Reference Manual

Trial and Hardware Control in EthoVision XT

Version 16

Noldus
Information Technology

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For EthoVision XT version 16

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Contents

1 Introduction to Trial Control

THIS MANUAL

This manual is meant for all users of EthoVision XT:

- For users of the **Base** version. You can find extensive information on the Trial Control conditions (see page 67) and analysis of Trial Control data (see page 166). This information is not present in the EthoVision XT Help.
- For users of the **Base** version plus the **Trial and Hardware Control** module. You find all the information you need in this guide.
- If you use EthoVision XT in combination with hardware devices, you can find the relevant information in Section 7 (page 103).

EthoVision XT Help

For extensive information on EthoVision XT or for troubleshooting, see the EthoVision XT Help. In EthoVision XT, choose **Help** > **EthoVision XT Help** or press **F1**.

Other documentation

On the Apps screen, choose **Noldus** > **EthoVision XT 16 Other documentation**. There you find:

- **Application Manual - EthoVision XT 16.pdf** for information on common tests.
- **Reference Manual - PhenoTyper.pdf** for information on PhenoTyper.
- **Service Manual - PhenoTyper.pdf** for information about PhenoTyper, and devices such as the Noldus Pellet dispenser, the Noldus L**5**ickometer and PhenoWheel.
- **Reference Manual - DanioVision DVOC-0041.pdf** if you use Trial Control with the DanioVision Observation Chamber.
- **Quick Start Guide - EthoVision XT with the Ugo Basile Fear Conditioning System.pdf** for connection schemes and procedures for using that system.

WHY USE TRIAL CONTROL?

Trial Control allows you to automate your experiment. For example:

- You want to set a **maximum duration** for your trials.

  See page 40.

- You want to **automate the start and/or stop of data acquisition**. For example:
  - Start recording when the rat is first detected in the open field.
  - Stop recording when the rat has reached the platform in the Morris water maze.
  - Start recording at exactly 12:30:00.
  - Stop recording after the animal has been in the closed arms of the plus maze for 5 minutes.

  See page 46 and also page 23 for general information.
- You want to **automate conditioning schedules**, or **operate hardware devices**. For example:
  - When the animal presses a lever, have the food dispenser drop a food pellet.
  - When the animal leaves the shelter, use an External Command action to start a recording with Media Recorder.
  - When the animal enters the shelter, turn on a light.
  - Close the door of a radial maze when the animal has exited that arm.

  See page 23 for general information and page 54 for making sub-rules.

To use Trial Control

1. Open the Trial Control screen (see page 16).
2. Define the conditions that, when met during your trial, trigger specific actions. Organize conditions and actions in sequences (see page 23).
3. Before starting data acquisition, you make sure that those Trial Control Settings are active.

For information on how to manage Trial Control Settings, see also **Settings and profiles**, under **File management** in the EthoVision XT Help.

CONDITIONS AND ACTIONS

A Condition is a statement that EthoVision evaluates. An Action is a command executed on a variable or a hardware device. You can therefore control your experiment by linking conditions with actions.

- **EXAMPLE 1** In a Morris water maze test, stop tracking when the rat reaches the platform (provided that the platform has been defined as a zone). *Stop tracking* is the action and *Rat on the platform* is the condition.
- **EXAMPLE 2** In a Fixed Ratio schedule, have EthoVision send a command to drop a pellet when the mouse has been detected in the Trigger zone. *Drop one pellet* is the action and *Mouse in the Trigger zone* is the condition.

You define and link conditions with actions in a graphical form. Example 2 can be represented with the following:

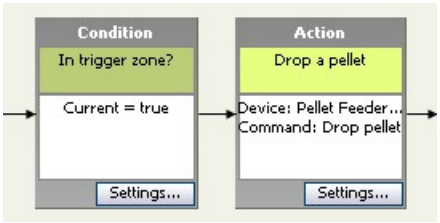


Figure 1 *A condition is followed by an action. The condition checks that the animal is in the zone named "Trigger zone". The action "Drop a pellet" is taken when the condition is met.*

- For more information on conditions, see page 26 and page 67
- For more information on actions, see page 27.

You can copy, move, connect and delete Trial Control elements such as actions and conditions. For how to do so, see the EthoVision XT Help.

TRIAL CONTROL RULES

A Trial Control rule is a set of conditions and actions organized in a logic sequence. It can be viewed as a set of instructions executed during a trial. There are two types of Trial Control rules:

- *Start-Stop trial rule*. For starting and stopping data recording. This rule cannot be repeated or deleted.
- *Sub-rules* (available only in EthoVision XT with the Trial and Hardware Control Module, see page 14). Subroutines of the Start-Stop trial rule, which are meant to carry out specific actions. Sub-rules can start at specific times and be repeated according to user-specified conditions.

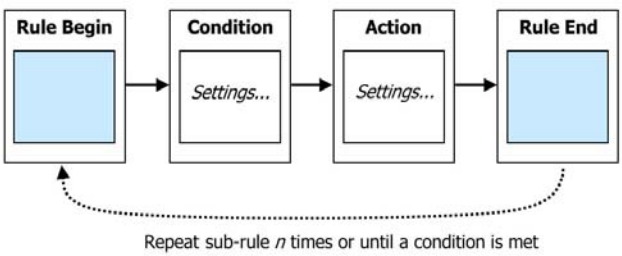


Figure 2 *Schematic representation of a generic sub-rule.*

- Sub-rules are called by specific control boxes called *References*.

For more information:

- On the Start-Stop trial rule. See page 46.
- On sub-rules. page 54.

HOW TRIAL CONTROL INSTRUCTIONS ARE EXECUTED

The instructions contained in the Trial Control Settings are carried out from the moment you start a trial, to the moment the trial is stopped. Only the instructions in the Trial Control Settings currently active (that is, highlighted in blue in the Experiment Explorer) are carried out.

The program evaluates the Trial Control sequence at each **sample time**. The rate at which this happens depends on your chosen **sample rate**, not on the video frame rate.

For the Start-Stop trial rule and each active sub-rule, the program remembers which Trial Control boxes were evaluated (**active**) in the previous sample. Depending on the type of this box:

- For a **Condition** box. EthoVision XT waits until the condition is met. When this happens (condition *becomes true* - see 3 in Figure 3), the program passes control to the next box in the sequence. The condition becomes then *inactive* (see 4 in the same figure).
- For an **Action** box. EthoVision XT carries out the action (see 4 in Figure 3), and passes control to the next box, which *becomes active*. Then, the Action box *becomes inactive* (see 5).
- For a **Sub-rule Begin** box. Control is passed to the box that follows. The Sub-rule Begin box *becomes inactive*.
- For a **Sub-rule End** box. This marks the end of a sub-rule. The sub-rule *becomes inactive*. Control is passed to the Reference box that had called that sub-rule. This Reference checks whether the sub-rule must be repeated. If this is not the case, control is passed to the box following the Reference box.
- For a **Reference** box. Control is passed to the Sub-rule Begin box. The Reference box *makes the sub-rule active*. The Reference box stays active until the last sub-rule repeat is completed. Next, the Reference box *makes the sub-rule inactive* and passes control to the box that follows. Next, the Reference box *becomes inactive*.

The events marked in *italics* above are those you can define in the Analysis profile and analyze or visualize in a chart (see page 166). When a box becomes *active*, the previous one becomes *inactive*.

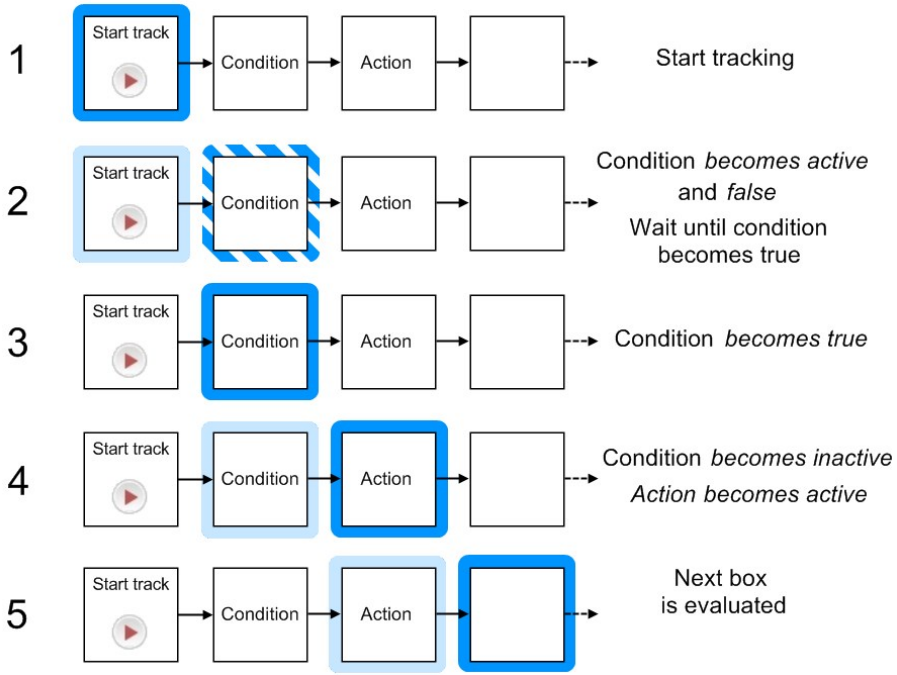


Figure 3 *Schematic representation of how Trial Control Instructions are executed. The scheme shows an example of a Start-Stop trial rule (see page 46).*
1 - Tracking starts, either manually or because a previous condition has been met.
2 - Control passes to a Condition box (for example "Is mouse on top of Shelter?"), which becomes active. *The condition is evaluated.*
*3 - The Condition is met (*becomes true*).*
4 - Control passes to the next box. In this case, it is an Action. Actions are taken immediately (but see the note below on actions on hardware devices).
5 - The Action box becomes inactive, *and the next box* becomes active.
For clarity, step 3 and 4 have been placed separately. In reality, when a condition becomes true it also becomes inactive *at the same time, and control passes to the next box.*
Hatched outlines - Condition box becomes active. *Dark outlines - Condition becomes true or Action is taken. Pale outlines - Box* becomes inactive.

When plotted against time, events like those in Figure 3 look like the following:

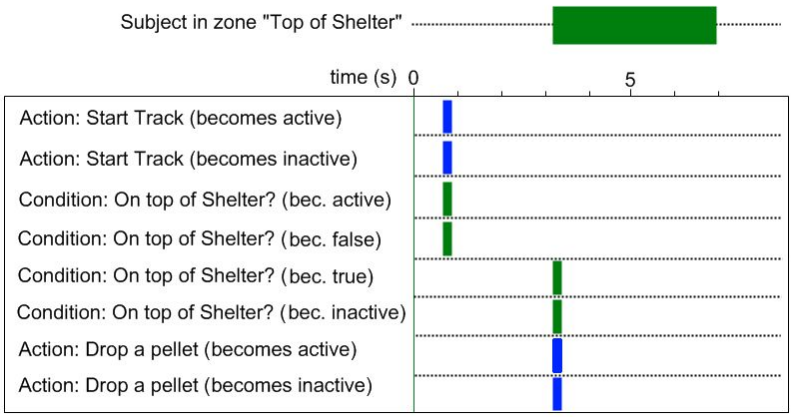


Figure 4 *Time plot of the events of Trial Control represented in Figure 3. The time that the animal is in the zone "Top of Shelter" is indicated by a horizontal bar.*
When tracking starts, the Action "Start track" becomes active (1st line) and immediately inactive (2nd line) as control passes to the next box. The condition "On top of Shelter?" becomes active (3rd line; see **2** *in Figure 3) and immediately false (4th line) since the animal is not in that zone. When the animal enters the zone, the condition becomes true (5th line), and becomes immediately inactive (6th line) as control passes to the next box (see* **4** *in Figure 3). The action becomes active (7th line) and immediately inactive (8th line) as the command "drop a pellet" is sent to the pellet dispenser.*

To plot Trial Control data, see page 166.

- Boxes combined in parallel using operators (see page 33) are evaluated at about the same time, in random order. This means that you cannot establish which condition is evaluated/which action is taken first.
- When your trial control is complex, for example when it includes sub-rules or conditions combined with operators, it is possible that two or more Trial Control boxes are evaluated at the same sample time.
- When a condition based on a dependent variable is evaluated (and therefore may or may not be met) also depends on the statistic used. See page 78 for details.
- If two or more boxes are evaluated at the same time, an action on a hardware device is taken only after all active boxes that must be evaluated have been evaluated.

- If the next box to be evaluated contains a condition that is fulfilled immediately, the program passes control to the next box. Therefore, within one sample time the program can pass control to two or more boxes to the right.
- When you stop the trial or the Maximum trial duration has been reached, all Trial Control boxes are deactivated.
- When the Rule End box of the Start/Stop trial rule is evaluated for an arena, data recording stops for that arena. From that moment, Trial Control is deactivated for that arena, even in those sub-rules that were ongoing in the meantime (see also the next page).
- A trial stops when trial control is deactivated in all arenas.
- Actions on hardware devices. A command is sent to a device after all boxes that must be evaluated have been evaluated. However, it may take some time for the outcome to be seen. For example, when a pellet dispenser is almost empty it may take a few seconds before a pellet is released.

