The RAT-ROTADRUM: A reaction time task depending on a continuous stream of tactile sensory information to the rat

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HIGHLIGHTS

• Presentation of a novel method to study rat sensorimotor behavior.
• Design of a rotating drum serving continuous stimulation of the rat vibrissae.
• A flexible and affordable stimulus control system drives the complex protocol.

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ABSTRACT

Rats running in their natural habitat monitor the immediate environment with their micro- and macrovibrissae as if reading Braille. These sensory inputs can serve as a cue to change their ongoing motor patterns, for instance to avoid obstacles. To mimic this behavior in a laboratory setting we present a novel behavioral test design. It includes a self-controlled stimulus presentation with sensory discrimination acting as a cue to redirect motor behavior. To acquire the final paradigm, rats undergo a sequenced training protocol. Extracellular neuronal activity was recorded during task performance in the final paradigm. Together with this behavioral test box we present a flexible, easy to build and affordable modular stimulus control system.

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1. Introduction

Rats use their highly developed olfactory and whisker sensory system to navigate through narrow, dark spaces (Van der Loos and Woolsey, 1973; Whishaw and Tomie, 1989; Hermer-Vazquez et al., 2007a; Ahissar and Knutsen, 2008). With their micro- and macrovibrissae they can distinguish surfaces, palpate and locate objects, measure distances and achieve a performance equivalent to 'seeing' in the dark (Guic-Robles et al., 1989; Carvell and Simons, 1990; Brecht et al., 1997; Sachdev et al., 2001; Arabzadeh et al., 2003; Kleinfeld et al., 2006; Mehta et al., 2007; Diamond et al., 2008; Lottum and Azouz, 2008).

As a model system for tactile processing, the rat whisker somatosensory system has been extensively investigated using a variety of whisker stimulation devices (Waite, 1973; Simons, 1985; Krupa et al., 2001; Rajan et al., 2006; Jacob et al., 2010). Most of these studies are, however, limited to anesthetized or dead preparations (Lottum and Azouz, 2008; Jacob et al., 2008; Towal et al., 2011). Awake animals have been mostly used in head-fixed preparations to study, for example, encoding of natural whisker movements (De Kock and Sakmann, 2009; Khatri et al., 2009), tactile discrimination (Wolfe et al., 2008) or object detection (Bermejo and Zeigler, 2000). Very few studies have reported tactile stimulation of the vibrissa in freely moving, behaving rats with simultaneous registration of neuronal activity (Prigg et al., 2002; Leiser and Moxon, 2007; Von Heimendahl et al., 2007; Wolfe et al., 2008; Kralik et al., 2001). During navigation whiskers are used to control movement, suggesting a strong interaction between the sensory acquisition and motor control that is rarely studied. We therefore designed a tactile discrimination task for freely moving rats that combines a motor reaction time task with sensory discrimination by the vibrissa.

Together with this behavioral method, we also present a new stimulus control system, capable of handling the complex protocols required for behavioral and neurophysiological studies. It offers the advantage of being cheaper and more flexible than commercially available systems. It is based on a PC equipped with a digital I/O-board controlled with the graphical programming language LabVIEW (National Instruments, TX, USA).
2. Materials and methods

2.1. Subjects

Eight adult female Long Evans rats were used in this study. During training, rats were group housed at the university animal housing facility under standard housing conditions. Training sessions took place on a maximum of 4 consecutive days a week. Rats were water deprived 24 h before the first training session of a week. During training rats received 40–50 μL water droplets when rewarded and got free access to water 1 h following the training session. Food was available ad libitum after training sessions and in their home cages. Rats maintained >85% of their free drinking weight and showed no signs of distress.

Animal handling and experimental procedures were based upon the European guidelines (86/609/EEC) to minimize animal pain and discomfort and were approved by the local ethics committee of the university.

