect, and duration of effect was performed using Kruskal-Wallis test and post hoc Tukey tests (hypothesis testing was two-tailed). Duration of effect was defined as the time at which the effect of an individual animal crossed the 95% CI of saline treatment.

The function of the injured paw was expressed as a percentage of the function of the sham animals. Gait analyses were performed in postoperative weeks 1, 3, and 4. Treatment effects were assessed by Kruskal-Wallis and post hoc Dunn's tests (hy-

pothesis testing was two-tailed). The data are expressed as mean ± SEM or median ± 50% quartile range for side effects (typical behavior and level of activity) unless otherwise stated. Statistical analyses were performed with SigmaPlot (version 11; Systat Software, Inc., Chicago, IL). P values less than 0.05 were considered statistically significant.

Results

Acute Pain Model

All three tested NMDA receptor antagonists produced dose-dependent acute antinociception (Figure 1). Model parameter estimates are $PWT_B = 5.3 \pm 0.2$ s, $PWT_0 = 1.1 \pm 0.3$ s, ketamine $X_5 = 7.6 \pm 0.9$ mg/kg, norketamine $X_5 = 12.5 \pm 1.3$ mg/kg, and Traxoprodil $X_5 = 37.9 \pm 2.6$ mg/kg. Corresponding values for γ are 0.9 ± 0.2, 1.9 ± 0.5, and 1.7 ± 0.3, respectively.

Stereotypical behavior and activity level scores are given in Figure 2. Both ketamine and norketamine produced dose-dependent side effects. Traxoprodil showed no signs of side effects across the dose range tested. Accordingly, no quantitative analysis was performed on Traxoprodil data. Model parameter estimates were for stereotypical behavior ($S_0 = 0 \pm 0$ [median ± SEM]): ketamine $Y_{1.5} = 3.7 \pm 0.4$ mg/kg and

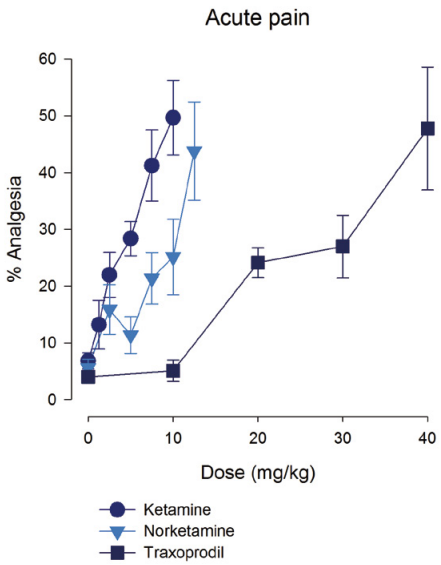


Figure 1: Dose-response relationship of ketamine, norketamine, and Traxoprodil versus paw withdrawal latencies. Percent analgesia was calculated as: (drug latency at dose d - latency after saline)/(cutoff latency - latency after saline). Values are presented as mean ± SEM.

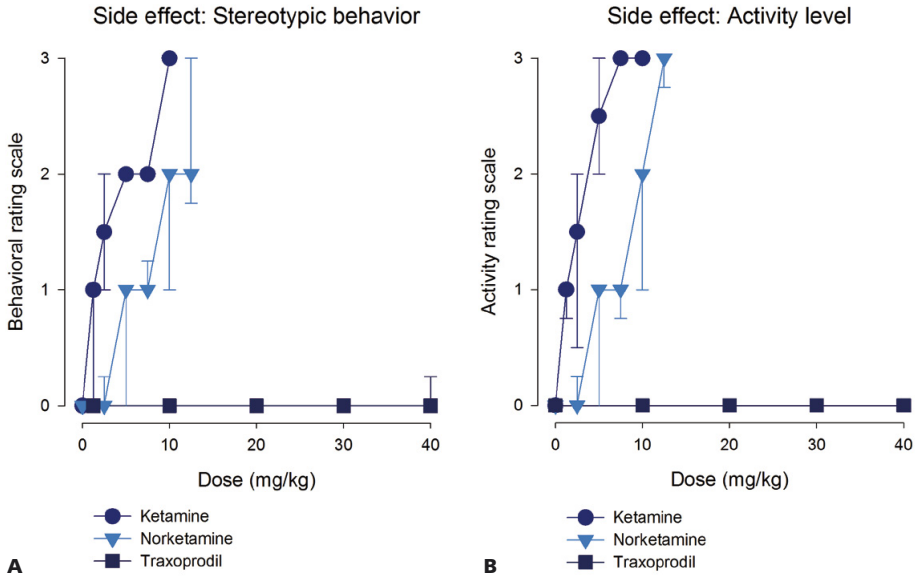


Figure 2: A: Dose vs. Stereotypical behavior. B: Dose vs. level of activity. Displayed for the three test drugs, ketamine, norketamine, and Traxoprodil. Values are presented as mean ± SEM.

norketamine $Y_{1.5}$ = 9.4 ± 0.6 mg/kg, with respective values for γ of 0.6 ± 0.1 and 1.3 ± 0.3. For activity level (S_0 = 0 ± 0): ketamine $Y_{1.5}$ = 2.8 ± 0.5 mg/kg and norketamine $Y_{1.5}$ = 9.4 ± 0.4 mg/kg, with respective values for γ of 0.6 ± 0.1 and 2.1 ± 0.3.