  IMPORTANT When two or more actions on hardware devices follow each other, it is possible that they are evaluated at the same sample time. This happens because the computer is fast enough to analyze multiple Trial Control boxes in the sample interval. To make sure that actions are taken in the correct sequence and do not interfere with each other, add a Time condition (see page 142) between the action boxes, so after the first action is carried out EthoVision XT waits for a short time (for example, 0.1 s) before carrying out the next one.
- Sub-rules. A sub-rule is completed when the Rule End box has been evaluated, or when it is interrupted (see page 60 for more information).
  - If two or more sub-rules are evaluated at the same time, it is not possible to establish which sub-rule is evaluated first at a specific sample.

Trial Control in multiple arenas

If your experimental setup includes two or more arenas, Trial Control is applied to each arena separately. This means that, if a condition is met in one arena, EthoVision XT takes the corresponding action in that arena, not the others.

EXAMPLE See Figure 5. The setup includes four cages, each defined as an arena. A Trial Control In zone condition (see page 84) has been defined so that tracking starts when the animal is first detected in the arena. When you first put an animal in Arena 2, the condition is met in this arena and tracking starts for that arena. When you release the second animal in Arena 4, 2 seconds later, tracking in that arena starts 2 seconds later than in Arena 2.

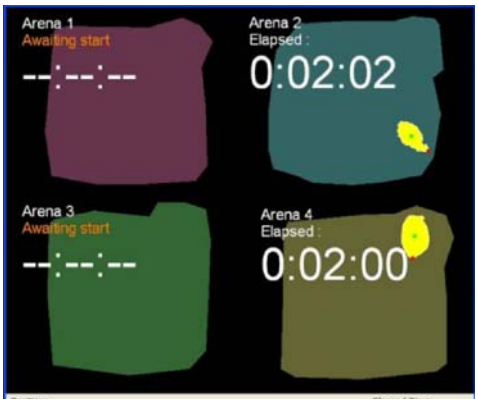


Figure 5 *Trial Control in multiple arenas. The time values displayed on the monitor are the time elapsed since the start of tracking in a particular arena. Tracking started earlier in Arena 2 than in Arena 4 (see text), therefore at any time the Elapsed time (duration of tracking) is longer in Arena 2 than in Arena 4.*

The advantage of Trial Control in multiple arenas is that you can put one animal at a time into the arenas, and EthoVision XT will start tracking in each arena at the appropriate moment.

If your setup includes multiple arenas, you cannot define a condition/action specific to one arena. This means that the zones and hardware devices specified in the condition/action must be present in all arenas, and have the same name.

- If a zone or hardware device is not present in an arena, and a condition is based on that zone or device, Trial Control cannot progress for that arena. Therefore, tracking does not stop unless you set a maximum trial duration or tracking reaches the end of the video.
- Trial Control executes the instructions for each arena separately, however one cannot establish which arena is evaluated first at a specific sample time.

YOUR ETHOVISION LICENSE AND TRIAL CONTROL

Your EthoVision XT license determines which type of Trial Control you can set up.

- **EthoVision XT Base license**. You can define a rule to start and stop data recording (Start-Stop trial rule; see page 46), not sub-rules. You can also use an External command action. You cannot control hardware devices.
- **EthoVision XT Base + Trial and Hardware Control Module**. You can define a Start/Stop trial rule (see page 46), and in addition sub-rules (see page 54) that serve as subroutines of the Start-Stop trial rule. Moreover, you can control hardware devices.

To acquire data in an experiment made with the Trial and Hardware Control Module, you must have a hardware key enabled for Trial and Hardware Control plugged in your computer.

ANALYSIS OF TRIAL CONTROL DATA

The Trial Control function also allows you to easily analyze events that occurred during the trial.

For example, in a conditioning experiment:

- **Number of rewards**. Calculate the number of pellets dropped during a trial.
- **Latency between action and reward**. Calculate the time interval between the time the animal pressed the lever and the time the animal approached the pellet dispenser.
- **Number of failures**. Calculate the number of times the animal approached the lever during the period between 'dropping the pellet' and 'animal approaching the pellet dispenser'. That is, how many times the animal pressed the lever when a reward was already available.

You analyze Trial Control data the same way as for the behavioral variables. In the Experiment Explorer, open an Analysis profile, and specify the data you want to analyze (Trial Control event for point events, Trial Control state for time intervals between two events).

For the detailed procedure, see page 166.

2 The Trial Control screen

To access the Trial Control screen, in the **Experiment Explorer**, click **Trial Control Settings 1** in the Trial Control Settings folder, or from the **Setup** menu, select **Trial Control Settings**, then click **Open** and select **Trial Control Settings 1**. The Trial Control screen appears, showing the default Trial Control settings.

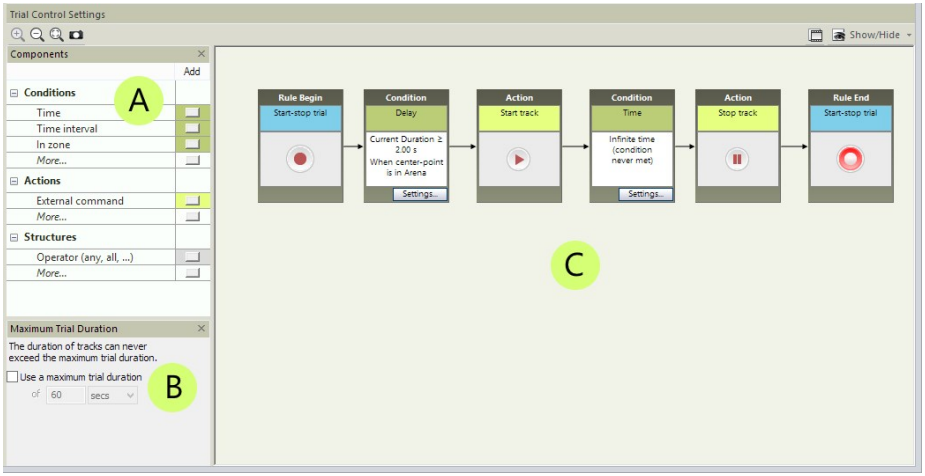


Figure 6 *The Trial Control Settings screen.* ***A*** *- Components pane,* ***B*** *- Maximum Trial Duration pane,* ***C*** *- Trial Control window.*

To access the Trial Control screen, you can also create a new Trial Control Settings, or open one other than Trial Control Settings 1.

- The Components pane, listing the conditions on which you can base your actions and the operators which you can use to combine conditions. See the next page.
- The Trial Control window, showing the Trial Control Settings that are active. It contains a sequence of boxes connected by arrows. See page 19.
- The Maximum Trial Duration pane that allows you to define a maximum duration for all trials. See page 21.

  You can show/hide the Components pane and the Maximum Trial Duration pane by clicking the **Show/Hide** button on the components tool bar and selecting/deselecting the corresponding option in the menu.

THE COMPONENTS PANE

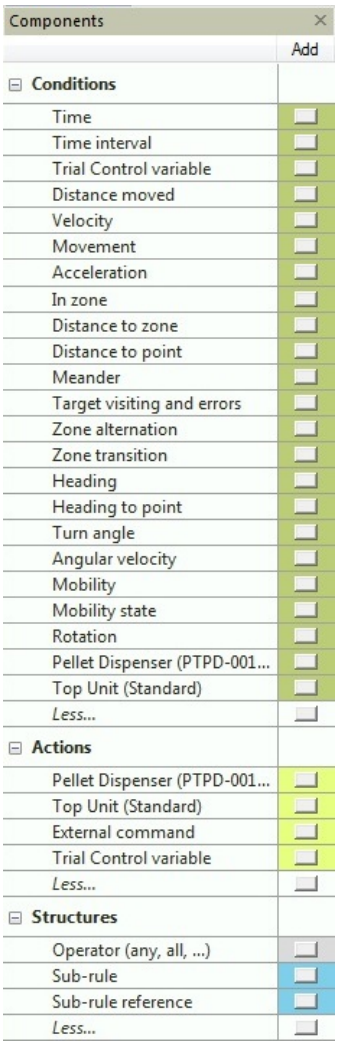


Figure 7 *The* **Components** *pane for Trial Control, with its main groups* **Conditions**, **Actions** *and* **Structures**. *Under* **Conditions** *and* **Actions**, *you see examples of hardware devices controlled by EthoVision XT: a Pellet dispenser and a PhenoTyper Top Unit.*

With the **Components** pane you choose the blocks that build up your trial control rules. Not all the components listed below may be available on your screen, depending on what EthoVision XT license you have on your computer (see page 14).

NOTE If you do not see the option you require, click the **More** button to view the complete list of options available.

Conditions

- **Time**. See page 67.
- **Time interval**. See page 69.
- **Trial Control variable**. See page 71.
- **Dependent variables**. To define a condition based on a variable that describe the animal's behavior, for example velocity, presence in a zone, movement etc. See page 72.

  NOTE You cannot define a condition based on behaviors scored manually and behaviors scored with Behavior recognition.
- **Hardware**. To define a condition based on the state of a hardware device. See page 79. If devices are not listed, you must first configure EthoVision XT in such a way it recognizes the devices. See page 103.

Actions

- **Trial Control variable**. See page 28.
- **External command**. See page 30.
- **Hardware**. See page 116.

Structures

- **Sub-rule**. To define a subroutine that can be called from a specific point of the Trial Control sequence. See page 54.
- **Operator**. To combine two or more conditions in such a way that an action is taken when *All*, *Any* or *N of All* conditions are met. See page 33.
- **Reference**. To insert a call to a sub-rule within a sequence of instructions. See page 56.

To define a sub-rule, condition, action or operator

Do one of the following:

- Double-click its name.
- Click the button next to it.
- Drag the name from the Components pane to the middle of the screen.

A new Trial Control box appears in the top-left corner of the Trial Control window. Insert the new box in the sequence of boxes.

For the complete procedure for programming Trial Control, see page 23.

THE TRIAL CONTROL WINDOW

The Trial Control window contains the sequences of instructions (rules) currently present in the Trial Control Settings. When you create a new Trial Control Settings profile, the Trial Control window contains the default Start-Stop trial rule, consisting of six boxes connected by arrows (see page 46).

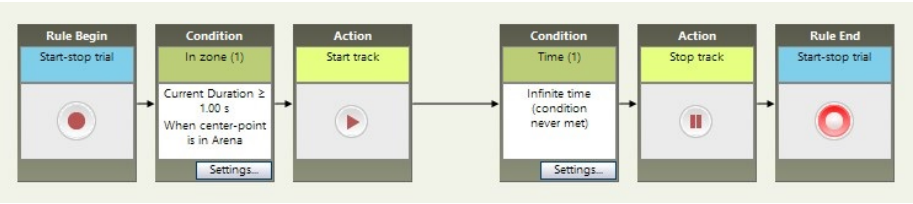


Figure 8 *The Trial Control window. By default, it contains the* Start-Stop trial rule.

You can then define:

- Your own conditions that determine the start and stop of data acquisition.
- If your EthoVision license includes Advanced Trial Control (see page 14):
  - Additional sub-rules, that is, sequences of actions and conditions that work like subroutines in a program. They can be called from another point in the Trial Control sequence.
  - Control hardware devices, like a food dispenser or a door.

For details on how to move, copy, connect and delete Trial Control boxes, see the EthoVision XT Help.

Grid

The trial control boxes automatically snap to a grid. You can change this by clicking the **Show/Hide** button on the component tool bar and selecting/deselecting the two Grid options (**Snap to Grid** and **Show Grid**).

Zoom

The component tool bar of the Trial Control Settings shows three zoom icons:

- **Zoom in**. You can keep zooming in until all trial control boxes fit in the window.
- **Zoom out**.
- **Zoom to fit**. Clicking this button fits all trial control boxes into the window.

The Trial Control window is 'dynamic': this means that it expands when you move trial control boxes to the right. In this case, you can navigate 'from left to right' in the Trial Control

window by using the scroll bar at the bottom. Use the **Zoom to fit** button in the component tool bar to make all trial control boxes visible.

Trial Control in special cases

- If you selected **Activity analysis** in the **Experiment Settings**, the first condition box is removed from the default Start-Stop rule. To carry out tracking and activity analysis simultaneously, and start tracking when your subject is detected in the arena for a specific time, insert a new **In Zone** condition box in the Start-Stop rule. For more information on **Activity analysis**, see the EthoVision XT Help.
- If you selected **Behavior recognition** in the Experiment Settings, the default Trial control rule also contains a Time condition box (3 seconds). This means that tracking starts 3 seconds after starting the trial.
- If you selected both **Activity analysis** and **Behavior recognition** in the Experiment settings, the same trial control rule is used as for only **Behavior recognition**. For more information, see Behavior recognition in the EthoVision XT Help.

To export Trial Control Settings

You can export an image of the Trial Control Settings:

1. Click the **Export image** button on the tool bar  .
2. Select a location to save the image to, type in the File name or accept the default one and select an image type from the **Save as type** list.
3. Click **Save**.

   The complete Trial Control window is exported, irrespective of the zoom factor.

