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A Comparison of Prilocaine 1% versus 1.5% for Ultrasound-guided Axillary Brachial Plexus Block.

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Abstract:	Background and Objectives: To compare the anesthetic characteristics of prilocaine 1% and 1.5% in terms of on- and offset time of sensorimotor block and methemoglobin levels, when used for ultrasound-guided axillary brachial plexus block. Methods: The prospective, randomized, double-blinded trial was conducted at the Universitair Ziekenhuis Brussel (Brussels University Hospital) on 60 patients (ASA I-III, age range 19-86) scheduled for ambulatory hand surgery. Axillary brachial plexus block was performed with ultrasound-only technique and 5 ml of prilocaine 1% or prilocaine 1.5% was injected around each nerve (20 ml in total). Onset time and duration of sensory and motor block were assessed as well as peak methemoglobin levels 2 hours after injection. Results: Mean onset time for sensory block of the median, ulnar and musculocutaneous nerves was 15, 13 and 11 min. respectively for the prilocaine 1% group compared to 14, 11 and 12 min. for the prilocaine 1.5% group (p = 0.437, 0.103 and 0.863). Mean duration of sensory and motor block was 265 min. and 247 min. respectively for prilocaine 1% versus 255 min. and 245 min. for prilocaine 1.5% (p = 0.731 and 0.201). The average methemoglobin level was 2.25% for the 1% group compared to 3.03% for the 1.5% group (p = 0.0005). Conclusions: We conclude that there is no statistically significant difference between prilocaine 1% and 1.5% in terms of on- and offset time of the sensorimotor function. However, significantly higher methemoglobin levels were found with prilocaine 1.5%. EUDRACT registration number: 2015-002744-14A

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Dear Dr. Huntoon:

Re: A Comparison of Prilocaine 1% versus 1.5% for Ultrasound-guided Axillary Brachial Plexus Block

I (we) are submitting the enclosed material for possible publication in ***Regional Anesthesia and Pain Medicine***. This material has not been submitted for publication or published elsewhere in whole or part. The undersigned listed have participated in the work and can publicly defend the manuscript's contents, and have read the manuscript prior to its submission for publication and agree with its contents.

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Abstract

Background and Objectives: To compare the anesthetic characteristics of prilocaine 1% and 1.5% in terms of on- and offset time of sensorimotor block and methemoglobin levels, when used for ultrasound-guided axillary brachial plexus block.

Methods: The prospective, randomized, double-blinded trial was conducted at the Universitair Ziekenhuis Brussel (Brussels University Hospital) on 60 patients (ASA I-III, age range 19–86) scheduled for ambulatory hand surgery. Axillary brachial plexus block was performed with ultrasound-only technique and 5 ml of prilocaine 1% or prilocaine 1.5% was injected around each nerve (20 ml in total). Onset time and duration of sensory and motor block were assessed as well as peak methemoglobin levels 2 hours after injection.

Results: Mean onset time for sensory block of the median, ulnar and musculocutaneous nerves was 15, 13 and 11 min. respectively for the prilocaine 1% group compared to 14, 11 and 12 min. for the prilocaine 1.5% group ($p = 0.437$, 0.103 and 0.863).
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The average methemoglobin level was 2.25% for the 1% group compared to 3.03% for the 1.5% group ($p = 0.0005$).

Conclusions: We conclude that there is no statistically significant difference between prilocaine 1% and 1.5% in terms of on- and offset time of the sensorimotor function. However, significantly higher methemoglobin levels were found with prilocaine 1.5%.

EUDRACT registration number: 2015-002744-14A

Introduction

Surgeries of the hand or wrist are common procedures performed on an outpatient basis. With the current evolution towards fast-track surgery an increasing number of these procedures are being performed under peripheral nerve block (PNB). This can result in a faster hospital discharge compared to general anesthesia.[1]

The choice of local anesthetic based on the duration of the surgery and the anticipated postoperative pain is an important factor determining the success of PNB as well as patient acceptance. In our experience a long duration of motor block is related to negative patient acceptance, especially in minor ambulatory surgery not associated with moderate to severe pain.

Prilocaine is an amide-type local anesthetic (LA) with a fast onset and short duration of action, comparable to lidocaine and mepivacaine but with significantly lower systemic toxicity.[2,3] These properties make it an excellent local anesthetic for intravenous (Bier's block) as well as loco-regional anesthesia.