2.2. Surgery and electrophysiology

Of all subjects used in this study, two were successful chronically implanted with electrode arrays in M1 and cerebellum. Anaesthesia was induced with isoflurane (4% in N2O/O2 (2:1), 2 L/min) followed by an intraperitoneal injection of ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (3.9 mg/kg). Atropine premedication (0.1 mg/kg) was given subcutaneously to reduce respiratory secretions. Toe-pinch reflexes were used to control the level of anaesthesia and supplementary doses (1/3rd of induction dose) were given intramuscularly as needed. The rat’s head was fixed in a stereotaxic frame with non-puncturing earbars. Body temperature was maintained at 37–38 °C using a homeothermic blanket system (Harvard Apparatus, MA, USA). A midline incision was made, the periorbit removed and the skull cleaned. Holes (1 mm) were drilled and 4–5 anchor screws, used to support the dental cement cap, were inserted in the skull. A small drop of cyanoacrylic glue was used to strengthen anchoring. One screw was used as a ground-reference electrode and was inserted through the skull until it touched the dura. Minimal-size craniotomies were made and the dura reflected over forelimb M1 and cerebellar paramedian lobule. Arrays (2 rows of 8 electrodes; electrode separation 250 μm within rows and 500 μm between rows) of 50-μm polyimide insulated tungsten electrodes (Tucker-Davis Technologies, FL, USA) were slowly inserted. In M1 the electrodes were lowered to record activity in layer V. Electrodes in cerebellum paramedian lobule were lowered until neuronal activity could be discriminated. During implantation, care was taken to keep the exposed brain surface moist. Surgical gel foam was put around the electrodes and the arrays were adhered to the skull and anchoring screws using dental cement. Implanted rats recovered in custom cages (length 500 mm, width 300 mm, height 300 mm) under infrared illumination. The same cages were used for individual housing till the end of the experiments. Analgesics (buprenorphine s.c. 0.05 mg/kg) were given twice a day for 3 days postoperatively. Antibiotics were given during 10 days through drinking water (Baytril 100 mg/L). Retraining started 2–3 weeks after surgery and recordings were performed as soon as performance reached pre-implantation levels.

All 32 channels were simultaneously processed (filtering: BP 400 Hz–20 kHz, digitized (sampling rate 25 kHz) and discriminated by a PC-controlled Multichannel Acquisition Processor (MNP; Plexon, TX, USA). Signals were also made audible to help discrimination of spike waveforms online using a time/voltage window (RASPUTIN; Plexon). Event states of the behavioral apparatus were registered with the MNP as TTL-pulses. Recordings of waveforms and spike trains, together with the behavioral events, were stored for further offline analyses (Offline sorter: Plexon; Neuropaxplorer: Nex Technologies, MA, USA). The signals presented are considered to be multi-unit neuronal activity. Peristimulus time histograms (PSTHs) are constructed with a bin width of 1 ms.

2.3. Behavioral apparatus

The behavioral apparatus and stimulus control system were custom-build. A schematic overview (Fig. 1) shows the different components of the setup that will be described in more detail. A detailed overview of the stimulus control system can be found in Section 2.4.

The test cage consisted of a plexiglas arena (length 42 cm, width 18 cm, height 35 cm) placed in an electrically shielded and sound-attenuated cubic. Rats were not restrained in the test cage. Windows in the left front corner accessed the sensory module and response lever. This design is further referred to as the ‘open arena’. In the first, second and third stage of the sequenced training protocol (see Results section), a Plexiglas sheet (20 cm × 20 cm) was positioned parallel to the left sideway at a distance of 6 cm, creating a corridor towards the left front corner. This design is further referred to as the ‘corridor arena’.

Fig. 1. Schematic drawing of the behavioral setup together with the modular stimulus control system. The input/output modules (I/O) are programmed using LabVIEW that is also used to create the experimental output reports. The opto-sensor is used to keep track of the drum’s position, more specifically the position of the sensory cue (presented here as the thickened part of the drum module). The other input module, the response lever, is programmed to control clockwise drum rotation (via a DC motor). Correct trials are water rewarded using the programmable solenoid valve. Neural signals of the freely moving rat can be recorded using the multichannel acquisition processor (MNP).

A response lever housed in a stainless steel standalone unit was custom made (Optitech, Belgium; supplementary material Fig. S1). The lever bar protruded 4 cm from the case and had a width of 1 cm. Lever displacement was detected using a microswitch (VXS-1A2: Omron, Japan). The minimum actuating force could be easily adjusted using an axial sliding-mechanism. In the present study the minimum actuating force was fixed to 25 g, comparable to commercially available systems.