Chronic Pain Model

Although, during treatment, side effects were observed for ketamine and norketamine, but not for Traxoprodil, no side effects were observed in the 8 weeks after treatment. One week after transection of the common peroneal and the tibial nerve, animals displayed overt mechanical allodynia with withdrawal responses induced by 0.004 – 0.02 g filaments versus 8 g in the sham operated group (Figure 3, table 1). Five-day treatment with ketamine, norketamine, and Traxoprodil, but not saline, resulted in alleviation of mechanical allodynia with maximum relief (peak effect) occurring at postoperative week 2 (ketamine and Traxoprodil) and week 3 (ketamine). No difference in efficacy of the three NMDA receptor antagonists on mechanical allodynia, at the doses tested, could be detected.

A significant main effect for treatment on $AUC_{1–10}$ was observed ($P < 0.001$). The $AUC_{1–10}$s of ketamine, norketamine, and Traxoprodil were significantly larger compared with saline, but did not differ among each other. Similarly, a significant main effect of treatment on peak antiallodynia was present ($P < 0.001$, Table 1). At peak antiallodynia, animals treated with the NMDA receptor antagonists ketamine

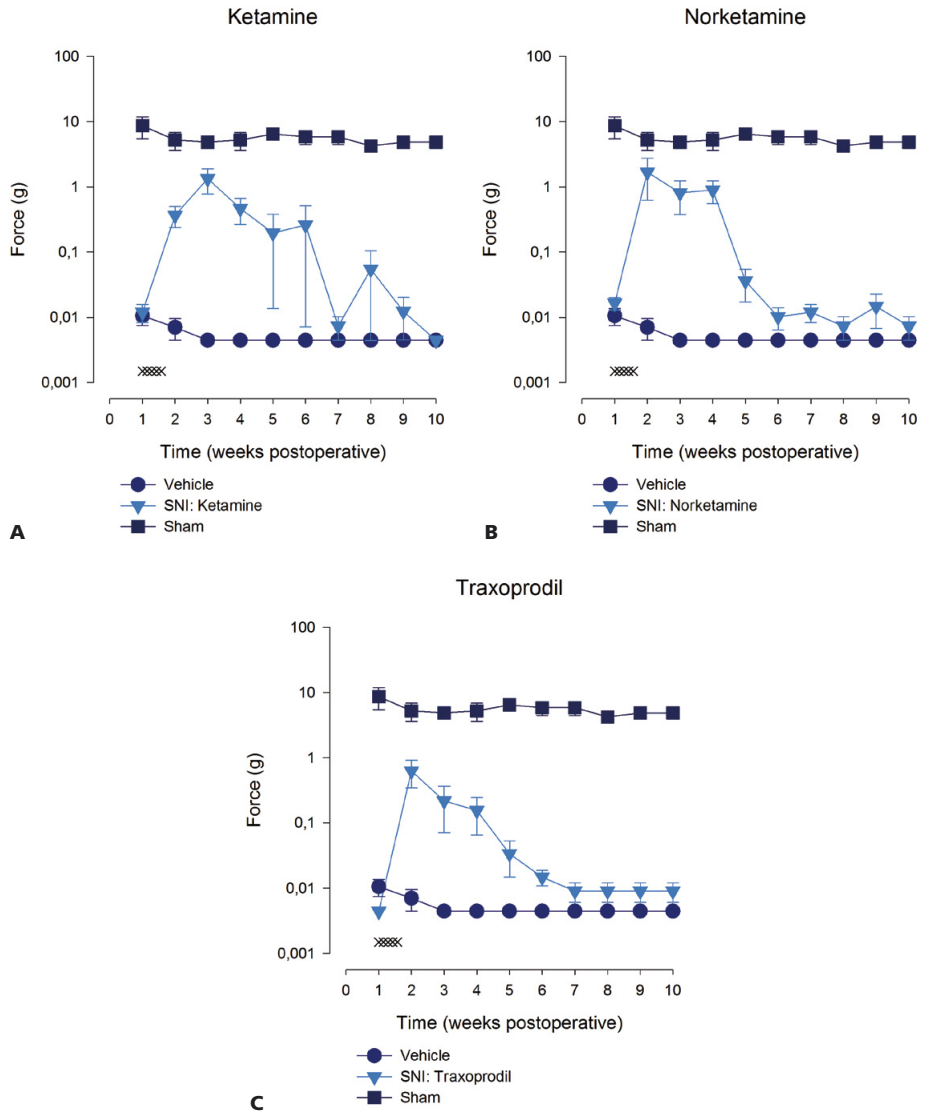


Figure 3: The effect of ketamine (A), norketamine (B), and Traxoprodil (C) on mechanical allodynia in the spared nerve injury model of the ipsilateral paw. Squares represent data from placebo-treated animals; circles, animals treated with N-methyl-D-aspartic acid receptor antagonists; triangles, sham-operated animals. X=treatment day. On the y-axis, the force used on injured paw with von Frey filaments to induce withdrawal response. Values are presented as mean ± SEM.