Although prilocaine has been associated with the formation of methemoglobin, doses required to produce clinically significant plasma levels are much larger than the doses used for loco-regional anesthesia.[2,4]

The introduction of ultrasound has changed the practice of loco-regional anesthesia allowing for an increased success rate[5] and a reduction in LA dose without compromising the quality of the PNB[6]. In past trials prilocaine has been successfully used for axillary brachial plexus block using concentrations from 1%to 2%.[7,8,9,10] In these studies axillary brachial plexus blocks were performed without ultrasound and rather high volumes of LA

(40-50 ml) were used.

In this trial we want to investigate the difference between prilocaine 1% and 1.5% when the axillary brachial plexus block is performed under ultrasound guidance and lower volumes (20 ml) are injected. Furthermore, the impact on methemoglobinaemia will be assessed.

Methods

This study was set up as a prospective, randomized, double-blinded trial. It was conducted at the Universitair Ziekenhuis Brussel (Brussels University Hospital) after approval by the local ethics review board (no. 2015/235) and the Federal Agency for Medicines and Health Products (EUDRACT no. 2015-002744-14A, 2015-09-24). A total of 60 patients, ASA I-III, scheduled for elective minor hand or forearm surgery were included. Exclusion criteria were: age < 18 years old, contra-indications for loco-regional anesthesia, and peripheral neurological disorders. Diabetic patients were included unless peripheral polyneuropathy was diagnosed or suspected. Patients were assessed for eligibility during the preoperative anesthesia consultation and written informed consent was obtained.

Study design

Patients were randomized into two groups with 30 subjects in each group. This number was based on a sample size calculation performed before the start of the trial. Study medication consisted of 20 ml of prilocaine 1% for group A and 20 ml of prilocaine 1.5% for group B. Due to unavailability of isobaric prilocaine at the commencement of this study, hyperbaric prilocaine (Tachipri®) was used (unlabeled use). Prilocaine 2% was diluted with normal saline to obtain 20 ml of the desired concentration.

Randomization and blinding

A computerized sequence was used for the randomization process generating two groups of 30 subjects and allocating every subject number to one of both study groups. This list

was concealed from the principal investigator (PI). Study medication was prepared by an anesthesiologist not involved in the data collection and handed to the PI responsible for performing the nerve block and the data collection. All nerve blocks were performed by the same anesthesiologist to avoid inter-individual variability due to potential differences in skill or experience levels between anesthesiologists. At the conclusion of the study the list of study subjects and their group allocation were disclosed to the PI.

Nerve block procedure

Benzodiazepine (alprazolam PO or midazolam IM) premedication was administered at the surgical ward at the discretion of the anesthesiologist at the preoperative consultation. Paracetamol and NSAIDs were administered to all patients, if not contra-indicated. Axillary brachial plexus block was performed with an ultrasound-guided, short axis, in-plane technique using a 60 mm, 23 gauge, short bevel ultrasound needle (TOP Neuropole, Malaysia). Nerve stimulation was not used. The ulnar, median, radial and musculocutaneous nerves were identified on the ultrasound image and blocked in this respective sequence. The needle was advanced using an in-plane technique up to the extra-neural space where 5 ml of study medication was injected around each nerve. Local spread of LA was evaluated during injection and if necessary needle position was adjusted to obtain optimal circumferential spread. Time 0 (t_o) was established at the time of conclusion of the nerve block procedure.

Outcome measures

The primary outcome measure was the comparison of prilocaine 1% with 1.5% in terms of block onset time and duration of sensory and motor block. Secondary outcome

parameters included methemoglobin levels 2 hours after injection, the time to first intake of pain medication and patient satisfaction with the used technique.

After injection of the study solution, onset of sensory block was evaluated every 3 minutes. Sensory blockade of musculocutaneous, median, ulnar and radial nerve was evaluated by ether skin testing and scored on a three point scale: 3 = normal sensation, 2 = decreased sensation, 1 = no sensation.
The onset time was defined as the time from t_o until a score of 1 was reached. If full sensory block wasn't obtained at 30 minutes after injection it was considered a failed block. In these patients a rescue block was performed and they were excluded from further analysis.

