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Correlation between shaking behaviors and seizure severity in five animal models of convulsive seizures

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Abstract

Wet dog shakes (WDS) and head shakes (HS) are associated with experimentally induced convulsive seizures. We sought to determine whether these behaviors are correlated or not with major (status epilepticus (SE) or fully kindled animals) or minor (non-SE or partially kindled animals) seizure severity. WDS are directly correlated with SE induced by intracerebral star fruit extract (*Averrhoa carambola*) injection and with kindled animals in the amygdala fast kindling model. On the other hand, WDS are inversely correlated with SE induced by intracerebral bicuculline and pilocarpine injections. Systemic pilocarpine in animals pretreated with methyl-scopolamine barely induced WDS or HS. The role of shaking behaviors may vary from ictal to anticonvulsant depending on the experimental seizure model, circuitries involved, and stimulus intensity. The physical presence of acrylic helmets may per se inhibit the HS response. Also, methyl-scopolamine, a drug incapable of crossing the blood–brain barrier, can induce HS in animals without acrylic helmets.

Keywords: Wed dog shakes; Seizures; Animal models; Rats; Kindling; Star fruit; Epilepsy; Anticonvulsant

1. Introduction

Shaking behaviors in rats may involve only the head (head shakes, HS) or the head and trunk, the so-called wet dog shakes (WDS) [1]. These behaviors are part of the normal behavioral repertoire of rats, and were first studied as behavioral manifestations of rats undergoing morphine withdrawal [1,2] or of pentobarbital-anesthetized animals immersed in cold water [3]. In experimental epilepsy, WDS have been described as behaviors associated with convulsive seizures in kindling [4–7] and acute [8–12] models. In amygdala kindling, the number of WDS is initially low but progressively increases and subsequently decreases when stimulation is pursued [4,6]. On the other hand, in septal [5] or hippocampal [6] kindling, the number of WDS is high initially and decreases with the progression of kindling. Although without great differences in dorsal or ventral hippocampal kindling, in this last site WDS are evoked very quickly after stimulation [6]. In acute models, intraperitoneal or intracerebral kainate, quisqualic acid, or cobalt injections elicit WDS and status epilepticus (SE) [8–10,13,14]. Specifically, lower doses of kainic acid induce more WDS than do doses sufficient to produce seizures [9,14].

Two main hypotheses are used to explain the circuitries involved in the expression of WDS and the potential

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role of WDS in seizures. First (generalization hypothesis), it has been suggested that WDS may be a marker of the progression of limbic seizures toward generalization [14]. In this sense, WDS would be expressed only when the activity of the limbic focus (e.g., in amygdala) has reached the hippocampus [14]. Second (anticonvulsant hypothesis), WDS may be the expression of inhibitory processes associated with limbic seizures [5]; the septal area [5] and somatostatin-related mechanisms [11] would be critical to this response. Curiously, the critical brain site for WDS evoked by morphine withdrawal and cold water stimuli is believed to be the transition between posterior diencephalon and rostral midbrain [3].

Testing both hypotheses (generalization and anticonvulsant) in different animal models of seizures may help to clarify the limits and characteristics of each. In this sense, it is important to verify shaking behaviors not only in animals with effective seizure development, e.g., animals with SE (SE animals) or fully kindled animals, but also in animals that do not exhibit an "adequate" seizure response to a convulsive stimulus, such as animals without SE (non-SE animals) and animals with limited kindling progression notwithstanding stimulation. This article describes the correlation between WDS and HS in SE and non-SE animals in four acute models of convulsive seizures: intracerebral microinjections of bicuculline (ICBIC), pilocarpine (ICPILO), and extract of star fruit, *Averrhoa carambola* (ICSTAR), and systemic administration of pilocarpine (SYSPILO). Also, amygdala fast kindling (AMYFK) was performed to compare shaking behavior between animals with good or bad seizure progression.

As many protocols involve surgical implants (electrodes or guide cannulas) with acrylic helmets, a control study was performed to count WDS and HS in animals without helmets to subtract any eventual effect of handling and systemic injections over the convulsant stimuli.

2. Material and methods

2.1. Animals

Male adult Wistar rats weighing 250–500 g (total $n = 68$) from the main animal house of the Ribeirão Preto Campus at the University of São Paulo were used. Rats were maintained on 12-hour light/dark cycle and given free access to food and water. All experiments were done in accordance with the recommendations of the Brazilian Society for Neuroscience and Behavior for animal experimentation, and all efforts were made to avoid unnecessary animal suffering and to reduce the number of animals used.