and norketamine responded to a higher force compared with saline-treated rats. However, between treatments, no difference was detected. In contrast, peak antiallodynic effect in the animals treated with Traxoprodil did not differ from peak effect in saline-treated animals. Duration of antiallodynia was similar between

Table 1: Effect of Ketamine, Norketamine, and Traxoprodil on mechanical and cold allodynia

	Ketamine	Norket-amine	Traxoprodil	Saline	Sham	Main effect#
Mechanical Allodynia						
Pre-treatment force (g) 95% c.i.	0.01 ± 0.01 (0.00 – 0.02)	0.02 ± 0.01 (0.01 – 0.03)	0.004 ± 0.00 (0.004 – 0.004)	0.01 ± 0.01 (0.003 – 0.017)	8.7 ± 3.2 (5.1 – 12.3)	P=0.08
AUC_{1-10} (g.weeks) 95% c.i.	2.7 ± 1.2 * (0.2 – 5.2)	3.5 ± 1.7 * (–0.06 – 7.1)	1.1 ± 0.5* (0.2 – 2.0)	0.04 ± 0.02 (0.03 – 0.05)	-	P<0.001
Peak anti-allodynic effect (g) 95% c.i.	1.4 ± 0.5 * (0.5 – 2.3)	1.8 ± 1.0 * (–0.3 – 3.9)	0.7 ± 0.3 (0.1 – 1.3)	0.004 ± 0.000 (0.004 – 0.004)	-	P<0.001
Cold Allodynia						
Pre-treatment score 95% c.i.	2.6 ± 0.3 (2.1 – 3.1)	2.6 ± 0.4 (1.7 – 3.5)	2.5 ± 0.3 (1.8 – 3.2)	3.1 ± 0.3 (2.5 – 3.7)	0.7 ± 0.3 (–0.1 – 1.5)	P=0.53
AUC_{1-10} (weeks) 95% c.i.	25.4 ± 1.4 * (22.3 – 28.2)	22.1 ± 1.0 * (20.1 – 24.1)	24.3 ± 1.1 * (22.0 – 26.6)	30.1 ± 1.8 (26.5 – 33.7)	-	P<0.001
Peak anti-allodynic score (95% c.i.)	1.9 ± 0.1 (1.6 – 2.2)	1.3 ± 0.2* (0.8 – 1.8)	2.1 ± 0.2 (1.7 – 2.5)	2.6 ± 0.4 (1.8 – 3.5)	-	P=0.03

All values are mean ± SEM (95% CI).
# Main effect (P-value), Kruskal-Wallis test. * P< 0.05 vs. saline, post hoc Tukey test.
AUC = area under curve.

treatments and lasted until postoperative weeks 5.6 ± 0.9 (ketamine), 7.1 ± 0.9 (norketamine) and 5.4 ± 0.6 (Traxoprodil). Relative potencies (calculated as dose ratio at which agents had identical AUC_{1-10}) were 1:2 (ketamine:norketamine) and 1:8 (ketamine:Traxoprodil).

Allodynia to cold stimulation occurred in week 1 after nerve injury in all animals (cold allodynia scores before treatment range between 2.5 and 3.1 in animals with SNI vs. 0.7 in sham operated animals, table 1). Ketamine, norketamine, and Traxoprodil induced relief of allodynia with peak antiallodynic effect occurring in week 2 (ketamine and Traxoprodil) and week 3 (norketamine).

A significant main effect for treatment on AUC_{1-10} was observed (Table 1 and Figure 4, P<0.001). The AUC_{1-10}s in animals treated with NMDA receptor antagonists were significantly smaller than the AUC_{1-10} observed in saline-treated animals by 17–27%. No differences in AUC_{1-10}s were observed among the three NMDA treatments. Although a significant main effect was observed for a difference in peak antiallodynic effect (P=0.03), post hoc analysis revealed that only norketamine was different from saline (Table 1). Duration of antiallodynic effect was similar between treatments and lasted until postoperative weeks 4.4 ± 1.1 (ketamine), 5.8 ± 0.7 (norketamine) and