Methemoglobinemia was assessed on a venous blood sample obtained 2 hours after LA injection. This measurement can be considered as an approximation of the peak methemoglobin level, since maximum levels are usually reached after 2–4 hours.[11,12]

It was not possible to perform postoperative sensory-motor testing due to surgical dressings/cast. Therefore patients were asked to note the time of regaining purposeful movement and first noticeable sensation or pain, as well as the time of first intake of pain medication. Duration of sensory-motor block was defined as the time from t_o until recovery of normal sensation and full motor function respectively.
Patients were visited at the postoperative ward to evaluate recovery time. In case of incomplete sensory-motor function recovery at discharge, patients were contacted within

24 hours for further follow-up.

Patients were also asked to grade the applied anesthetic technique on a scale of 1 to 10 in accordance to their satisfaction.

All patients were contacted again after 6 months to screen for nerve damage.

Statistical analysis

For statistical comparison of block onset time for the different nerves, overall sensory and motor block duration and methemoglobin levels between both groups the data were analyzed using a M-ANOVA test. A p-value of <0.05 was considered statistically significant.

Results

Sixty patients were enrolled, 30 in each group. A total of 5 failed blocks were reported: 4 subjects in group B (prilocaine 1.5%) still experienced cold sensation on ether skin testing after 30 minutes, therefore a rescue block was performed. One patient in group A (prilocaine 1%) experienced severe pain during surgery and had to undergo general anesthesia. These 5 subjects were excluded from further analysis.

Another 5 patients (3 in group A and 2 in group B) had satisfactory block on preoperative testing but complained of minor discomfort during the surgical procedure. Supplemental 5 μg of sufentanil was administered intravenously to each patient but they were not excluded from further analysis.

There were no significant differences between the 2 groups in demographic characteristics, ASA status, occurrence of diabetes mellitus and the types of surgical procedures performed. (Table 1). The time necessary to perform the nerve block was comparable between both groups.

No significant difference was found in the time to complete sensory blockade of the ulnar, median and musculocutaneous nerve between both groups. (Table 2)

Often, due to poor ultrasonic visibility of the radial nerve combined with the use of an ultrasound-only technique and the low volume of LA injected, failure to obtain a full sensory radial nerve block was not uncommon. On a total of 60 patients only 20 had full sensory block of the radial nerve, 29 patients had a partial sensory block and 11 had no sensory block at all. (Table 3)

Mean overall duration of sensory and motor block was 258 min. and 266 min. for group A compared to 261 min. and 242 min. for group B and did not differ significantly: $p = 0.731$ and $p = 0.201$ respectively. (Fig 1)

Patients in group A had an average methemoglobin level of 2.20% compared to 3.07% for patients in group B, a statistically significant difference ($p = 0.0005$). Data on methemoglobinemia are shown in Table 2 and Fig 2.

Patient satisfaction on a scale of 1 – 10 was comparable between groups: 8.1 ± 1.6 for group A and 8.3 ± 1.0 for group B. Due to limited extraction of data on the time to first intake of postoperative pain medication, no average was calculated.

None of the patients showed symptoms of local anesthetic systemic toxicity during the study procedure. All patients were contacted 6 months after the surgery to screen for postoperative nerve damage, none expressed any sign of short- or long-term neuropathy.

Discussion

With the introduction of ultrasound-guided techniques research is warranted to establish whether a reduction in concentration and/or volume of LA is justified. Our study shows no difference between prilocaine 1% and prilocaine 1.5% in terms of onset time or quality and duration of sensory and motor blockade.

We did however notice a difference in onset time between the individual nerves within the same group, i.e. a faster onset time of the ulnar compared to the median nerve. This could be explained by the sequential approach where LA was first injected around the ulnar nerve which results in a longer contact time at t_o. An explanation for the faster onset of the musculocutaneous nerve could be due to its smaller diameter compared to the other nerves.

Our results show that prilocaine 1% has a similar onset time and duration of action as mepivacaine 1.5%[13] and lidocaine 1.5% with epinephrine.[14] It can be recommended as an equal alternative for surgeries of short to intermediate duration where limited postoperative pain is expected. In these cases a short duration of nerve blockade with fast recovery of function is desirable.