The sensory module (Fig. 2) was custom made (Optitech, Belgium) and consisted of a stainless steel, 10 cm wide diameter, rotating drum. A sensory cue (2 cm-wide adhesive tape) was vertically positioned over the drum surface. The drum surface was cleaned with ethanol 70% at the start of every session. Drum rotational speed could be adjusted accurately using a 10-turn counting dial up to a maximum speed of 13.6 cm/s (Amax 16: maxon motor ag, Switzerland). An opto switch (OP8704: OPTEK, TX, USA) was used to determine the location of the sensory cue on the rotating drum. Axial movement of the drum up to 25 mm was possible (but not implemented in this study) using a servo-driven platform.

In the first three training stages water droplets (40–50 µl) were delivered to a drinking tube (2.0 mm orifice) from a gravity-driven water reservoir, positioned on top of the lever standalone unit. Droplet size was controlled using a solenoid valve (solenoid actuated 22 mm poppet valve, 1.6 mm orifice, QM/48: Norgren, CO, USA).

In the final training stage a drinking fountain (20 mm diameter, 3 mm orifice) was positioned 7.5 cm to the right side of the lever. A low-current contact circuit between the arena floor and the drinking fountain was used to detect onset of drinking behavior.

Every event-state of experimental interest (lever microswitch, drum opto switch, solenoid valve, drinking contact circuit) was monitored with all relevant in- and output modules looped to an event module using patch cables. The TTL-outputs of the event module were sent to the MNA for digital event recording and to a battery of IR-LEDs positioned above the rotating drum (see video in supplementary material). Toggle switches on the event module could switch IR-LED activation to an on- or off pulse. As such, the activation of the lever microswitch, the presentation of the cue (via the drum opto switch), the activation of the solenoid valve and the contact with the drinking fountain (via the low-contact current circuit) could be monitored on video. The IR-LEDS, an IR-video recording system and a video timer (VTG-33: FOR-A UK Limited, Switzerland) were used to record video data for the duration of each trial.

Fig. 2. Blueprint (upper panel) and picture (lower panel) of the sensory module showing the different components. The drum can be rotated clockwise and is fixed to a platform together with the opto switch, which monitors the drum's rotation. The servomotor can move the platform up to 25 mm perpendicular to the access window (inset lower panel).
UK) synchronized with the MNAp, allowed offline analysis of trial performance with a 40 ms resolution, limited by the video-capturing system (frame rate 25 fps).

Masking white noise was presented during all sessions.

2.4. Stimulus control system

The modular system was housed in an Eurocard compatible, 19 in. steel subrack (height 3 HE, depth 280 mm) equipped internally with a perforated reel for connectors and a backplane with guide rails for the printed circuit boards (Primus subrack: Knürr, Germany). An open plug-in unit type was chosen with front panel covers directly screwed onto the printed circuit boards. We used 6 TE and 12 TE units with a single 12 TE unit holding up to 4 (identical) function-blocks. To reduce building costs we did not use a real backplane. Instead, DIN 41612 (32-way) female sockets were screwed directly on the internal perforated reel with 2.5 mm screws. The few interconnections needed between sockets (power supply) were made with insulated wire (supplementary material Fig. S2). A ribbon cable was used to connect the PC acquisition card with the rack were it was connected to a connector block. From here, small ribbon cables connected to the (backplane) DIN 41612 sockets. Since the I/O ports were grouped in ports of 8 lines we kept the same scheme in the module configuration (8 lines with every output- or input module). On every line a status LED was added together with a microswitch for manual input/output testing (supplementary material Fig. S3). The schematic diagram and a blueprint of a module interface panel can be found in the supplementary material (Fig. S4).

The electronic circuits were built on Eurocard boards (160 mm × 100 mm) having predrilled holes for the DIN 41612 (32-way) male plugs.

The connections between modules were made with patch cables in 4.4 mm TT bantam jacks and plugs, the latter being screwed directly on the front panel covers (Fig. S4). Crossing of wires between both jacks made every jack carry both input and output (supplementary material Fig. S5C). This allowed bidirectional data traffic between connectors/modules. An additional advantage is that connections can be switched mutually without any danger for damage to the devices. Additional cable types (supplementary material Fig. S5A, B) were necessary to realize (optional) connections between a module and an external instrument with BNC connectors. To avoid damage to sensors or modules, different plugs were used (DIN, 2.5 mm audio, 5-pole plug, 7-pole plug, etc.). This way, inappropriate sensor connections were mechanically prevented.