MAXIMUM TRIAL DURATION PANE

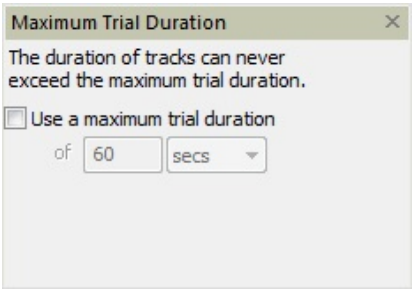
Aim

To define a maximum duration of the trial. This prevents EthoVision XT from waiting indefinitely that the condition to stop the trial (see page 46) is met.

EXAMPLE In a novel object test, in the Start-Stop Trial rule you have defined a condition 'stop the track when the mouse enters the zone with the familiar object'. it may happen that the mouse completely ignores the familiar object and only pays attention to the novel object. To prevent that EthoVision never stops tracking, set a maximum trial duration.

To access this option

In the Trial Control Settings screen, locate the Maximum Trial Duration pane, right below the Components pane. If you do not see it, click the **Show/Hide** button on the tool bar and select **Maximum trial duration**.



Notes

- Instead of using a Maximum trial duration, you can also define a condition based on time and place it immediately before the Stop track box (see page 46). However, there are two important differences:
  - If you use the maximum trial duration, the program counts the time from the start of the trial (this is indicated by the Start-Stop trial box). Instead, a condition placed immediately before the Stop track box considers the time from the start of data recording (this is indicated by the Start track box). The two starting points may not be the same if you have a condition between Start-Stop trial and Start track that makes data recording start some time later than the trial.
  - With a multi-arena setup, a maximum trial duration stops the trial (and thus data recording) in all the arenas simultaneously, even when data recording had started at different times. Instead, a time condition placed between the Start track and the Stop track box stops data recording in one arena when the condition is met in that arena.

This means that you can have data recording stop at different times in different arenas.

EXAMPLE You set to start data recording when the animal is detected for the first time in its arena. For this purpose, you make an *In zone* -based condition. Next, you define a 5-minutes Time condition, and place it immediately before the Stop track box. It the animals are detected for the first time at different times in different arenas, data recording stops at different times too, because of the 5-minutes interval set for all arenas. The trial ends when the recording stops in the last arena.

- The Maximum trial duration is not applied when you record video with the option **Save video only, track later**. You must stop video recording manually. When you track from the resulting video file, the Maximum trial duration is applied.

3 Program Trial Control

- If you just want record data for a specific time, you can do so by setting the maximum trial duration (page 21).
- If you want to start and stop data recording in relation to a few simple conditions (for example, start recording when the animal is detected in the water maze, and stop when the animal reaches the platform), define the Start/Stop trial rule (page 46).
- In other cases, see below.

PROCEDURE

1. Before defining Trial Control in the program, it is helpful to draw your experimental procedure as a flow diagram, where each block represents an action or a condition which, when met, triggers other actions or conditions. The logical order in the example below reads from left to right.

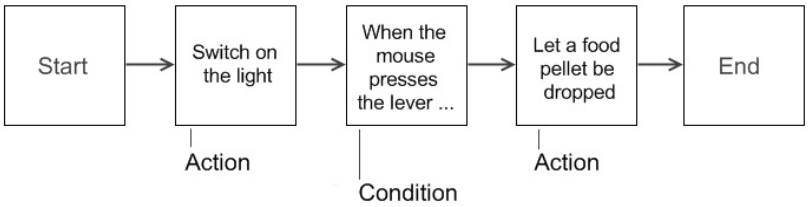


Figure 9 *An example of flow diagram representing the control of an operant conditioning test. The sequence is assumed to be repeated a number of times.*

2. From the **Setup** menu, select **Trial Control Settings**, select **New**, enter a name of the new Trial Control Settings or accept the suggested one, and click **OK**. The default Start/Stop trial rule appears on the screen.
3. Build the Trial Control sequence outlined in step 1, using the components available.
   - To define a Condition or an Action, click one of the buttons under **Conditions** or **Actions**. Insert the box in the appropriate place in the sequence.

     For conditions ➔ See page 26 and page 67

     For actions on hardware devices ➔ See page 116

     For actions on Trial Control variables ➔ See page 28
   - To define a set of actions and conditions to be repeated (Sub-rule), under **Structures** click the button next to **Sub-rule** (see page 54).

- To define the command that calls a sub-rule, under **Structures**, click the button next to **Reference**. Insert the box in the appropriate place in the sequence (see page 56).

4. Test the Trial Control sequence (see page 41).
5. To apply Trial Control to your trials, before starting data acquisition make sure that the Trial Control Settings are highlighted in blue in the Experiment Explorer (see page 44).

Notes

- When you create a new Trial Control element (action, condition, sub-rule or reference) and another of the same type has already been defined in this or other Trial Control Settings, a message appears asking you whether you want to create a new element or make a copy of the existing control. For more information, see page 35.
- A Trial Control variable is a variable that you can define to control your trials. For example, a counter variable that is incremented every time a specific event occurs. You can then define conditions and actions based on this variable. For more information, see page 28.
- If the experimental protocol is very complex, like in the case of a multiple-day recording session, it is handy to split the procedure in main blocks; then, for each block specify the basic actions and conditions, and whether the logical sequence should be repeated. This helps you identifying the main building blocks of your Trial Control logic, which will translate into Sub-rules.
- If you have inserted **Condition** boxes based on **Activity** in your Trial Control rule and then deselect **Activity analysis** in the **Experiment settings** (see Activity analysis in the EthoVision XT Help), the **Condition** boxes based on **Activity** are removed from your sequence and the connecting arrows are removed. This makes the rule invalid. Redesign your Trial Control rule and connect the arrows between the boxes.

  EXAMPLE A trial is to be run for a few days. On day 5 after the start, during the dark phase, an operant conditioning session must be repeated 10 times. On day 6, the same sequence must be carried out just once.
- If you choose to record video with EthoVision XT by selecting the option **Save video only, track later** in the Acquisition screen, Trial Control is applied when you do tracking, not when you record video. To start and stop video recording, do so manually.

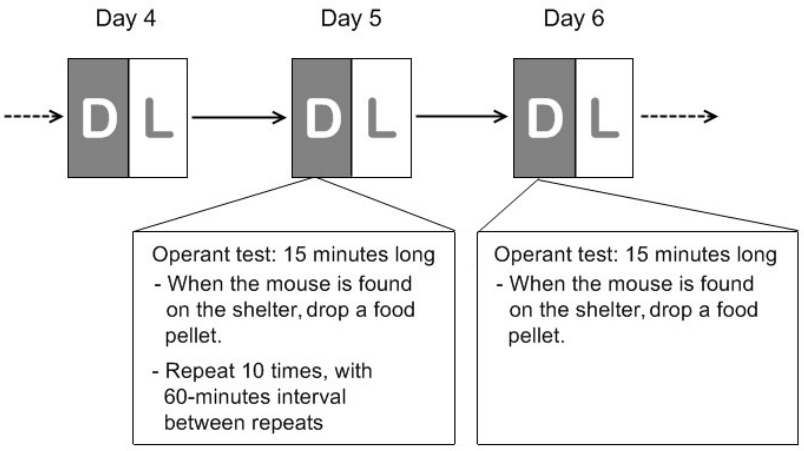


Figure 10 *An example of a block diagram representing a multi-day conditioning schedule.*

Procedures that must be repeated or represent a specific task must be defined as Sub-rules (see page 54). Sub-rules can be called at specific times or under specific conditions. In the main Start-Stop diagram, draw a block that calls each sub-rule.

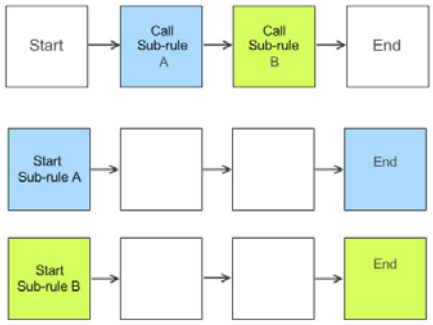


Figure 11 *An example of a block diagram with the main Start-Stop procedure (top) and two subroutines (sub-rules).*

See also the **Main cases** on page 37 for examples of logical blocks combined for a specific function.

CONDITIONS

A Condition is a statement that EthoVision checks during the trial. When the Condition is met (True), the program evaluates the next Trial Control element (another condition, an action or a reference to a sub-rule).

EXAMPLE Conditions are indicated in *italics*:

- *When the rat reaches the platform*, stop tracking.
- *When the mouse is around the novel object*, switch on the light.
- *When the animal presses the lever*, drop a pellet.

To define a condition

1. In the **Components** pane, under **Conditions**, locate the type of condition you want to define.
2. Double-click the condition name or click the button next to it.
3. If the **Add a condition** window appears, it means that there is at least one condition of the same type in your experiment. You are asked to choose between creating a new condition, or re-use an existing one (see page 35). Choose the option you require and click **OK**. If this window does not appear, skip this step.
4. Next to **Condition name**, type in the name you want to give to the condition, or accept the default name.
5. Specify the condition properties (for details see page 67).
6. Enter a **Comment** (optional), then click **OK**.
7. Insert the condition box in the sequence.

If the condition is complex (for example, Stop the trial *either if the rat has reached the platform or it has been swimming for 60 seconds*), then you must define separate conditions and combine them (see page 33).

IMPORTANT If you have inserted **Condition** boxes based on **Activity** in your Trial Control rule and then deselect **Activity analysis** in the **Experiment settings** (see the EthoVision XT Help), the **Condition** boxes based on **Activity** are removed from your sequence and the connecting arrows are removed. This makes the rule invalid. Redesign your Trial Control rule and connect the arrows between the boxes.

See also **Main cases** on page 37, and a detailed overview on page 67.

ACTIONS

An Action is a command that EthoVision carries out during acquisition and that influences the trial.

EXAMPLE Actions are indicated in *italics*:

- When the animal is detected in the arena, *start tracking*.
  This is an example of system actions (*start tracking* and *stop tracking*).
- When the animal enters the maze's left arm, *do C= C+1*.
  This is an example of actions on a Trial Control variable. See page 28.
- When the animal is found on the shelter, *drop a pellet*.
  This is an example of actions on hardware devices. See page 116.
- When the animal leaves the shelter, *play a sound or present a visual stimulus*. Or, when you start tracking, also start ultrasound recording with UltraVox XT.
  Those are examples of actions based on an external command. See page 30.

To define an action

1. In the **Components** pane, under **Actions**, locate the type of action you want to define:
   - **Trial Control variable** for an action on a Trial Control variable.
   - The device type name for actions on a Hardware device.
   - **External command** to start an external program or run a batch file.
2. Double-click the action name or click the button next to it.
3. If the Add an action window appears, it means that there is at least one action of the same type in your experiment. You are asked to choose between creating a new action, or re-use an existing one (see page 35). Choose the option you require and click **OK**. If this window does not appear, skip this step.
4. Next to **Action name**, type in the name you want to give to the action, or accept the default name.
5. Specify the action properties:
   - See page 28 for actions on Trial Control variables.
   - See page 30 for actions on External commands.
   - See page 116 for actions on Hardware devices.
6. Enter a **Comment** (optional), then click **OK**.
7. Insert the action box in the sequence.

You cannot create additional actions of the "Start track" and "Stop track" type, nor can you delete the existing ones.

See also **Main cases** on page 37.

TRIAL CONTROL VARIABLES

You can define variables to be temporarily stored during a trial, that you can use to influence the control of the experiment.

- **EXAMPLE 1** Variable as a marker. The variable *Phase* has been defined to specify the various phases of a trial (*Phase* = 1 means Conditioning; 2 Testing, etc.). The researcher can then program EthoVision to follow specific Trial Control sequences according to the current value of Phase.
- **EXAMPLE 2** Variable as a counter. A researcher has defined the variable SR (successful response) to count the number of times the subject responds correctly to a stimulus. To increment the value of SR after a correct response, specify the formula SR= SR+1.

To define a Trial Control variable

1. In the **Components** pane, click the button next to Trial Control variable under **Conditions** or **Actions**. Next, click the **TC Variables** button.
2. The Trial Control Variables window lists the variables currently in the experiment (also those defined in other Trial Control Settings). To add a new variable, click **Add variable**.
3. A new row is appended to the table. Under **Name**, type in the name you want to give to the variable. Under **Initial Value**, enter the value of this variable at the start of the trial (default: 0).
4. Click **OK**. In the TC-variable action/condition window, define the action or condition you require. Click **Cancel** if you do not want to create a condition or action based on this variable at this point.

To delete a variable, click the variable name in the Trial Control Variables window and click the **Delete variable** button.

To rename a variable, click the variable name in the Trial Control Variables window and edit this name.

The variable name cannot contain blank spaces.

The default name of a new trial control variable is VarN, where N is a progressive number.

Actions on a Trial Control variable

To assign a new value to a variable in Trial Control, define a Variable action in your Trial Control rule.

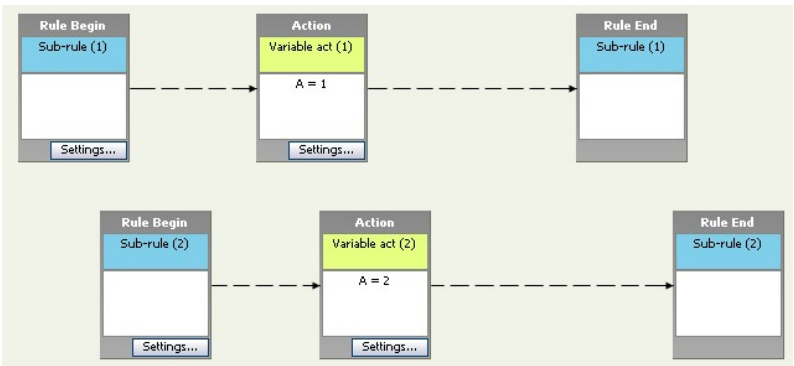
1. In the Components pane, under **Actions**, click the button next to Trial Control variable.