2.2. Technical procedures: behavioral analysis, stereotaxic surgery, drug microinjections, electrical stimulation, video/EEG, and histology

In all convulsive models (except AMYFK), animals were considered in SE after continuous clonic seizures or high-voltage electroencephalographic activity for more than 30 minutes after the stimulus, and were called SE animals. On the contrary, animals that did not exhibit SE after the convulsant stimulus (completely seizure-free) were called non-SE animals. Shakes (WDS and HS) were counted in all models.

When applicable, the presence of limbic seizures was evaluated with Racine's scale [15] as modified by Pinel and Rovner [16]: 1 = facial automatisms, 2 = head myoclonus, 3 = forelimb clonus, 4 = rearing, 5 = rearing and falling, 6 = three or more class 5 seizures, 7 = running and jumping, 8 = generalized tonic–clonic convulsions. For the AMYFK protocol, animals were considered fully kindled when they exhibited three class 4 seizures, after the stimuli. This decision was based on studies that consider class 4 seizures as a sufficient criterion for severe limbic seizures [17–19]. Indeed, the presence of three class 5 motor seizures has been assumed, by many authors, as the ultimate behavioral manifestation of amygdaloid kindling in the rat, referred to by some investigators as a "fully" kindled motor seizure. However, other experiments with additional stimulation have revealed that three class 5 seizures are not really the end of the kindling progression. If stimulation of the animals continues, they progress through several stages of epileptogenesis [16]. The literature is divided with respect to the criterion for fully kindled animals. For example, behavioral seizure scores of 4 and 5 are considered to represent kindled motor seizures by Lothman and Williamson [18]. Kokaia et al. [19] performed an analysis in animals that exhibited class 4 and 5 seizures. Ebert and Loscher [20] studied animals with one class 5 seizure. Lothman et al. [17] considered the occurrence of a stage 4 or 5 behavioral seizure as a criterion for severe limbic seizures. Moreover, the term *fully kindled* is also controversial in the kindling literature, and can refer to a situation when kindled responses are elicited with each stimulus, including the first stimulus after a prolonged stimulus-free interval [17].

When required by experimental protocols, stereotaxic implantation of guide cannulas, electrodes, or chemitrode (to inject and record at the same site) was done according to a stereotaxic atlas [21] and under ketamine/xylazine (0.1 and 0.007 mL/100 g rat wt, respectively), tribromoethanol (2.5%, 1 mL/100 g,) or thionembutal (25 mg/kg, i.p.) anesthesia. Cannulas, screws, and electrodes were fixed in the skull with zinc and dental acrylic cement. Three screws were used for mechanical support of the prosthesis. A screw posi-

tioned over the right frontal cortex was used as the ground electrode. After surgery, animals had 3 (ICSTAR model) or 5 (all other models) days for recovery before the experiments. The criteria to maintain animals in the ICSTAR group, despite the relatively short recovery period after surgery, were normal behavior and normal EEG recordings (without any polyspikes or spike-and-wave complexes). Intracerebral microinjections occurred on the day of the experiment. An injection needle was introduced through the preimplanted guide cannula, into an injection site always 1 or 2 mm below it. The desired volume of drug was delivered with a 10-μL syringe (Hamilton, Reno, NV, USA) with a PE10 polyethylene tube, coupled to a syringe pump (Harvard 2000, Harvard Apparatus, South Natick, MA, USA). The injection rate was 1 μL/min. On completion of tests, the correct positioning of injection sites and electrodes was evaluated. Briefly, rats were given a lethal dose of thionembutal (80 mg/kg, ip) and perfused through the heart with phosphate-buffered saline (PBS: phosphate buffer 100 mM + NaCl 0.9%, pH 7.4). After further fixation, brains were frozen and coronally sectioned in a cryostat (Zeiss, Oberkochen, Germany). Sections (40 μm) were mounted on chrome–alumin gelatin-coated slides, stained with cresyl violet, and checked visually under the light microscope.

Twisted monopolar Teflon-coated stainless-steel electrodes (0.008 in., AM Systems, Carlsborg, USA) were used for stimulation of brain nuclei. The same electrodes were used to record the electrical activity of target brain nuclei. Electrodes were connected to devices for electrical stimulation or video/electroencephalogram (video/EEG) recording. In AMYFK, electrical stimulation was performed with a GRASS-S88 stimulation system (Grass Instruments, Quincy, MA, USA). Signals sent to the rat brain were monitored with an oscilloscope (HP130C, HP, Palo Alto, CA, USA) with monopolar constant-current stimulation with biphasic square wave pulses (60 Hz, 1-ms duration). Video/EEG recording was performed as previously described [22]. Electrophysiological signals were recorded in personal computers using Acqknowledge 3.5.3 software (MP100 Manager, Biopac Systems, San Francisco, CA, USA) after amplification (sample rate: 500 Hz; CyberAmp 320, Axon Instruments, Foster City, CA, USA) and digitalization (MP100, Biopac Systems). Animal images were captured and digitalized with a video grabber (ATI All-in-Wonder Pro, USA). A field effect transistor (FET) source follower system was used to reduce movement artifacts [22].