Different module types were designed to control dataflow (supplementary material Fig. S6). An example of a unit converter can be found in the supplementary material (Fig. S7). A module converts the position of a switch into a debounced TTL-signal. When actuated, the switch moves a rather long way between contacts. The bouncing effect will generate a large amount of TTL pulses providing false experimental data. Using a set-reset (SR) latch, the output will be guaranteed bounce-free. A LED completes the circuit with visual feedback. Another type of unit conversion is required when components (i.e. mechanical stimulator, DC motor) need more power than can be delivered by TTL lines. The schematic electronics diagram of the amplifier used can be found in the supplementary material (Fig. S8).

Several supply-voltages are needed for the signal-conditioning units. Together with the current demand to power motors or electromagnets, this requires a large power supply, best housed in a separate cabinet. As with the modular system, we used a 19 in. cabinet. Speakon connectors (Neutrik, Liechtenstein), capable of handling large currents, were used to connect both cabinets. The power supply itself was made with 2 standard ATX-type computer power supplies (supplementary material Fig. S9). One was used to deliver +5 V and +12 V, the other unit for −7 V and −12 V. Both power supplies were connected in series, requiring a disconnection between ground and the metal chassis implemented by removing the extended ground plane on the printed circuit board. Since the supply may require a load to start-up we wired 2 LED’s to the +5 V, also giving visual feedback of operation. Depending on the computer supply used, a connection between DC,ON (green) and GROUND (black) might be necessary.

The system was controlled with LabVIEW-written software (http://www.ni.com/labview/) via a 24-bit programmable peripheral interface (PCI-6503, National instruments, TX, USA). All
LabVIEW VI's of the final paradigm can be found in the online supplementary material.

3. Results

We present a behavioral apparatus controlled by a custom stimulus control system, used with simultaneous registration of extracellular neuronal activity in motor cortex to strengthen the paradigm's potential (Kralik et al., 2001). In the final paradigm, rats had to correctly discriminate a sensory cue on a rotating drum surface in order to perform a motor reaction task. To accomplish this, we trained rats to use a response lever to initiate drum rotation till detection of the sensory cue prompted release of the lever within a defined reaction time limit. Correct trials were reinforced with water rewards, false trials remained unanswered.

3.1. Behavioral apparatus and stimulus control system

With the LabVIEW-controlled, modular stimulus control system it was possible to set and easily adapt I/O definitions and parameter boundaries using a graphical user interface (Fig. 3). The complete LabVIEW VI can be found in the online supplementary material.

All of the fixed and variable behavioral data within a session (drum speed, parameter limits, trial time, reaction time, number of responses, random drum position, trial performance) was written to the output module and exported to a spreadsheet file for further analysis.

We tested the stability of 5 different drum rotational velocities between 5.6 cm/s and maximal speed (13.6 cm/s, 300 s). The CV-values ranging between 0.4% (at maximal speed) and 1.0% (at 7.6 cm/s) were considered adequate for the behavioral paradigm.

An automatic calibration procedure calculating the drum velocity was done before sessions commenced. This was done by measuring the elapsed time for 5 drum revolutions. Drum velocity was also used to calculate a new random position after trial completion in the final paradigm, to decrease the chance level performance.

3.2. Behavioral training protocol

Because of the complex nature of the final operant conditioning paradigm, we implemented a sequenced training protocol (chaining) as schematically visualized in Fig. 4.

Eight rats (weight 200–250 g) were habituated to general handling procedures and the behavioral apparatus for 5 consecutive days. During this period, habituation to the test arena was done by gradually increasing the time spent from 10 min to 30 min.

After water deprivation, rats were first trained to press the lever with a continuous reinforcement schedule (Fig. 4A). A linear (R = 0.98) learning curve was obtained. In the final (seventh) session an average of 176 (SD 64) responses was recorded during the 30 min training session (Fig. 5).