2. If the Add an action window appears, it means that there is at least one action of the same type in your experiment. You are asked to choose between creating a new action, or re-use an existing one (see page 35). If this window does not appear, skip this step.
3. Next to **Action Name**, enter the name of the action (for example, Increment Counter) or accept the default name.
4. Under **Action to perform**, select the variable from the list. You can also create the variable by clicking the **TC Variables** button if you have not yet done so.
5. Next to the "**=**" symbol, do one of the following:
   - To assign the same value of another variable (for example A = B), select the other variable (B) from the second list.
   - To enter a formula, click the double-arrow button.

     Select the operator from the list and specify the formula in the second and third lists. For example, A= A + 1.
   - To assign a random value, select **Random** from the second list, and enter the **Minimum** and **Maximum** limits (only integer numbers 1 up to 999) in which the random value must lay.
6. Enter a **Comment** (optional), then click **OK**.
7. Insert the resulting **Action** box in the Trial Control rule.

Notes

- If your setup includes multiple arenas, each arena receives an instance of the variable. Thus, a variable can have different values in different arenas.
- You can define several instances of variable-based actions and conditions in the same Trial Control profile. Such instances may well become active at the same time. Make sure that they do not interfere with each other. For example, two actions on the same variable are carried out in two different sub-rules (see page 54):



If the actions A=1 and A=2 are evaluated at the same sample time, the value of the variable A after the actions are executed depends on the order of the Reference boxes those sub-rules refer to. If the reference to Sub-rule 1 comes first, A=1 is executed first. If references are placed in parallel, the order is undefined. In the Visualization, you can check which action is executed first (see page 41).

- You cannot combine **Random** with a formula (for example, to compute *A= Random+1*). The equivalent solution is the following: define first an action *B= Random*, and then one more action *A= B+1*. Place the two boxes in sequence.
- To generate a random value, the maximum limit must be greater than the minimum.
- Use the **Random** option when programming **Variable ratio** schedules. Define a variable that specifies the number of rewards to be provided. Insert the resulting box in a sub-rule (see page 54). Every time the sub-rule is repeated, the variable gets a new value. Make sure that the food dispenser is activated as many times as specified by the variable.
- To analyze the values of a Trial Control variable, or visualize its values plotted against time, also for testing purposes, in the Analysis profile choose **Trial Control variable**.

EXTERNAL COMMANDS

Actions based on an External command

1. In the **Components** pane, under **Actions**, click the button next to **External command**.
2. Next to **Action Name**, enter the name of the action (for example, *start recording*) or accept the default name.

   Click the **Information** button to get additional information about defining an External command.
3. Under **Actions to perform**, select which file you want to run by clicking the ellipsis button.
4. Next, select one of the file types from the list:
   - **Executables (*.exe)**.
   - **Batch Files (*.bat, *.cmd)**.
   - **All Files (*.*)**.
5. Locate the file and click **Open**.
6. Optionally, enter a **Command line option**.
7. To test the command, click the **Test** button.

Example 1 - Trigger Media Recorder to record video

You carry out live tracking during a 24-hour period and you want to record video with Media Recorder, but only when the animal is not in the shelter (defined as a Hidden Zone, where it spends most of its time).

To do so, you must split the camera signal and send it both to EthoVision XT and Media Recorder. This is possible only if you have an analog camera. Please note that you need to adjust the dip switches on the Euresys Picolo H.264 encoder card that converts the analog video signal into a digital video file. See the Media Recorder Help for details.

1. First, start up Media Recorder using an External command box: select **MRCmd.exe** as the Executable to run and enter **/E** as a Command line option to launch Media Recorder.
2. Next, insert a Condition *Out of shelter* and combine this with a Time condition to make sure that Media Recorder is launched before recording starts (see Figure 12 for an example). Then, insert an External command box: select **MRCmd.exe** as the **Executable** to run and enter **/R** as a **Command line option** to start recording with Media Recorder. Similarly, you can stop recording (Command line option: **/S**) when the animal enters the shelter again.

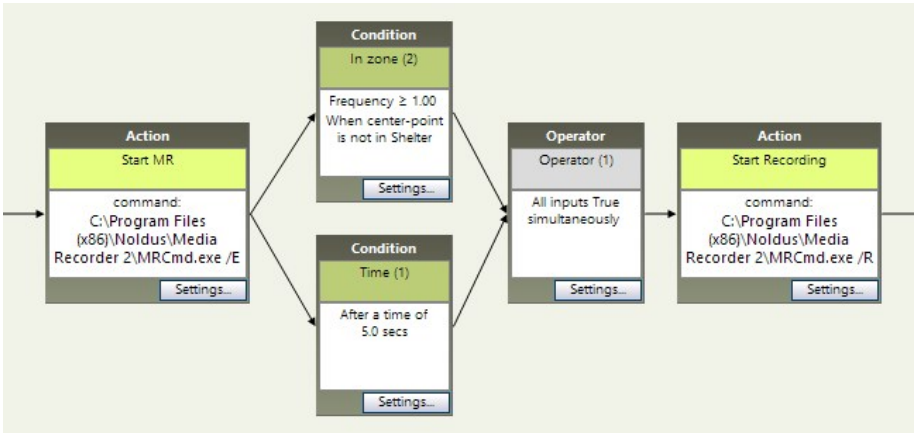


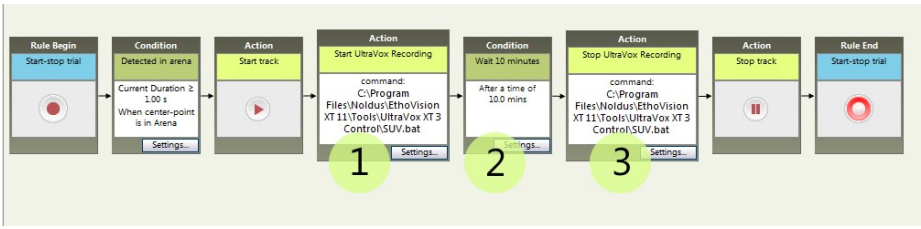
Figure 12 *Example of the External command action to start a recording with Media Recorder when the animal leaves a shelter. The left* ***Start MR*** *action box starts up Media Recorder. The* ***Start Recording*** *action box on the right starts the recording when both the Out of Shelter and Time(1) conditions are true, that is, the center-point of the animal has left the shelter at least 5 seconds after Media Recorder was started.*

NOTE There may be a delay between the command **Start Recording** and the moment Media Recorder actually starts recording. Run a test recording to measure that delay.

Example 2 - Trigger UltraVox XT to record ultrasound

You plan to video-track animals with EthoVision XT and, at the same time, record ultrasound with UltraVox XT. For ease of use you want to start and stop recording from one place — the EthoVision XT computer. Define an action based on the batch file SUV.bat (see 1 in the figure below). This file is located in C:\ProgramData\Noldus\Common\Tools\UltraVox XT 3 Control.

Note that you need to edit this batch file depending on your computer configuration. In the figure below, condition 2 determines the duration of the recording. Action 3 stops recording in UltraVox XT.



For more information, see **Control UltraVox XT from other software** in the UltraVox XT 3 Help.

Example 3 - Run a batch file

You want to play a sound during your trials. You can do this by running a batch file (*.bat or *.cmd) using an External command action in EthoVision. In the figure below, an action has been defined with the batch file **Play sound.bat**.

The batch file **Play sound.bat** starts Windows Media Player and plays the sound file **Stimulus_sound.wav**. Its content is:

```
start "%ProgramFiles%\Windows Media Player\wmplayer.exe"
D:\temp\Stimulus_sound.wav
```

- **Arena and Trial names**. When using a batch file, you can use the command line options **%an%** and **%tn%** to send information about the Arena and the Trial name in command lines from the Trial Control rule. For example, during a batch acquisition of trials you could send the trial name information to an external program, which starts a procedure when trial name (%tn%) becomes Trial 10.

In the following example, a batch file has been created to display the current Trial name and Arena name in the DOS window (one for each arena, in the case of multiple arenas). Echo %1 and Echo %2 listen to the values of %an% and %tn% in the order specified in the command line options. The DOS window is set to close 5 seconds later:

echo %1

echo %2

PING 1.1.1.1 -n 1 -w 5000 >NUL

In the **External command Action** window, specify the name and location of the batch file, and enter the command line options **%an% %tn%** (lower case only; parameters must be separated by space).

IMPORTANT Contrary to what worked in EthoVision XT 12 and older, **%an% %tn%** are not written with quotes. For example **%tn%** becomes Trial 1, not "Trial 1".

OPERATORS

Aim

The Operators help you combine actions, conditions and sub-rules in various ways.

EXAMPLES

- When *at least one of the two conditions A and B is met,* then do ... .

  This is an example of conditions combined by an operator of the "Any" type (OR logic).

- When two conditions *are met at the same time,* then do ... .

  This is an example of conditions combined by an operator of the "All" type (AND logic).

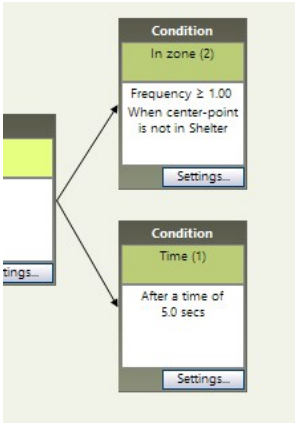
- When *at least/at most/exactly 4 of 8 conditions are met,* then do ...

  This is an example of conditions combined by an operator of the "N of All " type.

Procedure

1. Define the conditions/actions/rules that you want to combine. Place them in your Trial Control sequence as parallel branches.

   The connecting arrows must originate from the condition/action that precedes the combination of elements you want to define.



2. In the **Components** pane, under **Structures**, double-click **Operator** or click the button next to it.

3. If the Add an operator window appears, it means that there is at least one operator of the same type in your experiment. You are asked to choose between creating a new operator, or re-use an existing one. If this window does not appear, skip this step.
   - **Create a new operator**. A new operator is created.
   - **Re-use an existing operator**. Select the name of the operator already present in your experiment. See page 35 for more information.

   Click **OK**. The Operator window appears.

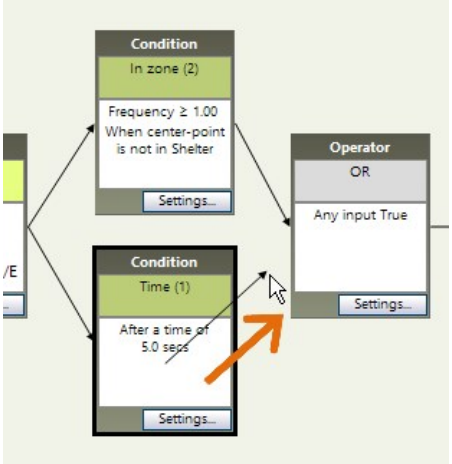
4. Under **Name**, enter the **Operator name** or accept the default name Operator (n), where n is a progressive number.

5. Under **Operator triggers when**, select the option that applies:
   - **Any (at least one) of the inputs is 'true'**.
   - **All inputs are simultaneously 'true'**.
   - **N of All inputs are simultaneously 'true'**.

   Where 'true' means a condition met, an action carried out, or a sub-rule finished (depending on the elements you want to combine).
   - If you choose the third option, specify how many inputs must be 'true': **=** (exactly equal to), **not=** (not equal to), **>=** (at least), **<=** (a maximum of), etc. Specify the number in the box.

6. Enter a **Comment** (optional) to describe this operator, and click **OK**.

7. A new Operator box appears in the Trial Control. Place the box right of the elements defined in step 1, and connect each element (or ending element, in the case of a sequence) to the operator.



8. Connect the operator to the next element that should be activated.

Notes

- Names of operators must be unique in your experiment. You cannot define two operators with the same Operator name, even if these are defined in two different Trial Control Settings.
- An Operator can also have just one input box. In that case the operator is of no use, because control passes immediately to the next box as soon as the input condition becomes true or the input action is carried out. EthoVision informs you about this.
- Operators can also combine sequences of elements. For example:
  - **Sequence 1** - When the animal presses a lever (Condition), then drop a food item (Action).
  - **Sequence 2** - Time= 5 minutes (Condition; this equals to waiting 5 minutes).

  If you want the program to take an action when either sequence has been completed, combine the two sequences with an "Any" operator.

RE-USE TRIAL CONTROL ELEMENTS

The elements of Trial Control (conditions, actions, operators, sub-rules and sub-rule references) that you have defined in other Trial Control Settings can be duplicated and re-used in the current Trial Control Settings to reduce your time spent editing.

To re-use all the elements defined in your current Trial Control Settings profile, make a copy of it: right-click the profile in the Experiment Explorer and select **Duplicate**.