However, it is important to note a significant inter-patient variability in the duration of sensorimotor block with values ranging from 150 to 504 min. in group A and 130 to 540 min. in group B. On further detailed analysis of our data we failed to identify any clear underlying cause or factors predictive for the duration of the block.

An issue related to our study was the poor visualization of the radial nerve in a significant number of patients, a phenomenon also described by Frkovic et al.[15] and Wong et al.[16] With poor visibility of the radial nerve on ultrasound, adequate perineural spread is difficult to appreciate and surgical anesthesia of the radial nerve is often not obtained. This can be especially problematic when the total volume of LA is reduced as was the case in this study. We do note that in this study only in 3 cases blockade of the radial nerve was required for surgery. In the other patients the radial nerve was not a primary nerve of interest, which might be an inadvertent cause of bias.

A number of 5 failed blocks on a total of 60 patients corresponds with a failure rate of 8%. This is lower than the studies of Janzen[7] and Dunlop[8] where respectively 25% and 34% of patients needed additional infiltration of LA. This is in accordance with the findings of Chan et al.[5] and the meta-analysis of Qin and colleagues[17] that show a higher success rate when ultrasound is used.
Since 4 of the 5 failed blocks in our study were seen in the group with the higher concentration (prilocaine 1.5%) we may conclude that concentration was not a causal factor. In our experience a clear explanation for block failure cannot always be identified. Failed block might occur in spite of perfect perineural spread of the LA on ultrasound image. This might suggest that there may be other factors that are currently not fully comprehended.

The only outcome measure where a significant difference was objectified between both groups was the methemoglobinemia. The highest registered methemoglobin level was

6%. This was our youngest patient: a 19 years old female from the prilocaine 1.5% group. This corresponds to the findings of Vasters et al.[4] who found that the most important risk factors for developing methemoglobinemia are young age, female sex, and high concentration of prilocaine. None of the patients were symptomatic or clinically cyanotic at any moment during or after the surgery. Symptoms like cyanosis, dyspnea, and low oxygen saturation only start to manifest at levels higher than 15%. Therefore, this statistically significant difference is not clinically relevant.

A limitation of this study is the absence of registration of postoperative pain scores as well as the failure to collect reliable results for time of first postoperative intake of pain medication in some patients. However, with similar block offset time and satisfaction scores we do not expect a significant difference in postoperative pain experience between the groups.

A second limitation is the variable blockade of the radial nerve, although blockade of the radial nerve was not rigorously pursued in this study since it was not obligatory for most surgeries. Further research might be conducted to suggest an optimal strategy to reliably block the radial nerve in case of poor ultrasound visualization and injection of low volume.

Conclusion

In summary, prilocaine 1% produces equally satisfactory nerve blockade as prilocaine 1.5% with similar onset time and duration but lower methemoglobin levels. When ultrasound-guided axillary brachial plexus block is performed, 20 ml of prilocaine 1%

results in adequate anesthesia. It can be recommended for outpatient hand or forearm surgeries of short to intermediate duration with limited postoperative pain.

References

1. Bulka CM, Shotwell MS, Gupta RK, Sandberg WS, Ehrenfeld JM. Regional anesthesia, time to hospital discharge, and in-hospital mortality: a propensity score matched analysis. Reg Anesth Pain Med. 2014;39(5):381-6.

2. Lund PC. Citanest and methaemoglobinemia. Acta Anaesthesiol Scand. 1965;16:189-96.

3. Löfström B. Aspects of the pharmacology of local anaesthetic agents. Br J Anaesth. 1970;42(3):194-206.

4. Vasters FG, Eberhart LH, Koch T, Kranke P, Wulf H, Morin AM. Risk factors for prilocaine-induced methaemoglobinaemia following peripheral regional anaesthesia. Eur J Anaesthesiol. 2006;23(9):760-5.

5. Chan VW, Perlas A, McCartney CJ, Brull R, Xu D, Abbas S. Ultrasound guidance improves success rate of axillary brachial plexus block. Can J Anaesth. 2007;54(3):176-82.

6. Lo N, Brull R, Perlas A, Chan VW, McCartney CJ, Sacco R, El-Beheiry H. Evolution of ultrasound guided axillary brachial plexus blockade: retrospective analysis of 662 blocks. Can J Anaesth. 2008;55(7):408-13.