In the second training stage (Fig. 4B) we incorporated the sensory module. Drum rotation was initiated with every lever response but interrupted upon lever release. Rats were reinforced with every trial were they rotated the drum until the sensory cue reached the access window where it automatically halted for 2 s, independent of the lever state. Lever release was required between trials to prevent the accumulation of correct trials without a press-release cycle. Different strategies were possible to the animal: a single, longer lasting lever response could result in the same outcome as multiple brief responses. The Maximal Number of Lever Presses (MNLP) was not limited and drum rotational speed was set to its maximum (13.6 cm/s).

From this stage on we will consider two equal-sized groups of subjects (group A and group B), since different parameters and criteria were used for those groups. Group A was transferred to the next stage after 3 training sessions. On average, 2.0 (SD 1.0) responses were needed with every reinforced trial in their third training session. In contrast, subjects of group B were trained for 13 sessions in the second stage. On average, 3.0 (SD 0.7) responses were needed with every reinforced trial in their last training session. Behavioral observation showed that 3 out of 4 group B subjects, but none of the group A subjects, used the presentation of the sensory cue to change their body orientation towards the water supply.

The rotating drum was not automatically stopped in the third stage (applies only to group A) and final stage of training (all groups, Fig. 4C). To be rewarded (with every correct trial reinforced), rats had to halt the drum after presentation of the sensory cue through release of the lever within a time constraint. The time constraint was 0–600 ms (group A) or 0–1000 ms (group B) from appearance of the sensory cue in the access window. In the third stage the ‘corridor arena’ was used (see Section 2.3 for details) whereas in the final stage rats were required to collect the water reward at a distance (‘open arena’). For group A, the MNLP was first set to 10 and after five training sessions to 3. Fig. 6 shows the performance (calculated as the percentage of correct trials to the total number of trials) for all group A subjects in the third (‘corridor arena’) and final stage (‘open arena’). There was a high variability between subjects when training started in the third stage. Subjects did not perform better in the final stage when performing more sessions in the third stage. Instead, some subjects’ performance dropped in the third stage, but, when taken to the final stage, improved up to levels better than the maximal ones obtained in the third stage. Fig. 6 also shows that performance did not drop after an interruption of training in the final stage (>2 months). Performance of the 2 subjects chronically implanted with electrodes is also shown during registration of neuronal activity (‘implanted’). Retraining was started after the surgery recovery period. Performance reached pre-implantation levels after 3–4 sessions.

Subjects of group B were taken to the final stage immediately after stage two. The MNLP was set to 10 only in the first session; thereafter it was set to a maximum of 3. Performance of all subjects of group B in the final stage is shown in Fig. 7A, with 3 out of 4 subjects (R1, R2 and R3) reaching high performance levels (arbitrary set to >80%) within 2 sessions. The other subject (R4) never acquired the task but applied an alternative strategy. To investigate maintenance of the active repertoire of the acquired behavior we interrupted training in all group B subjects by 5 months (Fig. 7B ‘T1 vs. T2’). We compared the average performance of the last 3 sessions at T1 versus T2 (8 training sessions in T1, 7 in T2). The long interruption did not alter performance.

Although different parameters and criteria were used in the two groups of subjects, they all mastered the final paradigm. However, the protocol applied to group B, with more training sessions in the second training stage, reduced the total number of sessions required to learn the final paradigm (Fig. 4C). Probably, it is in this second training stage that the subjects learn the operant conditioning to keep palpatting the sensory surface with their whiskers until a sensory cue can be discriminated. A single subject (of group B) did not care about the discriminative stimulus and applied a chance strategy to get reinforced. However, this subject never reached the arbitrary set performance level of 80%. With RT limited to 1 s, maximal drum rotational speed (2.3 s for full rotation), a random drum position of 0–100° (equivalent to 0–0.64 s) and with MNLP set to 3, our data suggest a chance level below 80% (see Section 4).

The online supplementary material features a 60-s video clip of a subject performing a series of correct trials.

To quantify the detection of the sensory cue in the final paradigm we analyzed data of the final 4 consecutive daily recording sessions
of both chronically implanted (and tethered) subjects (RAT1 and RAT4 in Fig. 6). For every session, most of the trials were executed in the first fifteen minutes with a mean of 78% (SD 8) for RAT1 (left panels) and 79% (SD 14) for RAT4 (right panels). For both rats, cumulative plots of correct trials in these 4 sessions are shown in Fig. 8 (top panels).