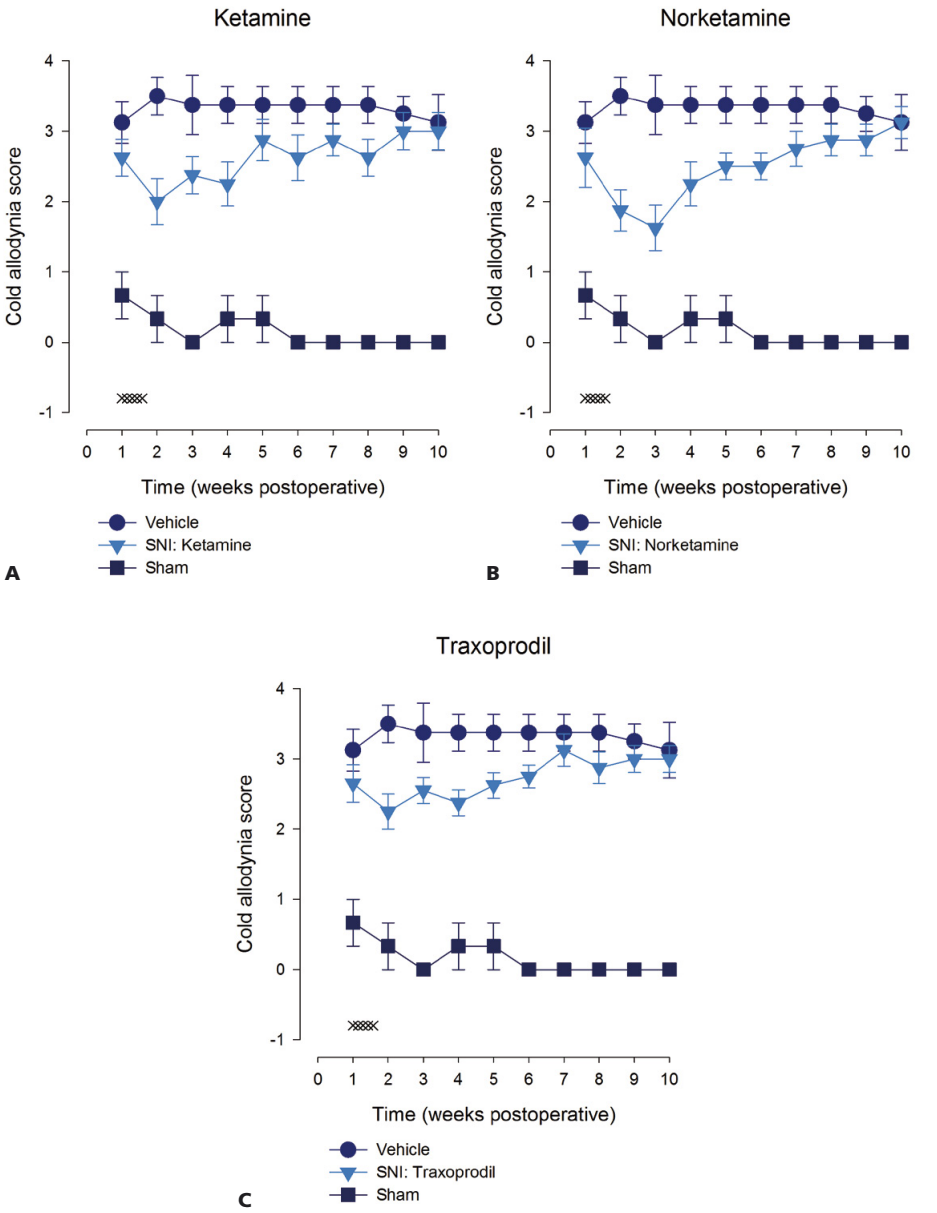


Figure 4: The effect of ketamine (A), norketamine (B), and Traxoprodil (C) on cold allodynia in the spared nerve injury model of the ipsilateral paw. The y-axis reflects the 4-point scale used to calculate cold-temperature stimulation of the injured paw via acetone spray test: 0 = no withdrawal, 1 = startle response lasting less than 1 s, 2 = withdrawal lasting 1–5 s, 3 = withdrawal lasting 5–30 s (with or without paw licking), and 4 = withdrawal lasting longer than 30 s (with or without licking and repeated shaking). Squares represent data from placebo-treated animals; circles, animals treated with N-methyl-D-aspartic acid receptor antagonists; triangles, sham-operated animals. X = treatment day. Values are presented as mean ± SEM.

4.8 ± 0.6 (Traxoprodil). Relative potencies (calculated as dose/AUC) were 1:3.4 for ketamine:norketamine and ketamine:Traxoprodil.

At postoperative week 1, nerve injured animals displayed severe dysfunction of the affected paw (Figure 5). At week 1, the surface of the injured paw used during a step cycle (area) was 2.6–9.6% (range) of that of the sham operated animals. Time spent on the injured paw during a step cycle (stand) was 19–33%. Pressure on the injured paw during a step cycle (intensity) was 32–37%. In the 2 weeks after treatment, saline-treated animals displayed a further gradual deterioration of gait-related parameters. Significant main effects of treatment were detected for all three tested indices (stand, $P=0.02$; area, $P=0.03$; intensity, $P=0.045$). Post hoc analysis revealed that neither ketamine nor Traxoprodil treatment had a significant effect on any of the measured gait parameters. Only after norketamine treatment improved responses (relative to saline) were observed for all three indices (all $P<0.05$). However, these effects were limited in magnitude and duration of effect (Figure 5).
Peak effect occurred at postoperative week 3, showing an improved value versus saline (area, 17 ± 2% vs. 3.4 ± 0.9%; stand, 52 ± 7% vs. 27 ± 5%; and intensity, 60 ± 4% vs. 33 ± 2%). At week 4, however, gait responses had returned to values observed before treatment (i.e., week 1).