To re-use a Trial Control element

1. Click the button next to the category of element that you want to re-use.
2. The Add window appears. Select **Reuse an existing condition/ action/ sub-rule/ reference**. This window does not appear when the experiment contains only one Trial Control Settings profile, or the experiment contains more Trial Control Settings profiles but none of them contains an element of the same type as that you have chosen.
3. Select the name of the existing element from the list next to the option.

   The second list shows the Trial Control Settings profile that contains that element. If the element is present in multiple Trial Control Settings, choose the appropriate one from the list.
4. Click **OK**.
5. A window appears for the type of element chosen. The Name and settings specified here are the same as in the element chosen in step 3.
   - To create an identical copy of the element, click **OK** and go to step 7.
   - In all other cases, edit the settings and click **OK**, then go to step 6.
6. If you have changed any property of the new element (including name and comment), a window appears showing two options:
   - Apply the new settings only in the current Trial Control profile.
   - Apply the new settings in all writable Trial Control profiles.

   The program asks you whether you want to apply the properties only to the new copy, or to extend those changes to the original elements in all Trial Control Settings that are writable (that means, not locked after acquisition). Choose the option you require and click OK.
7. Insert the resulting box in the Trial Control sequence.

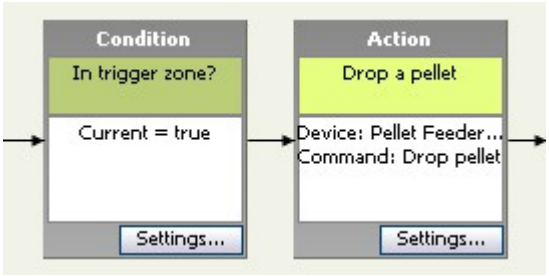
Notes

- If you choose the option **Apply the new settings** in all writable trial control profiles, changes are not made in those profiles made read-only after data acquisition.
- The program does not ask you whether you want to re-use the Trial Control element if no element of the same type has been defined in the other Trial Control Settings.
- You cannot re-use a Trial Control element from the same Trial Control Settings. This is because the Trial Control elements must be unique in order for correct analysis to be done.

FREQUENTLY-OCCURRING CASES

Have an action taken when a condition is met

EXAMPLE A food pellet is dropped (Action) when the mouse is detected in the zone that marks the top of the shelter (Condition).



The condition must precede the action. In the example above, the condition is based on a zone. The action is performed on the Pellet dispenser.

Have two actions taken at the same time

EXAMPLE In a radial maze experiment, make sure that the device Door 1 opens at the same time when Door 2 closes.

Define two action boxes, one for each door device. Next, do one of the following:

- Place the two action boxes in parallel, and combine them with an **All** operator (see page 33).
- Place the two action boxes one after the other. The time between the two actions is negligible.

Specify a time interval between actions/conditions

EXAMPLE Wait one minute between the action *Cue Light On* and the action *Drop one pellet*.

Insert a Time condition box between the two existing boxes (see also the next case).

Make multiple instances of an action

EXAMPLE In a conditioning experiment, *drop two food pellets in sequence*.

The two actions are placed in a linear sequence. If the action is performed on a hardware device, insert a Time condition between the two action boxes, for example wait 10 s before proceeding to the second action. If you do not do this, EthoVision XT interprets the twin actions as one. In the example above, it would deliver one food pellet only.

If the pellet dispenser is almost empty, it may take up to 30 seconds to deliver a pellet. make sure that the time specified in the Time condition exceeds the time that it takes the action to be carried out.

Another possible solution is to place a condition based on hardware between the two actions, that checks that the **Number** of pellet drops is **>=1** before taking the next *Drop pellet* action.

Have a sequence of conditions and actions repeated

EXAMPLE Make sure that the sequence *Mouse on Shelter --> Drop one pellet* on page 37 is repeated every time the animal is found on the shelter.

Define a **Sub-rule** (see page 54) and insert the sequence of conditions and actions in it. Next, create a **Reference** to that sub-rule, and specify when and how many times the sequence must be repeated (see page 56).

Give a reward after multiple instances of the subject's action

EXAMPLE In a Fixed Ratio schedule, it is required that the animal has to *press the lever three times* (Action) before getting a reward.

- Define a Trial Control variable C that counts the number of lever presses. The action *C=C+1* is triggered every time the condition *Lever pressed* is met.
- Define a **Sub-rule** that includes the sequence Condition *Lever pressed* ➔ Action *C=C+1*.
- Define a **Reference** for that sub-rule, and specify that the sub-rule must be repeated until *C* reaches the value required 3. Place the Action box for *Drop a pellet* immediately after the sub-rule's Reference box.

For more information:

- On Trial Control variables. See page 28.
- On sub-rules and references to sub-rules. See page 54 and page 56.

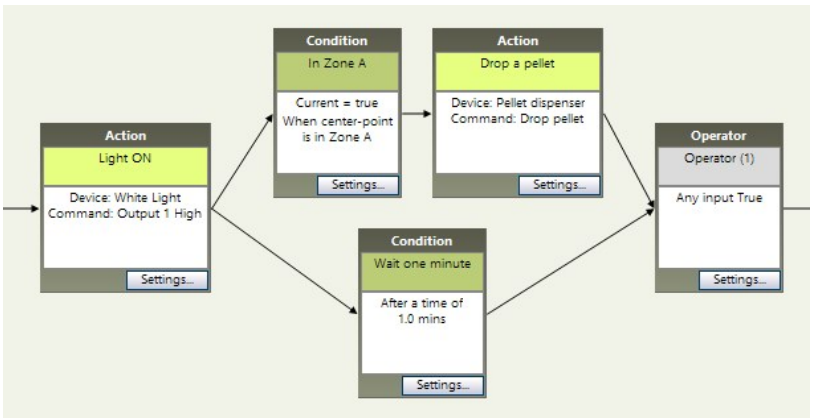
Have an action taken when a condition is met within a specific time

EXAMPLE Have EthoVision XT *drop a food pellet* when the *animal visits zone A within 1 minute from the release of a light stimulus*.

- Define the main condition (in the example above, *animal in zone A*. Specify that the **Current value** of **In zone** must be *true*.
- Define the action that must be taken (*Drop a pellet*).
- Define the accompanying Time condition (*Wait one minute*).
- Place the main condition followed by the action. Place those two boxes in parallel with the **Time** condition, using an operator of "Any" type.

With such a sequence, the food pellet is dropped as soon as the animal is found in zone A. If the animal does not go to zone A, one minute after the light was switched on the Time condition is met, and the operator becomes active. Therefore, control passes to the box following the operator, and the In zone condition is no longer evaluated.

With this solution, the action *Drop a pellet* is not taken when the animal enters zone A more than one minute after the light has been switched on.



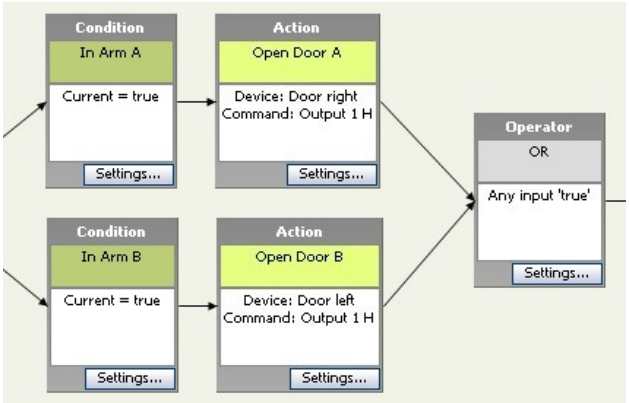
IMPORTANT If you combine the two conditions above with an operator of **All** type, the program may wait indefinitely until both conditions are met.

Have different actions taken according to different conditions

EXAMPLE A T-maze is provided with two doors, one in the middle of arm A and one in the middle of arm B. It is required that *door A is opened when the animal enters arm A*, and *door B when the animal enters arm B*.

- Define two conditions, one for arm A and one for arm B. For both conditions, specify that the Current value of In zone must be true.

- Define two actions, one for opening door A and one for opening door B.
- Connect each condition to the corresponding action. Next, connect the resulting sequences through an operator of the "Any" type.



Define phases in a trial

EXAMPLE It is required that tracking occurs in different phases of a trial, for example, Baseline, Conditioning, and Testing. In each phase, different trial control rules must be applied.

- For each phase, define a **Sub-rule** (see page 54) with its actions and conditions. Specify when the sub-rule must start.
- For each sub-rule, define a **Sub-rule reference** and insert it in the Start/Stop rule. Make sure that the references are in the order specified in your experimental protocol.

TEST THE TRIAL CONTROL SEQUENCE

It is not easy to make a complex Trial Control sequence work right the first time. To check that Trial Control works as expected, follow this procedure:

1. Make sure that the Trial Control Settings to be tested are highlighted in blue in the Experiment Explorer. If this not the case, click the **Trial Control Settings** you want to test.
2. Open the Acquisition menu (Choose **Acquisition** > **Open Acquisition**.
3. Click **Show/Hide** and choose **Show Dependent Variable**.
   - Choose **Trial Control Event** for simple events like Action *Drop a pellet* becomes active, or Condition *Subject in zone A* becomes true.
   - Choose **Trial Control State** to select the time between events occurred during the trial. for example **From Action *Light On* To Condition *In Trigger zone* True**.
     This will show the time interval from when the light switches on to when the animal enters the Trigger zone.
4. Start a trial. Watch the Trial Control Events or States in the **Analysis Results and Scoring** Pane at the bottom of your screen.

Trial Control event	Trial Control event
...	...
Start-stop trial / becomes ac	In zone (1) / becomes true
Frequency	Frequency
1.000000	0.000000

5. Create an Analysis profile and use the Trial Control event /state/variable and other dependent variables to specify the events you want to visualize. See page 166 for details.
6. To visualize the trial, choose **Analysis** > **Results** > **Plot Integrated Data**.

   Trial Control data are visualized as vertical segments (for events, with no duration) or horizontal bars (for states, with a duration) along the time line. Examine the data and check that EthoVision XT has responded as expected.

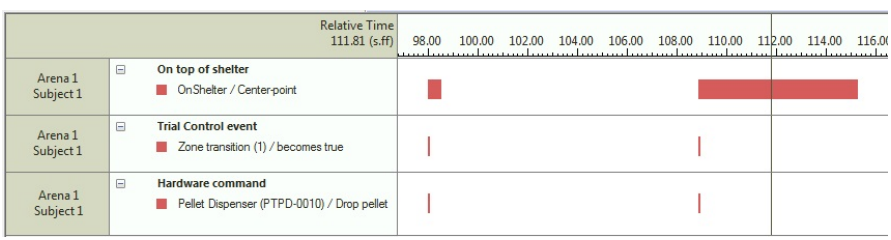


Figure 13 *Visualizing Trial Control data. Top: Dependent variable In zone "On Shelter". Middle: Trial Control event that marks the time when the Zone transition condition is met. Bottom: Trial Control event that marks the time when a food pellet is dropped.*

7. You can also check when exactly a condition becomes active or true, or when a command is given by looking at the **Trial Control Events** pane at the bottom-right corner of your screen. Select an Arena name from the list to visualize the sequence of Trial Control for that arena.

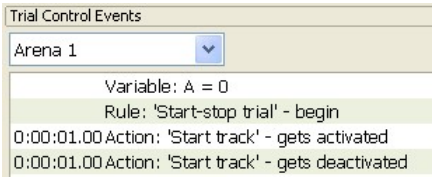


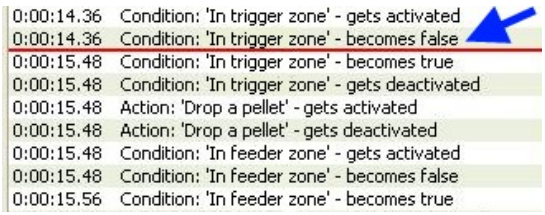
***Figure 14** The **Trial Control Events** pane.*

Play the track. The red hairline moves along the sequence and shows at what point Trial Control is. Play the track frame-by-frame to see when exactly a condition becomes true, or a command becomes active.

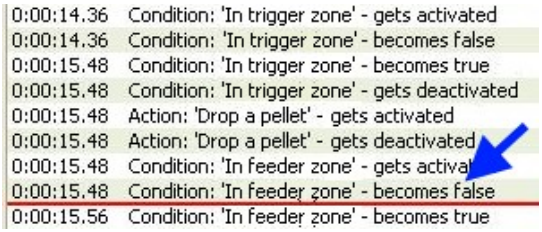
In the following example, a Trial Control sequence is defined:

Condition: In trigger zone ➔ Action: Drop pellet ➔ Condition: In feeder zone.

Play the track until the cursor gets between **Condition: In trigger zone - becomes false** and **Condition: In trigger zone - becomes true**. This is the time when the condition is activated and the program waits until the animal enters the trigger zone.



At 15.48 s the condition becomes true. The program executes the instructions that follow (Action: Drop pellet) and positions at the next condition (In feeder zone) until that becomes true too.



8. Make the necessary corrections to the Trial Control rules and start again from step 2.

Notes

- For a clear overview of the Trial Control events occurring during a trial, for each Trial Control box (except the Rule End box) define three dependent variables (step 3 above):
  - **Trial Control event** - under **Element**, choose which box to test, and under **Event**, choose **becomes active**. This marks the time when an action is taken, a condition is evaluated, or a sub-rule is activated.
  - **Trial Control event** - under **Element**, choose which box to test, and under **Event**, choose **becomes inactive**. This marks the time immediately after an action is taken (this is usually not distinguishable from the time the action is taken), a condition is met, or a sub-rule is completed.
  - **Trial Control state** - Select From when the box **becomes active** To when the box **becomes inactive**. This will display a colored bar that marks the time that the box is activated up to when it is deactivated.