7. Janzen PR, Vipond AJ, Bush DJ, Hopkins PM. A comparison of 1% prilocaine with 0.5% ropivacaine for outpatient-based surgery under axillary brachial plexus block. Anesth Analg. 2001;93(1):187-91.

8. Dunlop DJ, Graham CM, Watt JM. The practical use of axillary brachial plexus block for hand surgery. J Hand Surg Br. 1995;20(5):677–8.

9. Freitag M, Zbieranek K, Gottschalk A, Bubenheim M, Winter R, Tuszynski S, Standl

TG. Comparative study of different concentrations of prilocaine and ropivacaine for intraoperative axillary brachial plexus block. Eur J Anaesthesiol. 2006;23(6):481-6.

10. Saritas A, Sabuncu C. Comparison of clinical effects of prilocaine, dexamethasone added to prilocaine and levobupivacaine on brachial plexus block. J Pak Med Assoc. 2014;64(4):433-6.

11. Tryba M, Kurth H, Zenz M. Clinical and toxicologic study of axillary plexus block with prilocaine or mepivacaine. Reg Anaesth. 1987;10(1):31-6.

12. Nolte H, Dudeck J, Hultzsch B. Studies of the dose dependency of methemoglobin development after administration of prilocaine (Citanest). Anaesthesist. 1968;17(11):343-6.

13. Fenten MG, Schoenmakers KP, Heesterbeek PJ, Scheffer GJ, Stienstra R. Effect of local anesthetic concentration, dose and volume on the duration of single-injection ultrasound-guided axillary brachial plexus block with mepivacaine: a randomized controlled trial. BMC Anesthesiol. 2015;15:130.

14. Nishikawa K, Kanaya N, Nakayama M, Igarashi M, Tsunoda K, Namiki A. Fentanyl improves analgesia but prolongs the onset of axillary brachial plexus block by peripheral mechanism. Anesth Analg. 2000;91(2):384-7.

15. Frkovic V, Ward C, Preckel B, Lirk P, Hollmann MW, Stevens MF, Wegener JT. Influence of arm position on ultrasound visibility of the axillary brachial plexus. Eur J Anaesthesiol. 2015;32(11):771-80.

16. Wong DM, Gledhill S, Thomas R, Barrington MJ. Sonographic location of the radial nerve confirmed by nerve stimulation during axillary brachial plexus blockade. Reg Anesth Pain Med. 2009;34(5):503-7.

17. Qin Q, Yang D, Xie H, Zhang L, Wang C. Ultrasound guidance improves the success rate of axillary plexus block: a meta-analysis. Rev Bras Anestesiol. 2016;66(2):115-9.

Table 1 Baseline characteristics

	Group A (n = 30)	Group B (n = 30)
Sex (no. M / F)	14 / 16	13 / 17
Age (yr)	57 ± 17	58 ± 14
Weight (kg)	77 ± 13	80 ± 14
ASA physical status (no. I / II / III)	12/13/5	9/18/3
Diabetes mellitus (no.)	3	4
Benzodiazepine premedication (no.)	24	24
Duration of nerve block procedure (min)	11.5 ± 2	12 ± 2
Duration of surgery (min)	31 ± 20	25 ± 14
Type of surgery (no.)		
- carpal tunnel release	10	10
- digital flexor tenolysis	9	10
- carpal tunnel release + tenolysis	1	3
- cyst wrist or finger	3	4
- intramedullary pinning metacarpal fracture	5	0
- ulnar collateral ligament repair thumb	0	1
- thumb extensor tendon repair	0	1
- corrective osteotomy finger	1	0
- corrective osteotomy ulna	0	1
- wrist fracture	1	0

Group A = 20 ml prilocaine 1%. Group B = 20 ml prilocaine 1.5%. Values are mean ± SD

Table 2 Comparison of PNB characteristics between both groups

	Group A (n = 29)	Group B (n = 26)	p-value
Onset sensory block (min)			
- ulnar nerve	13 ± 6	11 ± 6	0.103
- median nerve	15 ± 6	14 ± 6	0.437
- musculocutaneous nerve	11 ± 6	12 ± 6	0.863
Duration of block (min)			
- overall sensory	258 ± 67	261 ± 81	0.731
- overall motor	266 ± 83	242 ± 71	0.201
Methemoglobin after 2h (%)	2.20 ± 0.64	3.07 ± 1.07	0.0005

Group A = 20 ml prilocaine 1%, Group B = 20 ml prilocaine 1.5%. Values are mean ± SD

Table 3 Comparison of radial nerve block

Radial nerve	Group A (n= 30)	Group B (n= 30)
- full sensory block (no.)	14	6
- partial sensory block (no.)	12	17
- no sensory block (no.)	4	7

Group A = 20 ml prilocaine 1%, Group B = 20 ml prilocaine 1.5%.