Because the MNLP was set to 3, false positive responses are likely to occur. Since the Number of Lever Presses (NLP) was recorded, a classification of trials with respect to this parameter was possible. For all sessions analyzed for both rats, 79.2% (SD 10.0) of the correct trials were executed with 1 LP, 17.1% (SD 8.5) with 2 LP, 3.6% (SD 2.5) with 3 and 1.3% (SD 0.5) with >3 LP (Fig. 8, middle panels). Considering all trials, including missed detected (MD), these numbers changed to 68.0% (SD 9.4), 14.6% (SD 7.0), 3.1% (SD 2.2) and 1.3% (SD 0.5), respectively.

We applied a box plot statistical analysis on the reaction times (RT) of classified trials (Fig. 8, lower panels). For RAT1 with 1 LP (n = 419) an average RT of 489 ms (SD 192) was recorded with 10th percentile at 265 ms and 90th percentile at 728 ms. The average RT of RAT4 with 1 LP (n = 320) was 519 ms (SD 152) with the 10th percentile at 376 ms and the upper one at 728 ms. Considering all trials with 1 LP, on average 74 trials (SD 32) per session were performed with an RT within the 10–90th percentile boundaries.
3.3. Electrophysiology

For illustrative purposes, we present extracellular neuronal activity in forelimb MI during 10 rewarded consecutive trials (Fig. 9). Variable responses of multi-unit activity can be observed in the PSTH with respect to lever release (RT 0–1000 ms) but also to other events. Other units (not shown) were more modulated to lever response or to presentation of the sensory cue.

4. Discussion

We present a novel method to study tactile sensory processing combined with a motor task in the behaving rat. Here we discuss limitations and possible improvements to the paradigm presented.

4.1. Behavioral apparatus and stimulus control system

The lever and rotating drum can be considered the core items of the behavioral apparatus, together with the water delivery system.

Since the response lever is acting on a microswitch, only two states (ON/OFF) are possible. As such, control of drum rotation is also limited to two states (rotate and still). This does limit the extent to which the rat can control the stimulus presentation, the rotat-
The surface of the rotating drum can be considered to be a continuous stimulus. A vertically attached strip of tape acted as a sensory cue that had to be discriminated on the rotating surface. Because the animals were not head-fixed and since no nose poke was used to force head position during training or recording sessions (Wolfe et al., 2008), rats developed their own behavioral strategies to scan the rotating drum. All of the rats correctly performing the final paradigm (Fig. 4C) positioned their nose close to the rotating drum surface, probably to use their microvibrissae instead of the macrovibrissae to detect the sensory cue. This would be in agreement with previous work showing the importance of microvibrissae, but not macrovibrissae, in object recognition (Brecht et al., 1997). In another study of freely moving rats performing a discrimination task with their macrovibrissae the animals did leave the nose poke from time to time to explore the detected rod with their microvibrissae (Mehta et al., 2007). Therefore, to use our paradigm with tactile discrimination restricted to the macrovibrissa, a head-fixed preparation would be necessary.
Fig. 9. Extracellular spike activity of 10 consecutive and correct trials in the final training stage (Fig. 4C). Perievent rasters and histograms (vertical scale bars: 5 counts per 50 ms bin) of 6 MI-units (recording A0606) are shown with modulated activity relative to lever release (dash line). Lever response (triangular marker) and presentation of the sensory cue (circular marker) are also indicated on the perievent rasters. Sorted waveforms (n = 100) of the units are displayed on top of the corresponding figurine (vertical scale bar: 50 μV; horizontal scale bar: 0.1 ms).