  Define also one or more a In zone dependent variable to monitor the position of the animal.
- Once you have carried out a trial, the Trial Control Settings used for that trial are locked. To edit them, either delete the trial acquired in step 2 or make a copy of the Trial Control Settings (right-click the Trial Control Settings in the Experiment Explorer and select **Duplicate**).
- To check at what time an action is taken or a condition is met, you can also export the Trial Control events specified in step 2 and the Hardware log data (see page 182).
- You can also test your Trial Control Settings by providing visual/audible feedback during the trial. Define an action box that calls up some feedback once the program has reached a specific point in the Trial Control sequence. Insert this box immediately right to the condition/action/rule reference box that you want to test.
- To test hardware devices, see page 114.

APPLY TRIAL CONTROL TO YOUR TRIALS

To apply Trial Control to your trials, make sure that the Trial Control Settings profile containing that logic is highlighted in blue in the Experiment Explorer.

Test your setup thoroughly before carrying out the actual trials (see page 41).

For setups with multiple arenas

Trial Control is applied to each arena independently.

Locked Trial Control Settings

When a Trial Control Settings profile is used for acquiring at least one trial, it becomes locked. Locked settings are indicated by a lock symbol in the Experiment Explorer, and cannot be edited. To edit a locked Trial Control Settings profile, make a copy of it and edit this copy.

Track from pre-recorded video files

When you track from video files, Trial Control checks conditions using video time instead of the real time.

- **Conditions based on time**. If you select the **Detection Determines Speed** option, Trial Control is carried out at the speed set by EthoVision in order not to skip video images). This results in the video playing faster or slower than normal (1x), depending on the processor load necessary to detect subjects. For example, if detection requires little processor work, the program tracks the subject faster than normal. A *time* condition (for example, 60 s) is therefore met earlier than at real time.
- **Using Clock time**. If you define a condition based on clock time, or schedule a sub-rule with Clock time, this is translated into the video start time, that is, the date and time the video file used for tracking was created.

  EXAMPLE 1 You set a *Time* condition to start tracking *After clock time 11:30*. The video file was created on March 6, 2008 at 11:00. once you start the trial, the condition is met half an hour later in the video. If you had set to start tracking After clock time 10:30, tracking would start immediately after starting the trial.

  EXAMPLE 2 You set a sub-rule to start at 10:00 (1st day). The video file was created on March 6, 2008 at 11:00. Once you start the trial, the sub-rule never starts, because the planned start occurs before the initial time of the video. To make a sub-rule start when tracking from that video, set the start time between 11:00 and the video end time.

Record video, then track the subject

You can choose to record video first and then acquire data from the resulting video file. You do this by selecting **Save video only, track later** in the Acquisition screen.

- When recording video, Trial Control is turned off. You get an appropriate message when selecting the option **Save video only, track later**.
- When you track from the video recorded in the previous stage, Trial Control for Start-Stop is activated, but you cannot control hardware devices.

Re-do a trial

For video files recorded with EthoVision, you can re-do the corresponding trial (see 'Redo trials' in the EthoVision XT Help). However, if you re-do a trial the Trial Control log files recorded with the previous instance of the trial are deleted.

Stop a trial

When you stop the trial, all rules active in the Trial Control Settings are ended immediately, and hardware devices are reset.

4 The Start-Stop trial rule

The Start-Stop trial rule is displayed on your screen when you create or open Trial Control Settings. It can be recognized by the first box with a green circle: With this rule, you control the start and stop of data acquisition (tracking).

You can only modify the initial Start-Stop trial rule.

You cannot:

- Delete the Start-Stop trial rule, including the **Rule Begin**, **Rule End**, **Start track** and **Stop track** boxes.
- Define an additional Start-Stop trial rule.

THE DEFAULT START-STOP TRIAL RULE

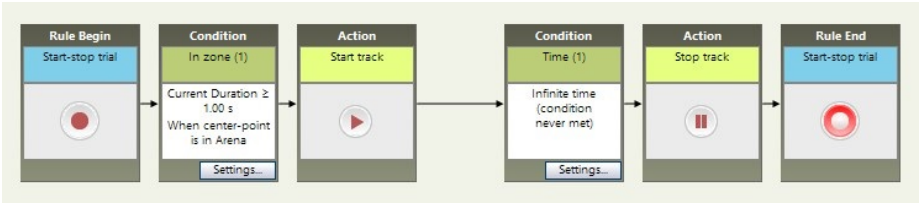


Figure 15 *The default Start-Stop trial rule is a sequence of six boxes:*

The default Start-Stop trial rule is a sequence of six boxes (but see a few exceptions, page 47):

- **Rule Begin - Start-Stop trial**. Activated when you start the trial (from the **Acquisition** menu, select **Start Trial**, or click the **Start Trial** button, or press **Ctrl**+**F5**). Once you start the trial, control passes to the next box.
- **Condition - In Zone - Cumulative duration >= 1 s**. This is the default Start track condition. It is fulfilled when center point of the subject (or of any subjects, in the case of an arena with multiple subjects) has been detected in the arena for 1 second after you started the trial.

  If you start the trial and the animal is not detected yet, the program waits until it detects the animal for 1 second, then it starts tracking.
- **Action - Start track**. Activated when the condition on its left side is met. Once this box is activated, data recording (tracking) starts. If the condition placed between the Start-Stop

trial box and this box is not met immediately, tracking starts later than the time you start the trial.

- **Condition - Time - Infinite time (condition never met)**. This is the default Stop track condition. This condition is never met. The trial stops when you give the Stop command or the time exceeds the maximum trial duration (when this has been set).

  IMPORTANT Always keep a Condition box that determines when the track is stopped right before the Stop track Action box, otherwise the trail stops immediately after you start the trial.

- **Action - Stop track**. Marks the end of all tracks (and trial).
- **Rule End - Start-Stop trial**. This box is just the delimiter of the rule, it does not take any action.

The Start-stop trial rule in special cases

- *Multiple arenas*. When working with multiple arenas, the rule is applied separately for each arena. This means that tracking can start at different times in different arenas in the same trial, depending on when the condition placed immediately before the **Start track** box is met in a specific arena. See also ***Multiple open fields (multiple arenas): Starting tracking when the subject is released in the open field. Tracking starts independently in each open field.***on page 51.
- *DanioVision and Activity analysis*. If you selected **Activity analysis** in the **Experiment Settings**, or created a DanioVision experiment, there is no box between the **Start-stop trial** and the **Start track** box. This means that tracking starts immediately when you click the **Start trial** button. For more information on Activity analysis, see **Activity analysis** in the EthoVision XT Help, or the Reference Manual - DanioVision DVOC-0041.
- *Behavior recognition*. If you selected **Behavior recognition** in the Experiment Settings the default Trial control rule contains a **Time condition** box set to 3 seconds. This means that tracking starts 3 seconds after starting the trial. This is done because the Behavior recognition algorithm needs 3 seconds of video images to make the first classification of behavior. With this condition you make sure that your track contains Behavior recognition data since the first sample.

  If you selected both **Activity analysis** and **Behavior recognition** in the Experiment Settings, the Trial Control Rule is the same as when you selected **Behavior recognition** only. For more information on Behavior recognition, see Behavior recognition in the EthoVision XT Help.

An important distinction: Trial vs. track

- **Trial**. A Trial can be viewed as a container for the data collected in one recording session. It starts when you give the Start command in acquisition and stops when the tracks for all arenas and subjects have stopped.
- **Track**. A Track corresponds to the actual recording of a subject's position and behavior. The start of a track may or may not coincide with the start of the trial. This depends on your Trial Control Settings. If you use the default Trial Control Settings, the track starts 1 second after the animal has been detected in the arena and stops when you stop the trial.
- A Trial may contain one or more tracks. For example, if you track two subjects simultaneously, each trial includes two tracks, one per subject. Similarly, if your setup contains four arenas with two subjects each, each trial includes 4 arenas x 2 subjects = 8 tracks.
- In a multiple-arena setup, the end of a track does not necessarily mean the end of the trial. The trial ends when all tracks come to an end.

CUSTOMIZE THE START-STOP TRIAL RULE

Modify the Start track condition

The default Start track condition is an *In zone* condition.

- To modify that condition, click the **Settings** button. In the window that appears, click **Settings** and specify the zone in which the animal should/should not be in order for the program to start tracking. (for details on In zone conditions, see page 86).
- To use another condition (for example: start recording exactly 1 minute after starting the trial), delete first the current condition (click that box and press **Delete**) and insert the new one. For an overview of conditions, see page 26 and page 67.
- To start recording as soon as you start the trial, delete the Start track condition. Click the box immediately before the Start track box and press **Delete**.

Modify the Stop track condition

The default Stop track condition is a *Time* condition.

- To modify that condition, click the **Settings** button, and choose the option you require (for details on Time conditions, see page 67).
- To use another condition, delete first the current condition (click that box and press **Delete**) and insert the new one. For an overview of conditions, see page 26 and page 67.

If you want to stop tracking when a specific time has elapsed, see page 21.

IMPORTANT Keep at least one condition between Start track and Stop track. If you do not do this, tracking stops immediately after tracking starts, resulting in no data.

IMPORTANT If you have inserted **Condition** boxes based on **Activity** in your Trial Control rule and then deselect **Activity analysis** in the **Experiment settings** (see Activity analysis in the EthoVision XT Help), the **Condition** boxes based on **Activity** are removed from your sequence and the connecting arrows are removed. This makes the rule invalid. Redesign your Trial Control rule and connect the arrows between the boxes.

Call a sub-rule

Sub-rules are subroutines of the Start-Stop trial rule (see page 54). You can program EthoVision XT in such a way that a subset of Trial control actions and conditions is called from a specific point in the Start-Stop trial rule. When this subset of instructions is finished (or repeated a number of times), the program resumes following the instructions of the Start-Stop trial rule.

1. Define a sub-rule (for details see page 54).
2. In the **Components** pane, under **Structures**, click the button next to **Sub-rule reference**. Enter a name for the reference, and select the sub-rule you have defined in step 1 above.
3. Insert the reference box in the correct place in the Start-Stop trial rule.

- For more information on sub-rules, see page 54. For more information on sub-rule references, see page 56.
- You cannot define a sub-rule reference without defining the sub-rule first.

EXAMPLES OF START-STOP TRIAL RULES

Start data recording at a specific time

You want to start recording at a time you are not in the lab, for example at 23:00 h.

Delete the default Start track condition (see page 48). Define a Time condition (see page 67). Select **At clock time** and enter 23:00:00. Click **OK** and place the resulting box before the Start track box. Before leaving the lab, click the **Start trial** button to start the trial. The program waits till 23:00 to start data recording.

For a similar example, see **Examples of Start-Stop trial rules** in the EthoVision XT Help.

Stop data recording after the maximum time has elapsed

Click **Settings** in the Condition box immediately before the Stop track box. Select **After** and enter the maximum time. **NOTE** Instead of using a *Time* condition, you can also use the **Maximum trial duration** option (see page 21).

Water maze: Stop the trial when the animal has found the platform

In the Arena Settings, make sure that the platform has been defined as zone. In the Trial Control Settings, delete the default Stop track condition (see page 48). Next, define a In Zone condition (see page 86).

- If you want the program to stop recording as soon as the animal is over the platform, select **Frequency** as Statistic and choose **>= 1**. Click **Settings** and select the platform zone.
- Sometimes the animal swims over the platform, but it does not stop there. In such cases the program would stop recording while the animal has not "found" the platform. Instead of selecting Frequency, choose **Current duration** and the minimum time the animal must stay on the platform (for example, 3 seconds). Click **Settings** and select the platform zone.

Click **OK** and place the resulting box before the Stop track box.

Water maze: Stop the trial either when the rat has found the platform, or when it has been swimming in the water maze for 60 seconds

The Arena Settings and the condition *In platform zone* are similar to those in the example above. The condition *subject swimming for 60 s* can be translated to *Time >= 60 s*.

The track stops when either condition is met. The two conditions are combined with OR logic (see Figure 16).

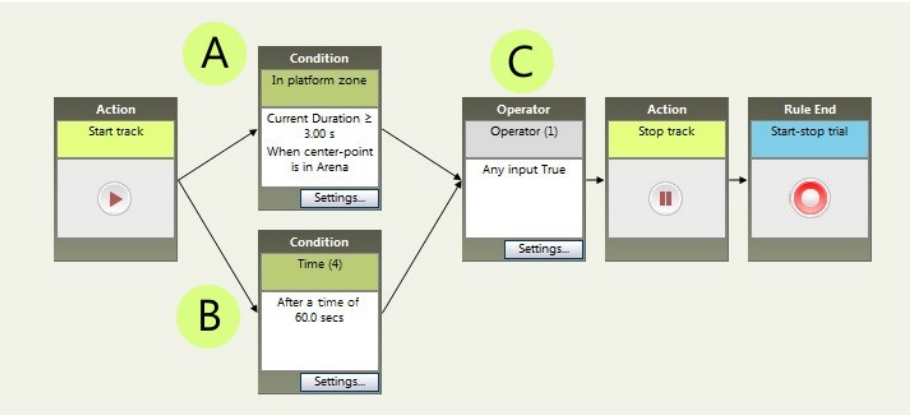


Figure 16 *Example of a Start-Stop trial rule for a water maze. The trial stops when the animal has been in the platform zone for at least 3 s without break, or the time since the start of tracking is 60 s.*
A *- In zone condition that specifies that the animal must be for at least 3 seconds over the* Platform zone. *Select Current duration >=3s.* ***B*** *- Time condition that specifies a time of 60 s since the track started.* ***C*** *- "Any" operator box.*

This solution results in tracks of different duration: less than 60 s for the animals that found the platform, and 60 s for the others.