Bicuculline methiodide was from RBI (Natick, MA, USA); thionembutal and sterile 0.9% saline from Cristália (Campinas, Brazil); and mono- and dibasic salts of sodium phosphate, from Merck (Gibbstown, NJ, USA). All other drugs were purchased from Sigma (St. Louis, MO, USA).

2.3. ICBIC: intracerebral bicuculline microinjection into the amygdalohippocampal area, posteromedial part (AHiPM)

Rats ($n = 9$) were implanted with three guide cannulas: two (bilateral) directed to the SNPR (Paxinos and Watson [21] atlas coordinates, in mm: anterior–posterior (AP): −5.8; lateral (L): ±2; dorsal–ventral (DV): 8.2) and one to the AHiPM (AP: −4.8; L: −1.4; DV: 9.2). On the day of the experiment, after being placed in an acrylic test cage (60 cm in diameter × 30 cm high) for 30 minutes (habituation), rats were gently held and received initially the 0.2-μL injection of PBS at the rate of 0.2 μL/minute bilaterally in the SNPR. Then, the injection cannula was introduced inside the guide cannula, but nothing was injected. WDS were counted for 10 minutes (controls). After this period, rats were held again and injected with 0.2 μL of bicuculline (20 nmol in 0.2 μL of PBS, pH 7.4; volume: 0.2 μL; rate 0.2 μL/minute) in the AHiPM. WDS and HS were counted for 10 minutes after bicuculline injection, and whether animals exhibited SE or not was also recorded. Because previous results had demonstrated that injection of 0.2 μL of PBS into the SNPR or AHiPM does not have any behavioral effect, what we are describing here is only the severe response to bicuculline microinjection into the AHiPM [23].

2.4. ICPILO: intracerebral pilocarpine microinjection into ventral hippocampus

Rats were injected with pilocarpine (2.4 mg in 1 μL of 0.9% sterile NaCl, pH 7.4; volume: 1 μL, rate: 0.5 μL/min, $n = 16$) or saline (0.9% sterile NaCl, pH 7.4, $n = 3$, controls) in the ventral hippocampus (AP: 6.3; L: −4.5; DV: 4.5). WDS and HS were quantified for 40 minutes after injection. The correlation between the numbers of WDS, HS, and SE animals was quantified.

2.5. ICSTAR: intracerebral star fruit extract microinjection into ventral hippocampus with video/EEG monitoring of ventral hippocampus

In six rats, a chemitrode was implanted into the ventral hippocampus (AP: 6.3; L: −4.5; DV: 4.5). We had previously observed that injection of PBS or even saline (0.9% NaCl) into the hippocampus does not cause any behavioral or electroencephalographic alterations, compared with controls [24]. Therefore, 3 days after surgery, animals were placed in the acrylic test cage and recorded for 30 minutes (without injection) to obtain control EEG records and behavior (number of WDS or HS). After this period, the star fruit extract was injected into the hippocampus, and experimental EEGs and behavior were recorded. The extract was obtained from lyophilized star fruit juice, resuspended in PBS. WDS and

HS were counted for 60 minutes, 30 minutes with no injection (control) and 30 minutes after star fruit extract injection. On the EEG, polyspikes or spike-and-wave complexes with a magnitude higher than the threshold of 1.02 mV, which is equivalent to 3.8 times the standard deviation SD of basal EEG activity, were considered high-voltage EEG seizure activity. The presence of high-voltage EEG seizure activity was used as a SE marker. EEG seizures were counted manually with the aid of *Acqknowledge* software. Correlations between the numbers of EEG seizures, WDS, and HS were quantified.