Figure 1 Duration of overall sensory (A) and motor block (B)

Figure 2 Methemoglobin levels after 2h.

Figure 1A

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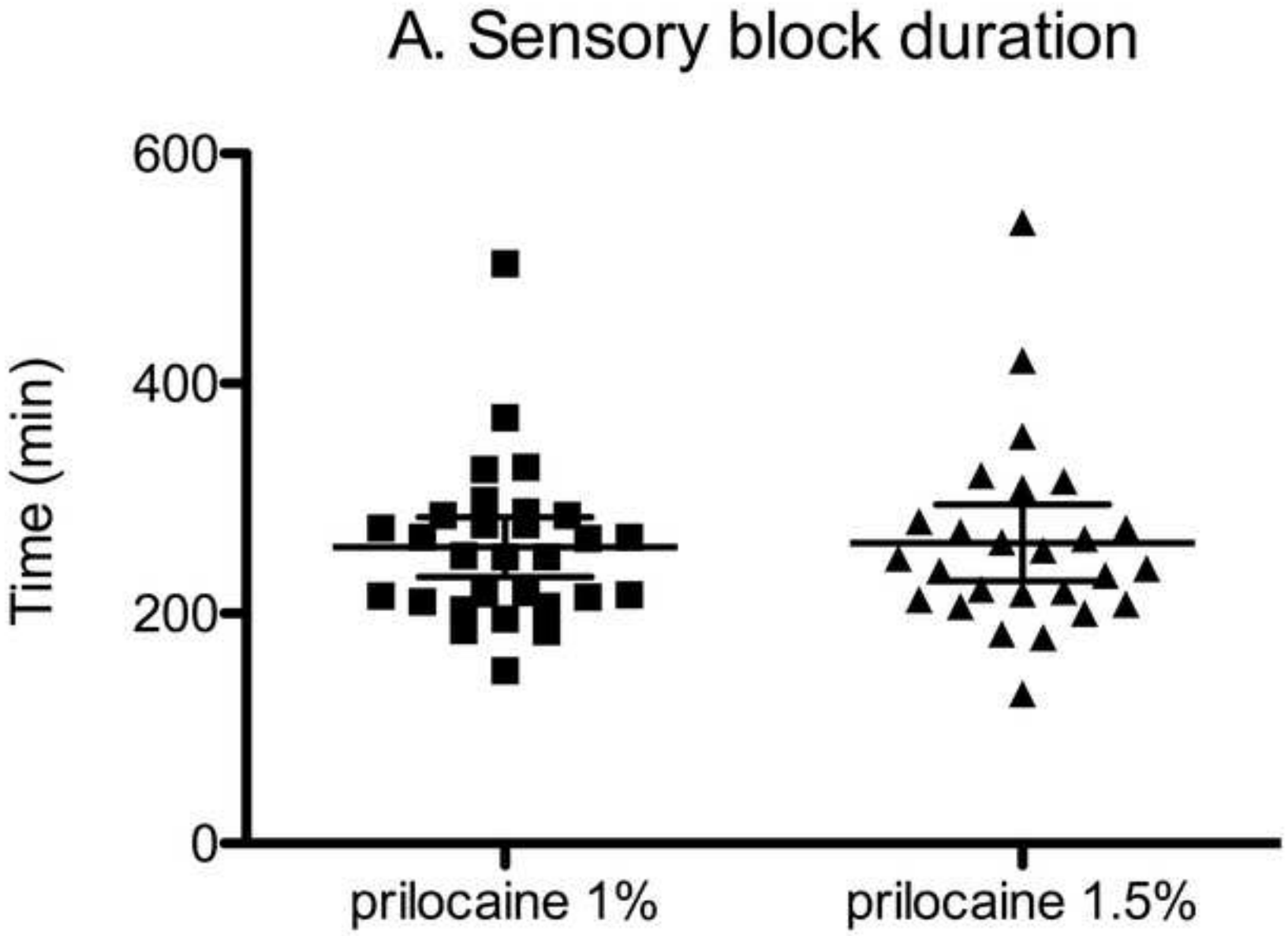