Besides tactile information, rats could use other available sensory cues to correctly discriminate the strip of tape. We can exclude visual and auditory cues because the behavioral apparatus was located in a sound attenuated and lightproof cabinet. The infrared illumination used did not interfere since rats are insensitive to these wavelengths. With respect to olfactory cues, we cannot exclude their contribution because the sensory cue and the metal drum surface are composed of different materials. With a comparable range of response reaction times for olfactory discrimination (Hermer-Vazquez et al., 2007b) and our observation that the rats positioned their nose close to the drum surface, we cannot exclude olfactory discrimination. However, our cleaning of the drum surface with ethanol 70% before every session should have limited the olfactory contribution of the sensory cue. Moreover, rotation of the drum is expected to diffuse any of the olfactory cues present. To exclude olfactory discrimination in the paradigm, control experiments with removal of all macro- and microvibrissa between two successive sessions could be done. As a future enhancement to
exclude olfactory discrimination the sensory cue could be engraved on the drum's surface. Another approach is to use real-time quantification of whisker movements and detection of initial whisker contacts (Bermejo and Zeigler, 2000) or to use high-speed video monitoring ( von Heimendahl et al., 2007).

Rats were rewarded on a continuous reinforcement schedule with water droplets. In order to maximize performance levels and/or to increase the mean number of trials executed per session, other schedules of reinforcement could be evaluated including variable or fixed ratio schedules (Skinner, 1958).

We report droplet size as being variable between 40 and 50 µL due to the limited precision of the solenoid valve (no specifications available on valve response variability) and the pulse controlling it (150.0 ms, SD 0.3). Since the water delivery system is gravity-driven, the size of the water reservoir matters. A large water reservoir (500 mL) was chosen to be a multitude of the amount consumed in one session (<10 mL) to prevent systematic diminishment of droplet sizes during a session. To minimize droplet size variability, the gravity-driven solenoid-controlled system could be replaced with a more expensive membrane dosing pump system.

The modular stimulus control system can be considered as a low-cost alternative to commercially available systems. The system can be upgraded at any time by adding extra modules or by interfacing with commercially available behavioral control units, although this requires the addition of appropriate unit (scale) converters.

4.2. Behavioral training protocol

In the final paradigm, the MNLP was set to 3. This automatically implies the occurrence of false positive trials. One rat applying a chance strategy (not scanning the drum surface) never reached an 80% performance level. Because of the complexity of the chance calculation in the paradigm, arbitrary parameters were used. If the maximal time the rat will press the lever is set to 4 s and the probability of lever release is independent within this period, the chance level will be maximally 25% with 1 lever press (LP) and RT limited to 1 s. Calculating chance levels with increased NLP requires knowledge of the average lever response time, which is dependent on the acquired behavioral repertoire.

Although most of the trials are executed with 1 LP, classifying trials into correct detections (CD), missed detections (MD), false detections (FD) and true negatives (TN) makes it possible to calculate the precision (CD/(CD + FD)) and accuracy (CD + TN)/(CD + TN + FD + MD). Because every trial comes with the presentation of the sensory cue, no true negatives responses could be recorded. With the current paradigm, we were also not able to classify a trial as being falsely detected (false positive). Trial-to-trial video analysis or changes to the paradigm could be used to improve the classification. In the final paradigm for instance, the maximum number of responses can be reduced to one, keeping the chance level as low as possible. We also presented a method using box plot analysis to classify trials with respect to reaction time. The RT percentiles can be used to narrow RT limits. Of all sessions analyzed in this study, a lower RT limit of 250 ms and an upper one of 750 ms would accept most of the correct trials (10th–90th percentile) but would half the reaction time window thus further reducing chance levels.

4.3. Electrophysiology

Of all animals trained in this study, two were retrained after successful implantation of wire electrode arrays in motor cortex and cerebellar cortex (paramedian lobule). Afterwards, tethered recording of extracellular activity was possible in both rats without altering task performance. The results shown of these recordings should be regarded purely illustrative (Fig. 9). However, we considered demonstration of successful chronic recording as an added value to the behavioral paradigm. Neural correlates of the behavior as present in this paradigm can be valuable to investigate how tactile sensory inputs guide ongoing motor behavior. The contribution of the cerebellum in this matter is of interest (Bowar, 1997). Unfortunately, our attempts to record extracellular activity in the cerebellar cortex with chronically implanted wire electrode arrays failed, the main issue being the dimpling effect caused by the dense structure of the cerebellar cortex. This dimpling effect could be minimized by using several individually lowered electrode wires (Hartmann and Bowar, 1998) or using single- or multis Shank silicon probes (Bragin et al., 2000; Hofmann et al., 2006; Tahon et al., 2011).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.jneumeth.2011.06.031.

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