2.6. AMYFK: Rapid electrical stimulation of the basolateral amygdala nuclei with video/EEG monitoring of ipsilateral hippocampus

One stimulation electrode was implanted into the left basolateral amygdala nuclei (AP: 2.3 mm; ML: 4.7 mm; DV: 7.1 mm) and a recording electrode was implanted into the left hippocampus (AP: 6.3 mm; ML: 4.5 mm; DV: 4.5 mm) of Wistar rats ($n = 8$), with video/EEG monitoring. Afterdischarge threshold was determined on the first experimental day. On the next 2 days, animals received 10 stimuli (500 μA for 10 seconds) per day every 30 minutes. A half-hour before the stimulus, rats were injected with vehicle (PBS, i.p.). On the fourth day, animals received the last stimulus and were perfused. Sham-stimulated control animals had the same electrodes implanted and, like experimental animals, were placed in stimulation cages, but did not receive electrical stimulation. All experiments started at 7:30 A.M. The presence and severity of limbic seizures [4] were registered during the kindling process. Whether animals were completely or incompletely kindled was noted. Kindling was considered complete if the animal exhibited progressive worsening of behavioral seizures, up to classes 4 and 5, after all stimuli. Any animals that did not reach these behavioral indexes, remaining predominately in seizure classes 1 and 2, were considered to be incompletely kindled. WDS and HS were counted in completely and incompletely kindled animals.

2.7. Control study of WDS and HS for peripheral injections: effects of handling, new environments, and intraperitoneal injections

Male Wistar rats ($n = 8$) were manipulated for 10 minutes and immediately placed into acrylic test cages for 40 minutes, once a day for 5 days (always starting at 9:00 A.M.). Two days later, the protocol restarted (manipulation for 10 minutes followed by 40 minutes in the acrylic test cages) for the sixth to ninth experimental days, but now the animals received no supplementary treatment (only manipulation again, sixth day), a sham intraperitoneal injection (seventh day: animals were held and stung with a 26-gauge syringe needle, containing no liquid), a saline injection (eighth day: NaCl 0.9%, 1 mL/kg rat wt), and injection of a drug that does not cross the blood–brain barrier (ninth day: methyl-scopolamine in saline 0.9%, 2 mg/mL, 1 mL/kg). The days were denoted HAB1 (first day), HAB6 (sixth day), SHAM (seventh day), SAL (eighth day), and MSCOP (ninth day). The time evolution of WDS and HS was registered in eight periods of 5 minutes on all days tested.

2.8. SYSPILO: systemic pilocarpine injection

Adult male Wistar rats ($n = 18$) were recorded once a day for 3 consecutive days, after the following stimuli: first day, sham injection (recorded for 40 min); second day, 0.9% saline (2 mL/kg, recorded for 40 minutes); third day, methyl-scopolamine (2 mg/kg, 2 mg/mL, peripheral cholinergic antagonist) and, 30 minutes later, intraperitoneal pilocarpine (340 mg/kg, 2 mL/kg). WDS and HS were counted in 5-minute intervals starting immediately after pilocarpine injection, for 40 minutes, for SE and non-SE animals.

2.9. Statistical analyses

Comparisons between multiple groups were executed with a one-way ANOVA with Dunnet's test and an ANOVA on ranks (Kruskal–Wallis) with Dunn's test for normal and nonnormal data, respectively. Comparisons in the same rats, before and after a treatment, were made with a paired Student t test and the Wilcoxon signed rank test, for normal and nonnormal data, respectively. Multiple comparisons on the same animals were made with the one-way repeated-measures ANOVA and Friedman repeated-measures ANOVA on ranks for normal and nonnormal data, respectively. Correlations between WDS and kindling evolution (in the AMYFK model) and between WDS and number of EEG seizures (in the ICSTAR model) were calculated with the Spearman and Pearson methods, respectively. Results were considered significant at $P < 0.05$.

3. Results

3.1. ICBIC

Injection of bicuculline into the AHiPM of nine rats evoked SE in four of the rats. The average number of WDS was significantly larger in non-SE rats (50.6 ± 2.01), compared with controls (1.77 ± 0.39) and SE animals (10.75 ± 1.95) (Fig. 1). In this model, animals did not exhibit HS.

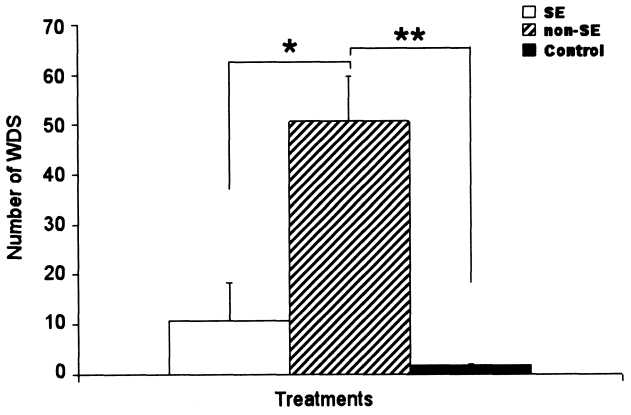

Fig. 1. Number of WDS (mean ± SEM) in 30 minutes of observation of rats after injection of PBS (controls) and bicuculline into the AHiPM. Non-SE rats had a significant increase in WDS compared with controls and SE animals. $^{*}P < 0.05$, Student t test; $^{**}P < 0.001$, Kruskal–Wallis with post hoc Dunn test.