TIP Instead of two Condition boxes in the example above, you can also define the In zone condition box and set a **Maximum Trial duration** (see page 21).

Multiple open fields (multiple arenas): Starting tracking when the subject is released in the open field. Tracking starts independently in each open field.

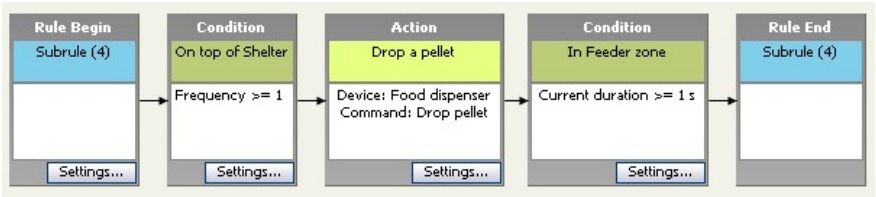
In this setup, four open fields are treated as separate arenas. You want to start acquisition when the animal is detected in the open field independent of what happens in other arenas. This can be achieved by using the default Start-Stop trial rule. As soon as an the subject is detected in an arena, tracking starts for that arena, not the others. This way you do not have to release all the animals at the same time.

Conditioning test: Stop the trial when the conditioning task has been repeated N times.

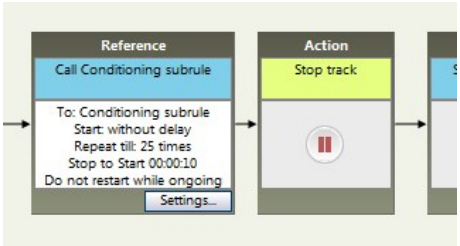
For example, when the animal has obtained 25 rewards.

1. Create a new sub-rule (see page 54) containing the instructions for the testing session. Sub-rule Begin ➔ Condition *Is subject on top of the shelter?* ➔ Action *Drop a pellet* ➔ Sub-rule End.

Since the sub-rule is restarted immediately after the pellet has been dropped, it is possible that the animal is still on the top of the shelter, and a new pellet is dropped before the animal eats the previous one. Therefore, you must make sure that the sub-rule ends when the animal has visited the reward zone, so the sub-rule can be repeated from that moment onward. You can do this by defining a condition "*In Feeder zone*" that is met when the animal has been in the Feeder zone for at least 1 s without break (Current duration >=1 s). Place this box to the left of Rule End.



2. Create a Reference to the sub-rule (see page 56) and specify to have the sub-rule repeated 25 times. Place the resulting box at the left side of the Stop track box.



Eight-arm radial maze: Stop the trial when the subject has been in four arms within 10 minutes.

Combine eight conditions, one for each arm. At least four conditions must be met, no matter which arm the animal visits.

1. Create an *In zone* condition (see page 86) and specify that the Frequency for Arm 1 must be >=1. That is, the animal must have visited Arm 1 at least once. Do the same for each of the other arms.
2. Connect the resulting eight condition boxes in parallel using the N of All operator (see Figure 17).
3. Set the **Maximum trial duration** (see page 21) to 10 minutes to stop tracking in the case the animal fails to visit four arms in the meantime.

For more information on operators of type **N of All**, see page 33.

IMPORTANT The frequency of In zone can become high very easily when the animal walks along the edge of a zone. This may result in the condition being met sooner than expected. If that happens, increase the Zone exit threshold. If that does not help, open the **Detection settings** and click **Advanced**, then under **Smoothing** set **Track noise reduction** to **On**. This smooths the track.

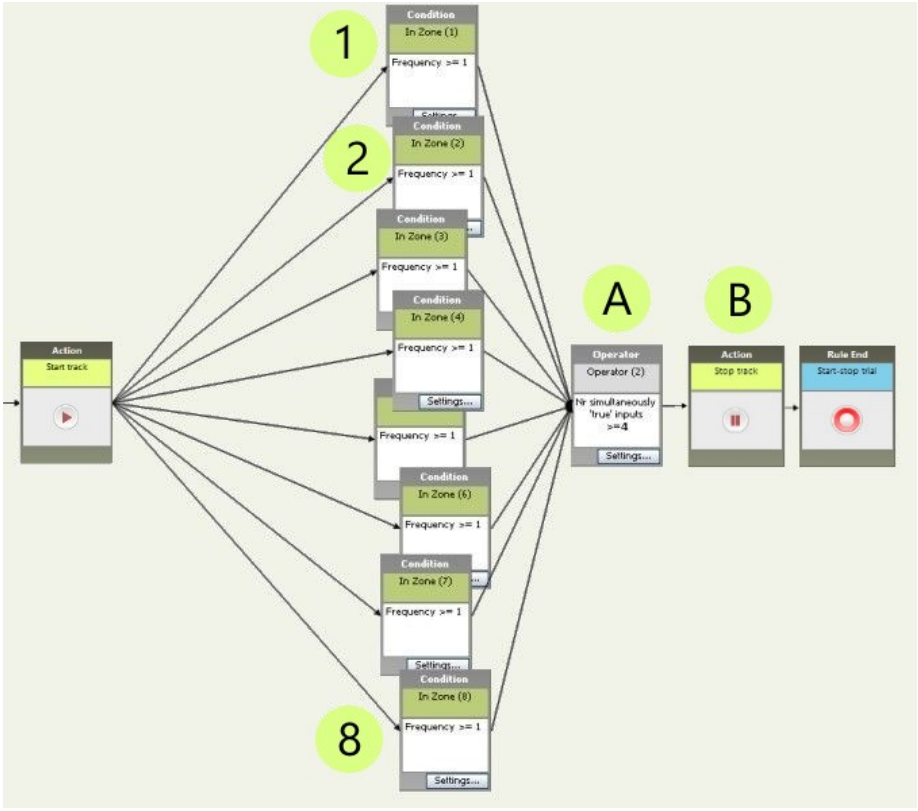


Figure 17 *A Trial Control sequence for an eight-arm radial maze. The trial must stop when the animal has visited four of the arms at least once.*
1, 2,...8-In zone *condition boxes for Arm 1, 2,... respectively. A condition is met when the* Frequency *of* In zone *for that arm is greater than or equal to 1.*
A *- Operator that checks that at least four of the eight conditions are met.*
B*- Stop track box. When four conditions are met, the trial is stopped.*

5 Sub-rules

USE SUB-RULES

A sub-rule is a sequence of actions and conditions separated from the Start-Stop trial rule. You can view a sub-rule like a subroutine in a larger program, where the program is your Trial Control.

- You can have the sub-rule start at specific times or under specific conditions. Each criterion defined is a Start condition of the sub-rule.
- A sub-rule can be repeated, that is, when EthoVision finishes executing the instructions contained in the sub-rule, the program can start the sub-rule again from the beginning, for a specified number of times or until a condition is met.
- You can also have the same sub-rule called from different points of the Trial Control sequence. To do so, define multiple References to the same sub-rule (see page 56).

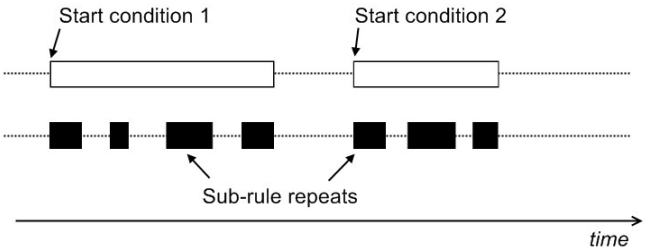


Figure 18 *Schematic representation of the functioning of a Sub-rule along the time line. You can specify a sub-rule to start at specific Start conditions (for example: Start at 12:00). For each start condition, a sub-rule can be repeated one or more times (black bars). The white bars represent the time from the start condition to the last repeat. Note that sub-rule repeats can vary in duration, for example when the behavior of the subject determines when a condition defined in the sub-rule is met. Also note that a sub-rule repeat can only start at least one sample after the end of the previous repeat.*

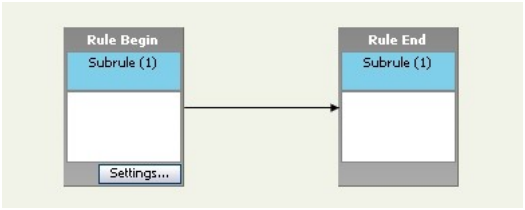
Why use a sub-rule?

A sub-rule is necessary when your trials involve repetition of tasks. For example, when the animal has to press a lever to obtain a reward, and you want to repeat that 50 times. This is not feasible within the Start-Stop trial rule.

A sub-rule is also handy when your Trial Control logic is very complex, because it helps you have a clear overview of the phases within your trial. For example, different tasks within a trial can be kept separated and managed more easily.

CREATE A SUB-RULE

1. In the **Components** pane, under **Structures**, double-click **Sub-rule**, or click the button next to it.
2. If the Add a sub-rule window appears, choose one of the following:
   - **Create a new sub-rule**. This creates a new sub-rule. You will be asked to enter a unique name for this sub-rule.
   - **Reuse an existing sub-rule**. Choose this option if you want to re-use an existing sub-rule. Select the existing sub-rule and, if necessary, the Trial Control Settings where it is stored. Note: You only copy the sub-rule name, the Rule Begin and the Rule End boxes, not the actions and conditions inside the existing sub-rule. See page 35 for re-using Trial Control elements.
3. Under **Name**, enter a name for the sub-rule.
   The default name is Sub-rule (n) where n is a progressive number.
4. Under **Comment**, enter a description of the sub-rule (optional).
5. Click **OK**. The sub-rule is displayed on your Trial Control screen. It consists of a Rule begin box and a Rule end box.



6. Complete the sub-rule by inserting the appropriate conditions and actions between the Rule Begin and Rule End boxes (see page 23).
7. Define a reference to this sub-rule (see the next section).

Notes

- **Sub-rule names**. Names of sub-rules must be unique in your Trial Control Settings. You cannot define two sub-rules with the same Name, even if these are defined in two different Trial Control Settings. However, you can create a sub-rule with the same name of an existing sub-rule stored in other Trial Control Settings by Re-using it (see below).
- **Re-using a sub-rule**. If you choose to re-use a sub-rule and then change any property of this copy (Name or Comment), a window appears with the message *Define the scope of your changes*. The program asks you whether you want to apply the properties only to the newly-created sub-rule, or to the sub-rules with the same name in all Trial Control

Settings. Choose the option you require and click **OK**. See also page 35 for information on re-using elements in Trial Control.

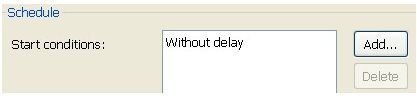
- **Deleting a sub-rule**. You cannot delete a sub-rule if at least one reference to that sub-rule is present in the Trial Control. You must first delete its reference.
- **If you create a sub-rule without a valid reference**, you can still acquire data but EthoVision informs you that trial control may not work as expected.

CREATE A REFERENCE TO A SUB-RULE

A Reference calls the instructions contained in an existing sub-rule.

1. Make sure that the sub-rule has been defined in the Trial Control screen.
2. In the **Components** pane, under **Structures**, double-click **Reference** or click the button next to it. 

   If the Add a reference window appears, choose one of the following, then click **OK**.
   - **Create a new reference**. To create a new reference to a sub-rule.
   - **Reuse an existing reference**. Choose this option if you want to make a copy of an existing reference. Select that reference and, if necessary, the Trial Control Settings where it is stored. For details on re-using elements, see page 35.
3. The Sub-rule Reference window appears. Under **Name**, enter a name for the reference. The default name is **Reference (n)**, where n is a progressive number.
4. Under **Reference**, from the **Reference to sub-rule** list select the sub-rule you have defined in step 1 above.
5. Under **Schedule**, the Start conditions box shows **Without delay**. This means that, by default, the sub-rule starts as soon as the reference box becomes active.



   You can define one or more start conditions. Once the sub-rule starts according to Start condition, you can have it be repeated one or more times (see the picture under point 2 on page 49).

   Click the **Add** button to add a Start condition. The Add Start Condition window appears.
6. From the **Select type** list, select one of the following:
   - **Without delay**. To start the subrule as soon as the reference is activated.

- **Delay (default)**. To start a specific time after the reference becomes active. Enter this time in the **Delay** field.
- **Clock Time**. To start at a specific day and time.
- **Trial Control variable**. To start when a Trial Control variable gets a specific value.

For details, see **SUB-RULE START CONDITIONS** on page 59.

7. Repeat steps 5-6 to add more start conditions. If you want to delete a start condition, select this and click the **Delete** button.