Figure 1B

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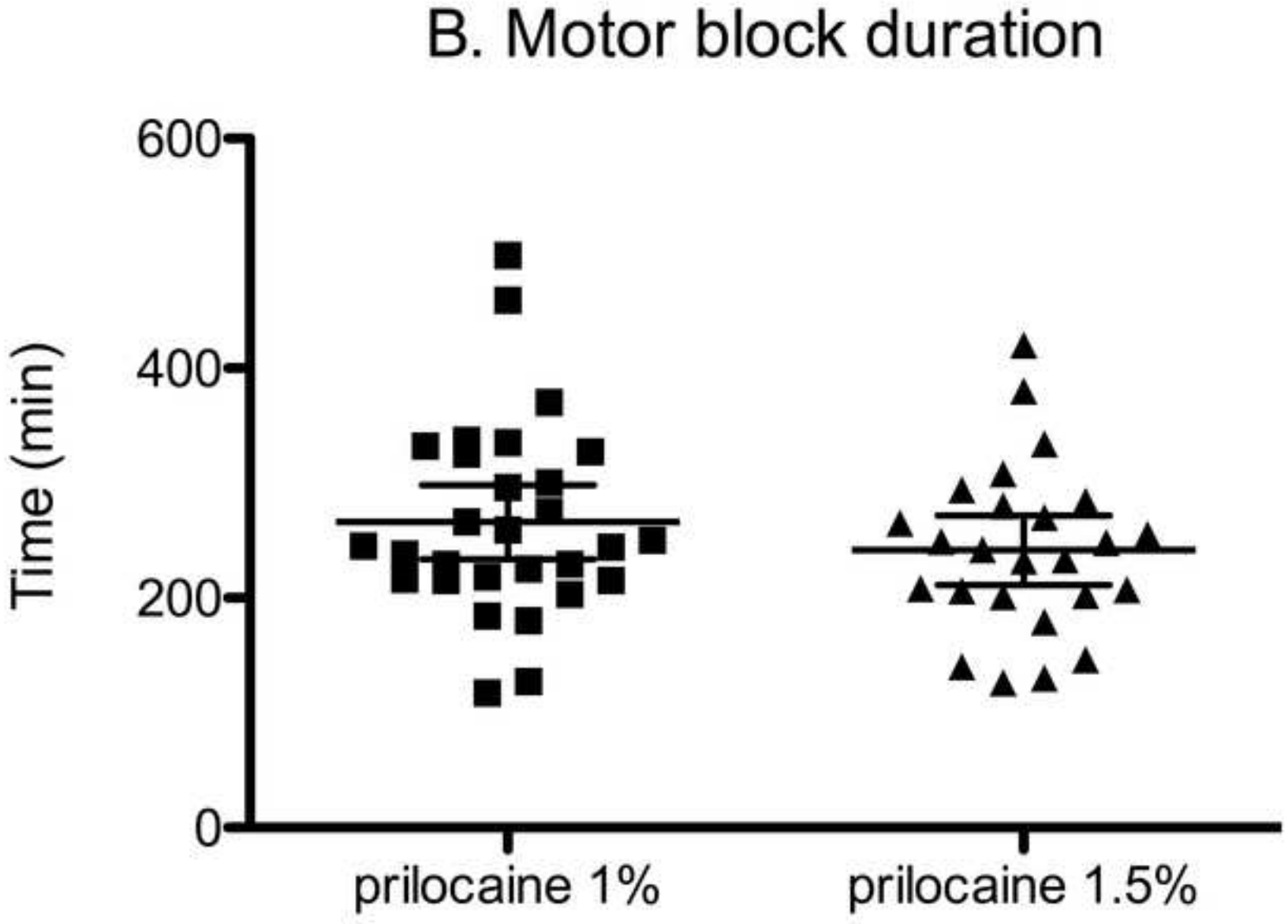

Figure 2

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