3.2. ICPILO

In this model, SE animals had a larger number of WDS (44 ± 8.2, mean ± SEM) when compared with non-SE and control animals (1.17 ± 0.88 and 0.58 ± 0.55, respectively, Fig. 2A). The number of WDS (but not of HS) increased with time and peaked 15 minutes after pilocarpine microinjection (Fig. 2B). The majority of animals exhibited SE 30 minutes after injection (68.7%, Fig. 2B). There was an inverse correlation between number of WDS and number of animals that entered SE (Fig. 2B).

3.3. ICSTAR

In all animals, intrahippocampal star fruit extract injection caused EEG seizure activity in the hippocampus, but almost no convulsive behavior. The number of WDS (but not of HS) increased simultaneously with the number of EEG seizures, with a positive correlation (Fig. 3B).

3.4. AMYFK

Four rats were not completely kindled and exhibited only facial automatisms or head myoclonia (limbic seizures classes 1 and 2, respectively [4]). Nevertheless, four of eight rats progressed to generalized seizures: forepaw myoclonia, rearings, and rearings with falling (classes 3–5, respectively [4]). There was a positive correlation between number of WDS and kindling evolution in all animals (Figs. 4A and B). But those rats that exhibited more severe seizure progression with generalized class 3–5 seizures in Fig. 4B had significantly more WDS than partially kindled rats with only class 1 and 2 seizures, seen in Fig. 4A (2.13 ± 0.96 and 0.55 ± 0.40, respectively, mean ± SEM; $P < 0.001$, Mann–Whitney).

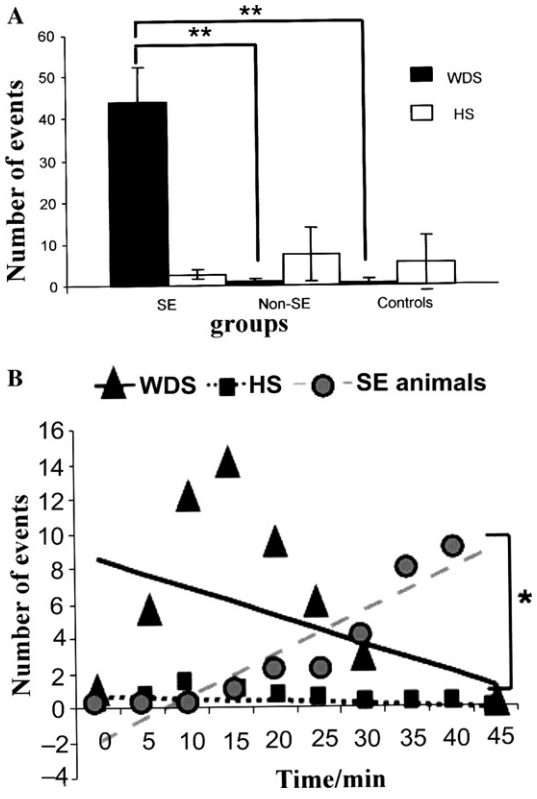

Fig. 2. Shaking behaviors in the ICPILO model. (A) Number of WDS (mean ± SEM) in 1 hour of observation of rats after injection of PBS (controls) and pilocarpine into the ventral hippocampus. SE animals had a significant increase in WDS compared with controls and non-SE animals. $^{**}P < 0.05$, Student t test. (B) Time evolution of WDS (▲), HS (■), and the cumulative number of SE animals (●) after intrahippocampal pilocarpine injection, in 5-minute intervals. Note that the number of WDS increases initially, but decreases with progression to SE. Lines: linear regression of WDS episodes (continuous) and SE animals (interrupted). There was an inverse correlation between number of WDS and number of SE animals ($r = 0.65$, Pearson). $^{*}P < 0.05$.

3.5. Control study of WDS for peripheral injections

In this protocol, the average number of HS was higher than that of WDS on all test days ($P < 0.05$, Kruskal–Wallis). HS diminished gradually from HAB1 to SAL (Fig. 5). Nevertheless, injection of methyl-scopolamine increased the number of HS, compared with SAL (Fig. 5). There were no differences in WDS (Fig. 5).