Discussion

Ketamine is the prototypical NMDA receptor antagonist, now in use for nearly 50 years.[21] It is widely applied in the treatment of therapy-resistant chronic pain, as an adjuvant to opioids in the perioperative setting, and in cancer pain management.[11,21] Side effects, occurring at analgesic doses, restrict its use and emphasize the need for an NMDA receptor antagonist with an improved therapeutic index.8
In this experimental study, we characterized and compared the effects of ketamine with its active metabolite norketamine and the NR2B-selective NMDA receptor antagonist Traxoprodil. Several important observations were made: (1) All three NMDA receptor antagonists were efficacious in a model of acute antinociception and a well-established model of chronic neuropathic pain. (2) Ketamine was most potent in acute and chronic pain models, followed by norketamine and Traxoprodil. (3) Pain relief in the chronic pain model persisted for weeks after treatment termination. (4) Side effects (stereotypical behaviors and loss of activity) were present after treatment with ketamine and norketamine. Although norketamine showed an improved therapeutic index compared with ketamine, side effects occurred over the entire analgesic dose range tested (0 –12.5 mg/kg) for both agents. A clear separation be-

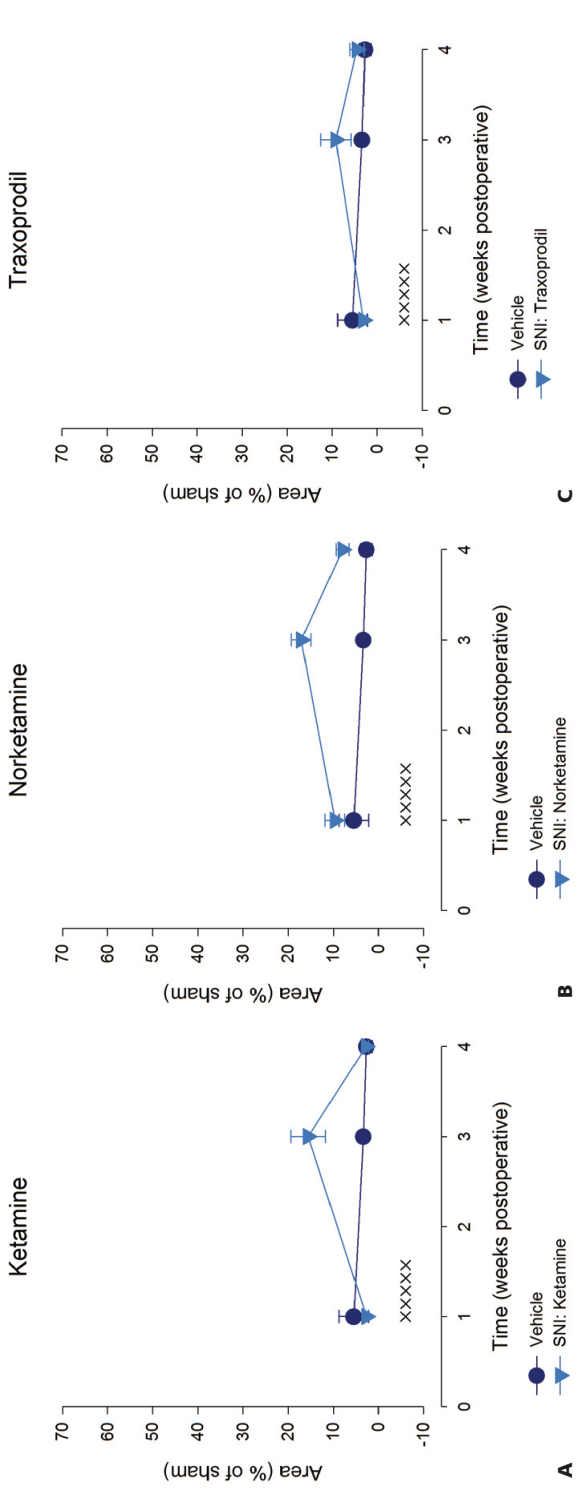
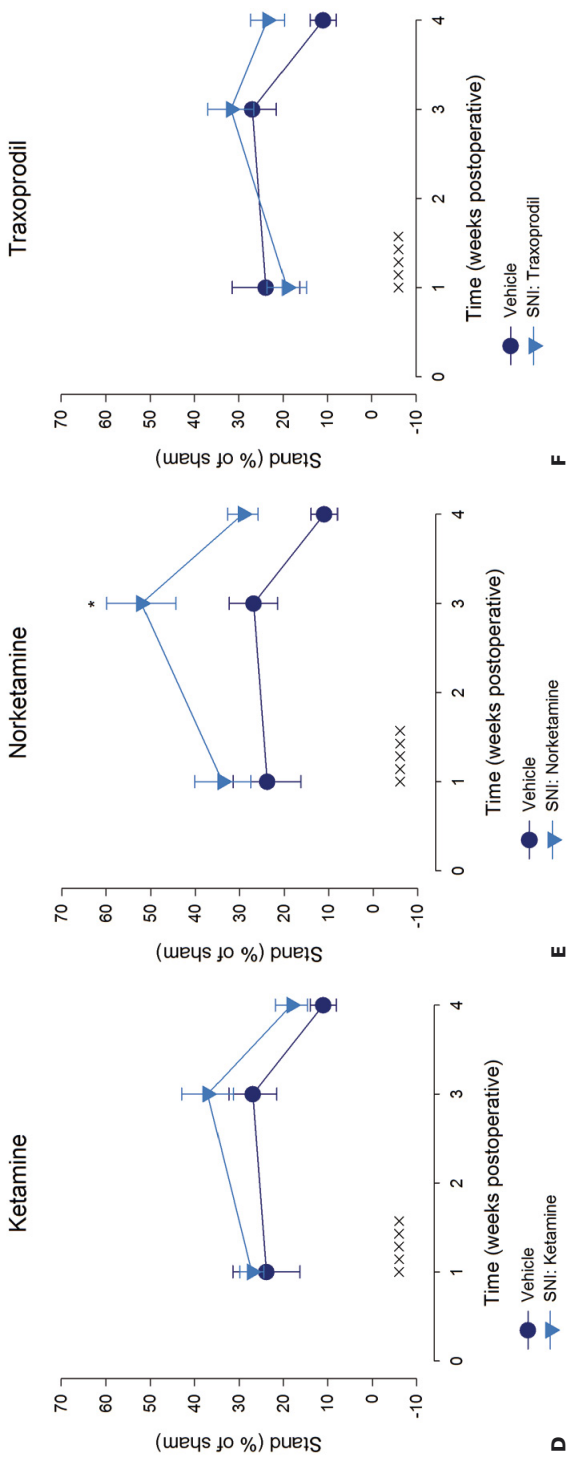
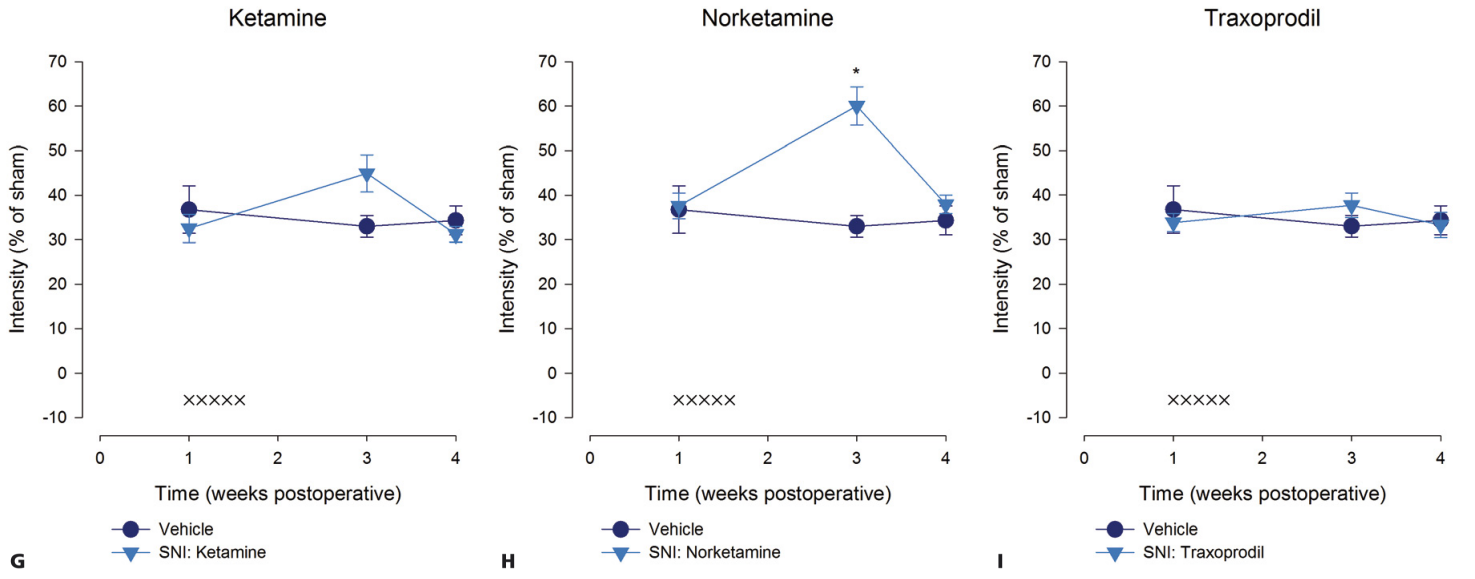


Figure 5: Treatment effect on gait response of ipsilateral paw in first 4 weeks after induction of spared nerve injury. A–C: Effect on area or surface of injured paw used during step cycle.



D–F: Effect on stand or time spent on injured paw during step cycle.



