Supplemental Material (SM)

Kv4 potassium channels modulate hippocampal EPSP-Spike potentiation and spatial memory in rats.

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RESULTS

Electrophysiology (SM Fig. 1 and 2)

Baseline and IO curves

**Baseline**

Injection of vehicle or AmmTX3 had no effect on the baseline measures for fEPSP slope (vehicle $F_{1,10}=0.00$, NS; AmmTX3 $F_{1,12}=0.01$, NS) (SM Fig. 1A and B) or PS amplitude (vehicle $F_{1,10}=0.81$, NS; AmmTX3 $F_{1,12}=2.64$, NS) (SM Fig. 2A and B).

**IO curves**

Across whole IO curves, two-way ANOVA showed no significant changes following injection of vehicle or AmmTX3 for fEPSP slope (vehicle $F_{1,10}=0.04$, NS; AmmTX3 $F_{1,12}=0.57$, NS) (SM Fig. 1C and D) or PS amplitude (vehicle $F_{1,10}=0.01$, NS; AmmTX3 $F_{1,12}=3.03$, NS) (SM Fig. 2C and D).

**Paired pulse ratio**

*fEPSP slope*

In all cases, PPR showed a clear paired pulse inhibition. Within-group comparison showed no changes were observed following injection (vehicle $F_{1,10}=0.02$, NS; AmmTX3 $F_{1,12}=0.01$, NS). Similarly, between-group comparison showed no differences before or after injection for EPSP slope ratio (pre-injection: $F_{1,11}=0.00$, NS; post-injection: $F_{1,11}=0.02$, NS) (SM Fig. 1E and F).

**PS amplitude**

PPR for PS amplitude showed an inhibition at all delays. Within-group comparison showed no effect of the injection in both groups (vehicle $F_{1,10}=0.01$, NS; AmmTX3 $F_{1,12}=0.38$, NS). Likewise, between-group comparison showed no differences (pre-injection: $F_{1,11}=0.55$, NS; post-injection: $F_{1,11}=1.17$, NS) (SM Fig. 2E and F).
SM Figure 1: Effect of AmmTX3 injection on fEPSP slope of baseline recording, Input/Output (IO) curves, and Paired Pulse Ratio.

A-B: fEPSP slope during 40 min baseline recording in vehicle (A) and AmmTX3 (B) groups. One single stimulation was applied every 30 s. AmmTX3 was injected after 20 min (arrow).

C-D: IO curves for fEPSP slope, obtained by a single stimulation across an intensity range of 5 to 200 µA in vehicle (C) and AmmTX3 (D) groups. Measures were repeated four times, every 15 s, for each rat (n=6) and averaged for each stimulation intensity. Data are expressed as mean ± SEM.

E-F: Paired Pulse Ratio of fEPSP slope. Data obtained across an interpulse interval range of 10 to 140 ms. Each measurement was repeated twice, every 15 s, and averaged. Ratio calculation was 2nd/1st pulse. Data are expressed as mean ± SEM (n=6).
SM Figure 2: Effect of AmmTX3 injection on Population Spike (PS) amplitude of baseline recording, Input/Output (IO) curves, and Paired Pulse Ratio.

**A-B:** PS amplitude during 40 min baseline recording in vehicle (A) and AmmTX3 (B) groups. One single stimulation was applied every 30 s. AmmTX3 was injected after 20 min (arrow).

**C-D:** IO curves for PS amplitude, obtained by a single stimulation across an intensity range of 5 to 200 µA in vehicle (C) and AmmTX3 (D) groups. Measures were repeated four times, every 15 s, for each rat (n=6) and averaged for each stimulation intensity. Data are expressed as mean ± SEM.

**E-F:** Paired Pulse Ratio of PS amplitude. Data obtained across an interpulse interval range of 10 to 140 ms. Each measurement was repeated twice, every 15 s, and averaged. Ratio calculation was $2^{nd}/1^{st}$ pulse. Data are expressed as mean ± SEM (n=6).
Locomotor and feeding activities

To determine the specific dose of AmmTX3, we first observed the effects of 1 and 0.75 μg of AmmTX3, injected i.c.v., on locomotor activity in the rat in an open field. During the first hour of the diurnal period, AmmTX3 or vehicle was injected immediately before beginning the locomotor task. SM Figure 3A shows a difference in the number of zones crossed during the first 30 min after AmmTX3 injection (F\(_{2,12}\)=5.78, P<0.05). The dose of 1μg AmmTX3 induced a significant decrease of locomotor activity (Scheffé, P<0.05) whereas 0.75 μg AmmTX3 induced no difference. During the first day following injection, the food and water intake was similar between the three rat groups (food intake: vehicle, 26.58±1.44g; 0.75μg, 19.90±2.59g; 1μg, 24.18±1.87g; water intake: vehicle, 45.68±3.57g; 0.75μg, 27.56±7.67g; 1μg, 36.8±2.82g) (F\(_{2,12}\)≤3.09, NS). The dose of 0.75 μg of AmmTX3, which did not modify locomotor, feeding or drinking activity, was therefore used in all attention and spatial learning tasks and electrophysiological experiments.