3.6. SYSPILO

Intraperitoneal injection of pilocarpine induced SE in 11 of 18 animals; the remaining seven were non-SE animals (completely seizure free). No difference was noted between HS and WDS among SE or non-SE animals (data not shown).

3.7. Histological control

All injection sites are illustrated in Fig. 6. An animal was included in the group only if the injection site was

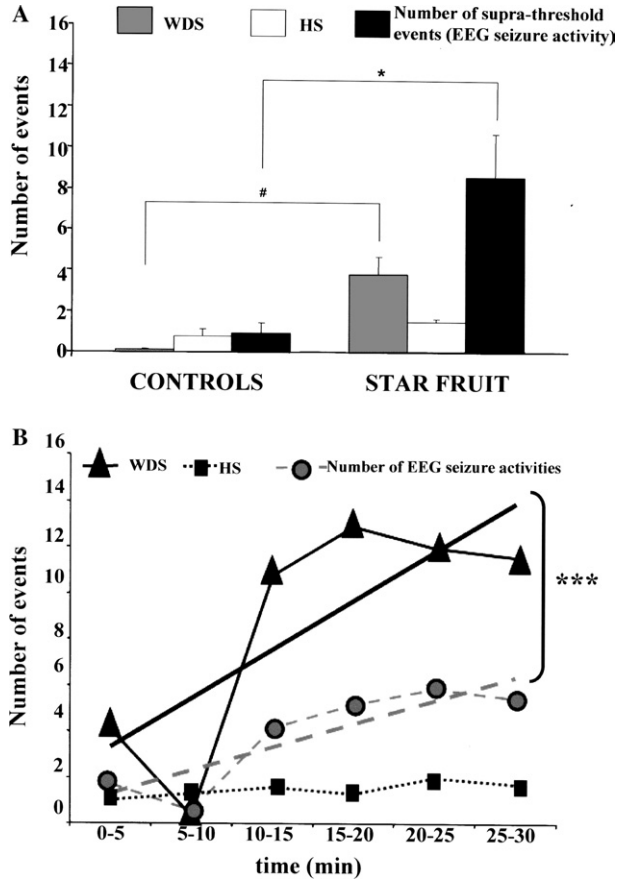

Fig. 3. Shaking behaviors after intrahippocampal star fruit extract injection. (A) Numbers of WDS, HS, and EEG seizures after PBS (controls) or extract injection. The numbers of WDS and EEG seizures increased in experimental animals. $^{*}P < 0.05$, Wilcoxon test; $^{\#}P < 0.05$, paired t test. (B) Time evolution of WDS, HS, and EEG seizures in a 30-minute period of observation of rats after injection of star fruit extract into the ventral hippocampus. Lines: linear regression of WDS episodes (continuous) and EEG seizures (interrupted). There was a positive correlation between the number of WDS (but not HS) and number of EEG seizures in SE animals. $^{***}P <$ 0.05, Pearson correlation.