G–I: Effect on intensity or pressure placed on injured paw during step cycle. Data are relative to responses of sham-operated animals. X=treatment day. * $P<0.05$ versus placebo. Values are presented as mean ± SEM.

tween effect and side effects was present for Traxoprodil with, across the dose range tested (0 – 40 mg/kg), the absence of any signs of agitation and motor dysfunction. (5) After induction of nerve injury, norketamine caused improvement in function of the injured paw during the period of pain relief, although the effect was short-lived and relatively small. In contrast, no improvement was seen after treatment with ketamine or Traxoprodil, despite significant relief of mechanical allodynia.

Potencies for acute pain relief – as determined by parameter X_5, the dose causing an increase in PWT of 5 s (an approximate ED_{50}) – indicate that ketamine was 1.6 times more potent than norketamine and 5 times more potent than Traxoprodil (derived from X_5 ratios). Side effects were present after the administration of ketamine and norketamine. Although norketamine showed an improved therapeutic index (defined as $X_5/Y_{1.5}$, equations 1 and 2) compared with ketamine (agitation, ketamine = 2.1 vs. norketamine = 1.3; motor dysfunction, ketamine = 2.7 vs. norketamine = 1.3), side effects occurred over the entire analgesic dose range for both agents. A clear separation between effect and side effect was present for Traxoprodil with (across the 0-40 mg/kg dose range tested) the absence of any signs of stereotypical behavior and loss of activity.

Our data indicate that Traxoprodil may be clinically useful in the treatment of acute pain, with a superior therapeutic index when compared with ketamine and norketamine. The observation of absence of side effects is in agreement with other studies.[10] With respect to motor function, this finding is likely related to the absence of NMDA receptors containing the NR2B subunit in the cerebellum.[6] The mechanisms by which NMDA receptor antagonists produce acute pain relief have been attributed to activity at non-NMDA receptors, such as the μ-opioid receptor.[22] However, there is evidence for the NR2B-containing NMDA receptors located presynaptically on primary afferent C-fibers in lamina I of the dorsal horn.[6] These receptors may modulate the presynaptic release of substance P and glutamate and consequently modulate acute pain transmission in the spinal cord.[6]

All three NMDA receptor antagonists produced relief of mechanical and cold allodynia in the SNI model. Relative potencies indicate that, for mechanical allodynia, ketamine is twice as potent as norketamine and eight times more potent than Traxoprodil. For cold allodynia, ketamine was 3.4 times more potent than norketamine and Traxoprodil. For none of the three agents did any side effects (agitation, motor dysfunction) occur during the period of testing. We observed that the magnitude and duration of relief of mechanical allodynia was more pronounced than that of cold allodynia (Figure 4). Previously, Qu et al.,[23] showed that pretreatment with ifenprodil, a selective NR2B antagonist, induced relief of mechanical allodynia, but not thermal hyperalgesia, in a spinal nerve ligation model. These findings suggest that the development of different pain expressions or modalities (e.g., mechanical

vs. cold allodynia) is due to activation of different pain pathways after peripheral nerve injury, each with a different (subunit) expression of NMDA receptors and, consequently, distinct sensitivities to different NMDA receptor antagonists.[23] For example, after peripheral nerve injury, sprouting of Aδ-fibers into the superficial layers of the dorsal horn is associated with mechanical allodynia but not thermal hyperalgesia.[23,24]

Several studies on the effect of NMDA receptor antagonists on neuropathic pain test the preemptive effect of treatment or the effect of treatment in the early stage of nerve injury.[6,23,25,26] We performed our infusion 1 week after the induction of nerve injury. This timing was used to induce an established neuropathic pain state. We assumed that 1 week after surgery (the late phase of nerve injury), NMDA receptor sensitization had fully developed with established structural changes in the affected pain pathways (such as up-regulation of NMDA receptors).[9] Our approach mimics the situation in neuropathic pain patients, who are often treated weeks or months after nerve injury has occurred. We infused the test agents for 5 consecutive days. This process was used because, in neuropathic pain patients, long-term or repetitive treatments with NMDA receptor antagonists (rather than short-term infusions) produce long-lasting analgesic effects.[11] For example, we previously showed that a 100-h infusion with S(+)-ketamine produced pain relief that lasted up to 12 weeks in patients with chronic pain as a result of complex regional pain syndrome type 1.[15] In patients with neuropathic pain from spinal cord injury or monoradiculopathy, a relatively short 24-h infusion with Traxoprodil produced pain relief during the infusion period only.[4,5,27] A similar short duration of analgesic effect (8 h) was observed in the rat after an intrathecal injection with ifenprodil given 7 days after dorsal root ganglion compression.[9] We believe that a prolonged analgesic effect is mandatory when treating neuropathic pain patients with intravenous NMDA receptor antagonists to reduce treatment costs and patient discomfort and increase treatment compliance.