Effect of AmmTX3 on attention processes

AmmTX3 (0.75 μg), was injected i.c.v. 30 min before the 10 min session and was tested for its effect on attention processes in an open field. Entry latency, time spent in contact with the object present in the central zone, and number of contacts with the object are presented in SM Figure 3B. ANOVA revealed no significant difference for any of the parameters between the first and second objects presented 48 h apart (F\(_{1,8}\)≤4.74, NS). Novelty was not a critical factor eliciting the investigator response, as performances were similar for the two object presentation sessions. After injection, comparison of the recorded parameters across groups revealed no significant difference (F\(_{1,8}\)≤2.04, NS). Thus, AmmTX3 had no effect on attention processes in comparison with vehicle rats.
Figure 3: Effect of AmmTX3 on locomotor activity and attention processes in an open field task.

A. The doses of AmmTX3 injected i.c.v. were 0.75 (n=5), 1 (n=5) or 0 μg (vehicle group; n=5). Frequency of movement of AmmTX3 and vehicle-treated rats were evaluated by number of areas crossed in open-field by 10-min blocks on 60 min. Insert: total area crossed during a 30-min period. * P<0.05 in ANOVA comparing the vehicle and AmmTX3 groups.

B. AmmTX3 (0.75 μg) (n=5) or vehicle (n=5) was injected 30 min before the 10-min session during which an object was placed in the central zone. Latency, total contact duration with the object, and total contact number with the object are recorded. Data are expressed as mean ± SEM.
MATERIALS AND METHODS

Electrophysiological studies

Surgery

Animals were mounted in a stereotaxic frame and were implanted unilaterally in the left hemisphere. The depths of the recording and stimulating electrodes were adjusted under electrophysiological control to maximize the slope of the positive-going field excitatory postsynaptic potential (fEPSP) evoked by stimulation of the MPP. Bipolar electrodes were made from 66-µm diameter formvar-insulated nickel-chrome wire (A-M Systems Inc.) running through beveled fine-gauge steel cannula, homemade insulated except at the tip (Cooper’s Needle Works LTD). Differential records were made between the two tips. The distance between the two tips was 1-1.5 mm for recording and 0.5 mm for stimulating electrodes.

Experimental parameters

To maximize the chances of observing changes on hippocampal LTP following AmmTX3 infusion, a HFS protocol was set up to obtain a moderate level of LTP, thus leaving more margins for synaptic plasticity to occur. This protocol was basically one-fifth of the protocol that had been used in previous studies (Truchet et al. 2002), and was itself derived from the “LTP saturation” protocol described by Jeffery and Morris (1993): 2 main trains at 1 min intertrain interval, each main train was set up with 5 subtrains at 1 Hz intersubtrain interval (Fig. 4). Each subtrain contained a burst of 10 biphasic (+250 µs/-250 µs) a pulse at 435 Hz. Stimulation intensity was the same for HFS and baseline. Animals were sacrificed immediately after the last record.

Figure 4: High Frequency Stimulations (HFS) Protocol.
The amplitude of the population spike was measured as indicated below:

\[
\text{PS amplitude} = \frac{a + b}{2}
\]

**Figure 5. Measure of PS amplitude**

**Locomotor and feeding activities**

We used the method of Mpari et al. (2008). Because the spatial learning task involved locomotion and feeding activities, it was necessary to test the effect of AmmTX3 on these behaviors. A standard circular open-field was divided into 41 equal areas. During testing the rats were video-tracked individually into the open-field arena for a period of 60 min. The arena was cleaned with 70% ethanol solution between trials. Locomotor activity was measured by counting the number of areas crossed, blindly, for each rat. An area was considered to have been crossed if at any point in time it contained both the head and the two front paws of the rat. Moreover, the number of central-area crossing was counted. To evaluate any possible alteration of the motivational state during a spatial-learning task, it was necessary to test the effect of AmmTX3 on feeding behavior. The food and water intakes of rats were determined by calculating the difference between the food and water given (20 g) and the food and water remaining over the time period concerned.
**Attention task**

To evaluate attention, we adapted a previously described test (Dai et Carey 1994; Kourrich et al. 2001; Mpari et al. 2008). The behavioral test was carried out in the same open-field apparatus used to evaluate locomotor activity. The central area was constituted, and a solid object could be fixed in the center of it. The setup was designed to create an environment in which the rats would have a low, but reproducible probability of entering the central zone, regardless of the presence or absence of an object (Kourrich et al., 2001). We minimized emotional reactivity, anxiety behavior, and novelty exploration by familiarizing the animals with the experimental environment and with the possible presence of an object in the central zone for 10 days before the i.c.v. injection of toxin or vehicle. Several parameters were assessed, including total contact time for examination of the object in the central zone and the number of contacts with the object. Two days were allowed to elapse between two object presentations to minimize novelty exploration. The data recorded during presentation of the first and second objects made it possible to evaluate the exploratory behavior of the rats. Moreover, the data recorded on the last three days of this period made it possible to establish two homogeneous behavioral groups before injection: an AmmTX3 group and a vehicle group. The i.c.v. injection was administered the next day (0.75µg of AmmTX3 or vehicle). Rats were tested 30 min after injection. The test involved the rats being placed in the presence of a third object in the central zone for 10 min.