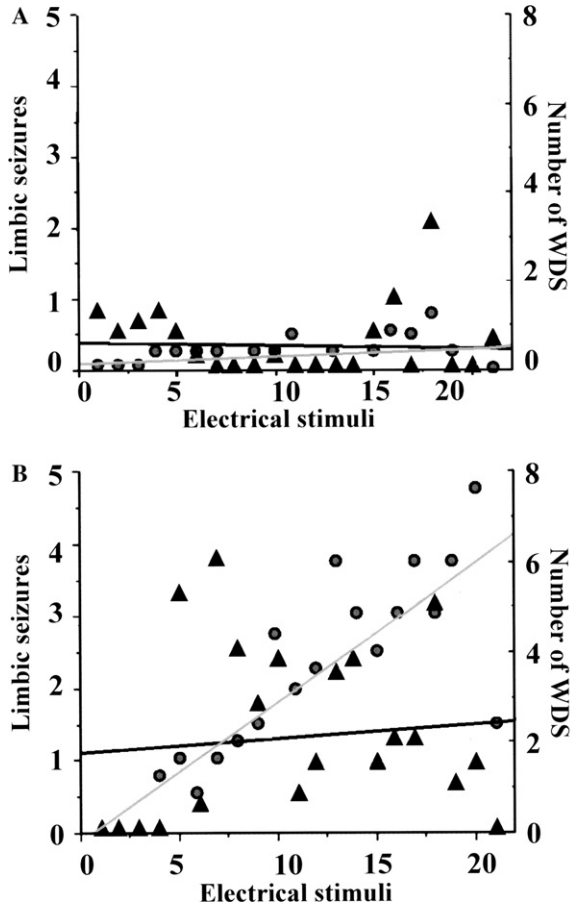

Fig. 4. Evolution of WDS and limbic seizures in the AMYFK model (21 stimuli). (A) Partially kindled animals (limbic seizure classes 1 and 2). (B) Fully kindled animals (classes 3–5). Lines: linear regression of WDS episodes (black) and seizure severity (gray). There was a positive correlation between number of WDS and number of limbic seizures in both (A) and (B) (A: $r = 0.24$, $P = 0.02$; B: $r = 0.39$, $P = 0.01$; Spearman correlation).

inside the boundaries of the nucleus. Some injections in the ICPILO group were near the border of the hippocampus, but the injection area, into which the drug spread, was mainly inside the target. In the ICSTAR group, all injection sites were also inside the hippocampus, although near the curvature of the dentate gyrus. The PBS injection sites in SNPR (ICBIC model) are not shown as it is believed that they did not influence the effects of bicuculline injection into the AHiPM, in SE and non-SE animals.

4. Discussion

This work confirms that HS and WDS are not uniform and not always directly correlated with the presence of major seizures (SE animals or completely kindled animals) in different models of experimental seizures.

Two of the models tested (AMYFK and ICSTAR) seemed to fit the generalization hypothesis. In the AMYFK model, we saw confirmation of standard amygdaloid kindling [4,6]. In this model, WDS were increased in fully kindled rats (Fig. 4B), although also present in animals with seizures of lower severity (Fig. 4A). In Fig. 4B, the first four stimulations did not evoke WDS, but animals also did not have any behavioral seizures. We recognize that the correlation factor r is very low in both graphics; however, r values are positive, corresponding to $P \leqslant 0.02$, and thus represent, without doubt, statistically significant increases in the number of WDS.

Similarly, in the ICSTAR model, the number of WDS increased concomitantly with the number of high-voltage events, above the threshold of 3.8 times the SD of basal electrical activity (EEG seizure activity, Fig. 3).

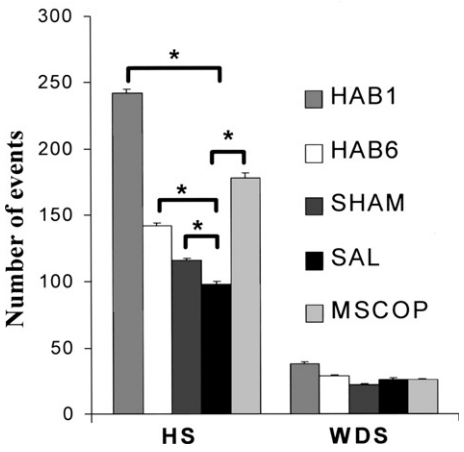

Fig. 5. Shaking behaviors (HS and WDS, average ± SEM) in 40-minute observation periods after handling only (HAB1 and HAB6: first and sixth habituation days, respectively), sham intraperitoneal injection (SHAM, seventh day), intraperitoneal saline injection (SAL, eighth day), and intraperitoneal methyl-scopolamine injection (MSCOP, ninth day). The number of HS was larger than the number of WDS. The average number of WDS did not differ among observations ($P = 0.0539$, Kruskal–Wallis). $^{*}P < 0.05$, Kruskal–Wallis. Note that the number of HS decreased with habituation and handling from HAB1 to SAL, and increased only after methyl-scopolamine injection. Results in this figure represent the total numbers of HS and WDS, as there were no differences in the time evolution of these behaviors on the days tested ($P > 0.05$, Kruskall–Wallis). HAB, habituation.

In two other models (ICPILO and ICBIC), the presence of WDS seemed to be explained by the anticonvulsant hypothesis. In ICPILO-injected animals that would have SE, there was, initially, a large number of WDS, but this number fell dramatically after SE (Fig. 2B). In the ICBIC model, the WDS abundance was a predicting factor that the animal would not exhibit SE (Fig. 1). In these two models, our interpretation is that shaking behaviors are not seizure manifestations; WDS and seizures may propagate by different pathways [25,26]. In some situations, WDS may be the expression of a central strategy to control seizures and, in this sense, would be anticonvulsant, similar to what was proposed by Dagci et al. [11] through somatostatin-related mechanisms, and may involve the septal-hippocampal area [5], an area rich in somatostatin [27]. A consequence of HS and WDS could be an increase in brain perfusion, thus safeguarding mitochondrial energy supply and preventing seizures [28]. This hypothesis must be carefully studied in future work.