We observed long-term relief of mechanical and cold allodynia lasting 3–6 weeks after the initiation of 5-day treatment. Because ketamine exhibits a rapid reduction in ketamine and norketamine plasma concentrations on infusion termination, it is not expected that any active agent was present in the rat during the test phase of our study. Apparently, ketamine initiated a cascade of events (of which the first step is NMDA-receptor desensitization) that caused long-term effective and continuing blockade for central trafficking of pronociceptive signals to the thalamus and cortex.[28,29] In agreement with this theory, Christoph et al.[30] showed that the antiallodynic effect of NMDA receptor antagonists (more than 3 h with ketamine) outlasts the in vivo NMDA receptor antagonism ($t_{1/2}$ = 10–12 min) in rats with chronic nerve constriction injury. In addition, a central reset of central glutamatergic brain circuits involved in pain transmission may play a role.[29] A supraspinal effect of NMDA recep-

tor antagonists is in agreement with studies that point to a role for NR2B NMDA receptors in the forebrain and amygdala in the development and enhancement of neuropathic and inflammatory pain.[31-33] Our findings indicate that long-term relief of neuropathic pain is possible despite a delayed therapy start with all three tested NMDA receptors. More prolonged effects may be feasible by adjusting dosing or duration of treatment, or by repetition of treatment at 4 –5 week intervals.

Injured paw use during locomotion was tested using Cat-Walk automated quantitative gait analysis,[16,17] which has been used previously to quantify tactile allodynia in the rat. Vrinten and Hamers[16] compared gait analysis using von Frey testing in rats with chronic constriction injury of the sciatic nerve. They observed a high degree of correlation between gait parameters and von Frey mechanical allodynia. In contrast, Gabriel et al.[34] were unable to find significant correlations in rats with chronic pain induced by intra-articular λ-carrageenan injection in the knee. Mogil et al.,[35] using a blinded scoring approach, observed differences in dynamic, weight-bearing (gait) changes between sham operated mice and mice with spared nerve (but not chronic constriction injury). However, in the SNI animals, there was a pharmacologic dissociation between mechanical allodynia and gait changes. Morphine, gabapentin, and EMLA cream (2.5% lidocaine ± 2.5% prilocaine) reversed mechanical allodynia but did not affect gait abnormalities of the injured paw. Our data indicate a profound and long-lasting effect of SNI damage on the gait parameters of area, stand, and intensity. In all animals with SNI, we observed reduced use of the affected paw with minimal floor contact during locomotion. We relate the reduced use to the perception of mechanical allodynia during contact of the paw with the surface (weightbearing allodynia).

We are the first to quantify pharmacologic treatment with NMDA receptor antagonists on gait patterns in chronic pain with the CatWalk system. The effect of treatment on gait abnormalities was disappointing. After treatment with norketamine, a significant effect on gait-parameters was observed, but the effects were short-lived (effect in week 3 only, Figure 5) and relatively small. No improvements were observed after treatment with ketamine or Traxoprodil, despite significant relief of mechanical allodynia (measured via von Frey test). The reason for absence of improvement of paw abnormalities (or just a limited effect, as seen with norketamine) may be that weight-bearing allodynia is less sensitive to pharmacologic intervention and requires higher doses. Testing mechanical allodynia with von Frey hairs may be less painful and more sensitive to NMDA receptor antagonism. Alternatively, diminished paw use and gait changes after nerve injury may not reflect (NMDA receptor–related) mechanical allodynia and are therefore not responsive to treatment with NMDA receptor antagonists. Both at the spinal and supraspinal level, gait and pain pathways are distinct. Gait is controlled by spinal networks under the direct influence of

descending pathways originating at the brainstem, which in turn receive afferent information from the cerebellum, basal ganglia, and sensorimotor cortex.[34] It is reasonable to assume that, after the inflicted nerve injury changes occur in these motor pathways (which do not involve NMDA receptor sensitization and up-regulation), a permanent inability to use the paw during locomotion occurs, possibly without spontaneous pain.[35]

The current findings are in agreement with our findings in patients with chronic pain from complex regional pain syndrome type 1.[15,36] After treatment with ketamine, spontaneous pain scores improved significantly. However, there was no improvement of function-related parameters. We argued that pain relief should coincide with improvement of function and use of the affected limb. Our current and previous data do not support this argument. It may well be that improvement of locomotor function and increase in use requires a different treatment approach (e.g., combining pharmacotherapy with physical exercise, physiotherapy, and/or surgical intervention (nerve reconnection or transplantation)).

Conclusion

All three NMDA receptor antagonists caused dose-dependent antinociception in the acute pain model. Likewise, they caused relief of mechanical and cold allodynia for 3–6 weeks after treatment in a chronic neuropathic pain model. In both pain tests, ketamine was most potent, with norketamine 1.5–2 times less potent and Traxoprodil 5–8 times less potent than ketamine. In contrast to nonselective NMDA receptor antagonists, treatment with Traxoprodil caused no side effects. Although all three agents produced long-term relief of mechanical allodynia in nerve-injured animals, improved use of the affected paw during locomotion, as tested by computerized gait analysis, was limited (norketamine) or absent (ketamine and Traxoprodil). These observations make Traxoprodil an attractive alternative to ketamine in the treatment of chronic neuropathic pain. Alternative treatment options are required to induce increase limb (paw) use.

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