In addition to the two hypotheses discussed in the present work, shaking and epilepsy could theoretically have a third interpretation: they could be an epiphenomenon. But even if these shakes were elicited secondarily, they would be markers of circuitry activation, maintaining their importance in experimental epilepsy.

In the SYSPILO model, almost no WDS or HS were observed. In fact, the number of shaking behaviors in this model was the lowest of all tested.

In protocols with stereotaxic implants, e.g., cannulas, electrodes (ICBIC, ICPILO, ICSTAR, and AMYFK), the number of HS may be decreased compared with that of animals without surgical helmets (intraperitoneal sham or saline injections and SYSPILO). This device may per se change the physical *momentum* of the head of the animal, and inhibit the HS response (Moraes, personal communication). The present results demonstrate that the number of HS fell gradually and daily with handling in animals without helmets, and even the relatively harmful sting of a needle (SHAM) did not alter this decrease (Fig. 5). Generally, when drugs are injected intraperitoneally, their effects on WDS are interpreted only as central effects [29,30]. But our data show that peripheral stimuli can also trigger shaking responses, as the injection of methyl-scopolamine, which does not cross the blood–brain barrier, enhanced HS even after habituation (Fig. 5). Maybe the specific peripheral cholinergic antagonism of methyl-scopolamine is responsible for the increase in HS, which is reinforced by the almost complete absence of shaking behaviors after systemic injection of pilocarpine into animals pretreated with the same dose of methyl-scopolamine (Fig. 5).

Only in the ICSTAR model were animals given 3 days to recover after stereotaxic surgery. In all other

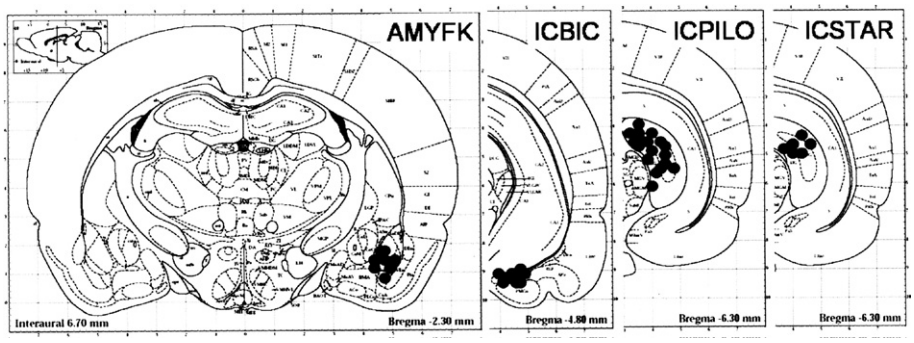

Fig. 6. Central injection or stimulation sites in the AMYFK, ICBIC, ICPILO, and ICSTAR models. Adapted, with permission from Paxinos and Watson [21].

models, animals were allowed 5 days to recover before experiment. However, in the ICSTAR group EEG recordings were absolutely normal (data not shown), without any polyspikes or spike-and-wave complexes. Certainly there may be some residual inflammation below the skin as a result of cannula implantation, but there was no apparent painful response to manipulation of the head of the animals. Also, the guide cannula never runs to the target nucleus, and always stops well above it. So, in general, brain parenchyma remains untouched until the day of the experiment.

It is believed that star fruit extract may contain excitatory neurotoxins (probably GABAergic antagonists) responsible for seizures in renal patients and experimental animals (present work); its isolation and characterization are underway [24]. This work was previously published in abstract form [31].

5. Summary

In conclusion, WDS are directly correlated with SE and fully kindled animals in the ICSTAR and AMYFK models, respectively, and inversely correlated with SE in the ICBIC and ICPILO models. In the AMYFK model, the total number of WDS was larger in completely kindled animals than in partially kindled animals (2.13 ± 0.96 and 0.55 ± 0.40, respectively, mean $\pm$ SEM; $P < 0.001$, Mann–Whitney). The role of shaking behaviors may vary from ictal (generalization hypothesis) to anticonvulsant (anticonvulsant hypothesis), depending on the experimental seizure model, circuitries involved, and stimulus intensity. In the present work, the ICSTAR and AMYFK models are apparently explained by the generalized hypothesis, while the ICBIC and ICPILO models are explained by the anticonvulsant hypothesis. The physical presence of acrylic helmets may per se inhibit the HS response. Also, methyl-scopolamine, a drug incapable of crossing the blood–brain barrier, can increase the number of HS in animals without acrylic helmets.

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