8. Under **Stop** conditions, select one of the options to specify whether you want the sub-rule to be repeated once or more times (see Sub-rule stop conditions on page 60).
9. If you select to repeat the sub-rule, the Repeat interval option becomes available. Select:
   - **Stop to Start (default)**. To keep a constant time between the stop of a repeat and the start of the next repeat.
   - **Start to start**. To keep a constant time between the start of successive repeats. For example, start repeats at every hour.

   Enter this time in the corresponding box (hh:mm:ss).
10. Select one of the options to specify what to do when a new start condition becomes true and a previous repeat is still ongoing. For details, see 'Options for starting a new repeat' on page 61.
11. Under **Comment**, enter a description of the sub-rule reference (optional).
12. Click **OK**. The reference box is displayed on your Trial Control screen.
13. Insert the reference box in the appropriate place in the Trial Control sequence (this can be either the Start-Stop trial rule, or another sub-rule). See also page 62.

Notes

- If you want the sub-rule not to start immediately as its Reference becomes active, select **Without delay** and click the **Delete** button.
- If you accept the default options (**Without delay** in the Start conditions box and one repeat), the sub-rule starts immediately and is repeated just once.
- You cannot edit a condition that you have added to the Start conditions box. Delete first the condition (select the condition and click the **Delete** button), then click **Add** to add a new condition.
- The Start conditions are listed in the following order: Without delay, Delay, Clock Time, Trial Control variable.
- **Stop to Start** of a repeat. There is always at least 1 sample difference between the time that the Rule End box is evaluated and the time that the Rule Begin box is evaluated for

the start of a new repeat. This means that a new repeat can never start exactly at the same time as the end of a repeat.

- **Reference names**. Names of sub-rule references must be unique in your experiment, unless you have re-used an existing reference. You cannot define two sub-rule references with the same Name, even when these are defined in two different Trial Control Settings. This is because analysis of Trial Control data (see page 166) is based on names of elements (sub-rules, references, actions and conditions). Elements with the same name would give ambiguous results.
- **Re-using a sub-rule reference**. If you choose to create a copy of a sub-rule reference and then change any property of this copy (Name, Reference options, or Comment), a window appears with a message *Define the scope of your changes*. The program asks you whether you want to apply the properties only to the newly-created reference, or to the references with the same name in all Trial Control Settings. Choose the option you require and click OK.
  If you re-use a Reference that is stored in another Trial Control profile, and this profile is locked after data acquisition, changing any of the Reference settings will not affect the Reference in the locked profile.
- **Modifying a sub-rule reference**. To modify a sub-rule reference, click Settings button in the reference box.
- **Multiple references to one sub-rule**. You can define multiple references to the same sub-rule, and place the corresponding Reference boxes in the same Trial Control sequence. In the case they are activated at the same time, the program executes the sub-rule in two parallel instances. Such instances are independent, however they can interfere with each other if they both contain actions on a Trial Control variable (see an example on page 29) or on hardware devices.
- **Endless loop** - EthoVision XT prevents the occurrence of an endless loop in the Trial Control, which would make EthoVision XT unresponsive. When in a Sub-rule Reference box the number of repeats is not set to a fixed value and the Sub-rule has a 0-second duration, this Sub-rule is evaluated endlessly from the Sub-rule Reference box. To prevent this, in EthoVision XT a Sub-rule is evaluated only once per sample from the Reference box. Sub-rules that are empty, only contain a Trial control variable Action box or Hardware Action boxes, are 0-second Sub-rules. With a 0-second Sub-rule, the Sub-rule Reference box never becomes inactive and, therefore, the Stop track Action box and Rule End box are never reached. In this case, the trial is stopped when you click the Stop trial button (for Live and offline tracking) or when the end of the video file is reached (for offline tracking).

SUB-RULE START CONDITIONS

To access those options, click **Add** in the sub-rule's Reference window.

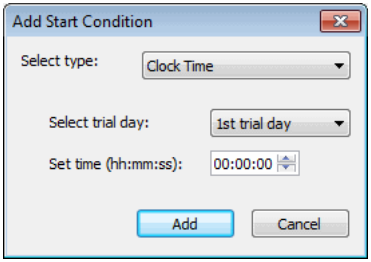
Without delay

Choose this option to start the sub-rule as soon as the sub-rule reference becomes active.

Clock Time

Choose this option to start the sub-rule at specific times of the day.

- **Select trial day**. Select the day from the start of the trial that the sub-rule should start. 1st trial day= the same day that the trial starts.
- **Set time (hh:mm:ss)**. Enter the time of the day that the sub-rule should start.



EXAMPLE If you want to start the sub-rule at 12:00 h of the fifth day of the trial, select 5th day and enter 12:00:00.

Delay

Choose this option to start the sub-rule with a specific delay from the moment that the Reference box becomes active.

EXAMPLE if you want to start the sub-rule one hour after the start of tracking, enter 01:00:00. Place the Reference box immediately to the right of the Start track box.

The time that the Reference becomes active is essentially the time that the previous box in the sequence becomes inactive (a condition being met, or an action being taken). See page 10 for more information on how Trial Control instructions are executed.

Trial Control variable

Choose this option to start the sub-rule when a Trial Control variable gets a certain value for the first time since the Reference becomes active.

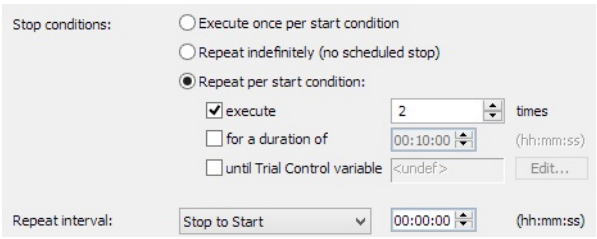
1. Click the **Edit TC-variable** button. The TC-variable condition window appears.

2. From the first list, select the **Trial Control** variable. If you need to create a new variable, click **TC Variables** (see page 28).
3. Select the comparison operator (=, >, etc.) from the second list.
4. Do one of the following:
   - If you want to compare the Trial Control Variable with another variable, select that variable from the third list.
   - If you want to compare the Trial Control variable with a numerical value, enter this value in the third list.
   - If you want to compare a Trial Control variable with a formula, click the double-arrow button and select the operator, then the variable and/or values to complete the formula.

   EXAMPLE You have defined the variable *Phase* which can have one of three values 1, 2 and 3 during the trial. You want to start a sub-rule when the current value of *Phase* is 2. Select *Phase* = 2.

If your variable is of the type true/false, and switches between true and false several times, the sub-rule starts only the first time that the variable gets the value required since the activation of the Reference. For more information on Trial Control variables, see page 28.

SUB-RULE STOP CONDITIONS



Execute sub-rule once per start condition (default)

The sub-rule is executed once when one of the Start conditions is met.

Repeat indefinitely (no scheduled stop)

The sub-rule is repeated until trial the trial stops (see page 62 for how this can happen).

Repeat per start condition

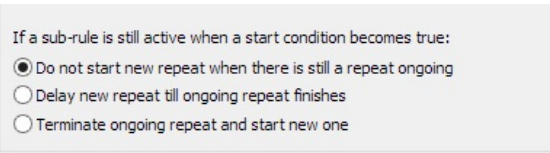
The sub-rule is repeated a number of times, or until a condition is met.

- **For a number of times**. To have the sub-rule repeated a fixed number of times for that start condition. Enter this number in the box (default= 2).
- **For a duration of (hh:mm:ss)**. To have the sub-rule repeated for a specific time from the corresponding start condition. Enter this time in the box. If you choose this option, when the time elapsed since the start condition exceeds the time set here, the ongoing repeat will continue till its end, but no new repeat will start.
- **Until Trial Control variable**. To have the sub-rule repeated until a Trial Control variable (see page 28) gets a certain value. Click Edit, specify the variable, its value and click **OK**. For example, repeat the sub-rule until the variable Count reaches 100. Specify Count <= 100.

If you select two or more Repeat per start condition options, no more new repeats are started as soon as any of the conditions is fulfilled.

A sub-rule is completed when the last repeat for the planned Start condition is ended. See also the note on Endless loops page 58.

Options for starting a new repeat



- **Do not start new repeat when there is still a repeat ongoing**. A new repeat is not started. A new repeat will start at the planned time, after the ongoing repeat has been completed.
- **Delay new repeat till ongoing repeat finishes**. The repeat starts as soon as the ongoing repeat finishes.
- **Terminate ongoing repeat and start new one**. The ongoing repeat is ended no matter at which point it is, and a new repeat is started.

EXAMPLE A sub-rule is planned to start every 10 minutes. The first Start condition is set to 12:00 h, the second to 12:20 h etc. Suppose that at 12:10, a repeat has not been completed yet, and it will be completed at 12:15.

- When selecting the first option above, the repeat planned at 12:10 is not started. The program will start the next planned repeat, at 12:20, provided that the first repeat has been completed.
- When selecting the second option, the repeat of 12:10 starts at 12:15 when the first repeat has been completed.

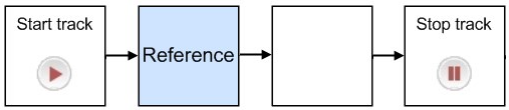
- When selecting the third option, the repeat of 12:10 starts at the planned time. The repeat of 12:00 is forcibly terminated.

If Trial Control switches on a light or sound device at the time the repeat is aborted, that device stays on until a switch off command is encountered in the next repeat (or another ongoing sub-rule).

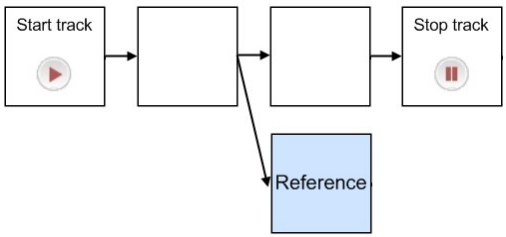
PLACE THE SUB-RULE REFERENCE IN THE TRIAL CONTROL SEQUENCE

The position of sub-rule's Reference box influences progress in the Trial Control sequence. Consider the difference between the following two cases:

- **Reference box in the linear sequence**. Control can only progress when the Reference box becomes inactive. This occurs when the last repeat of the sub-rule has been completed.



- **Reference box -n a dead branch of the sequence**. When the sub-rule's Reference box becomes active, the box placed in parallel becomes active too. Thus, control moves forward independent of whether the sub-rule has been completed.



For more information on activation of Trial Control boxes, see page 10.

END A SUB-RULE

A sub-rule is normally ended when the last repeat (see page 56) is completed. However, it can also be forcibly interrupted in the following cases:

- **When the maximum trial duration has been reached**.
- **When you stop the trial manually**.

- **When a condition passes control to the next box in the sequence**. Consider the following case, where the Reference box is combined with a Condition box through an operator of the Any type.

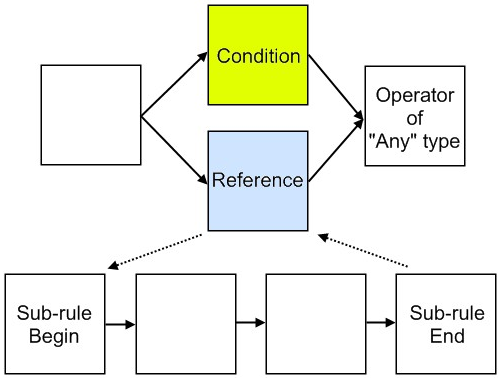


Figure 19 *Example of a sub-rule that is forcibly interrupted when a condition is met.*

When the condition is met, the Any operator applies the OR logic, and passes control to the next box. The sub-rule is interrupted.

- **When the Reference box is placed on a dead end of Trial Control sequence**. Consider the following example:

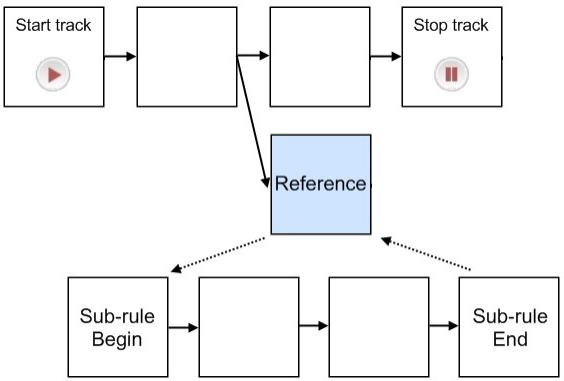


Figure 20 *Example of a sub-rule that is forcibly interrupted when a condition is met.*

The right-hand side of the Reference box is not connected to any box of the Start-Stop trial rule. Therefore, the Reference box poses no limit to control that passes through boxes up to the Stop box.

When the last repeat of the sub-rule is ended, the corresponding Reference box becomes inactive.

See also the note about Endless loops on page 58.

CALL A SUB-RULE FROM ANOTHER SUB-RULE

Within a sub-rule, you can define a reference that calls another sub-rule.

Why insert a sub-rule within another sub-rule?. Suppose that your Trial Control is made of four sub-rules that have at least some instructions in common. For example, sub-rules that start at different times, but include the instructions to have the program make a light flash. In such a case, you can define the instructions for the light flash in a separate sub-rule, and then in each of the four sub-rules insert a reference to this sub-rule.

1. Define the sub-rule that should contain the reference to another sub-rule (see page 54).
2. Define the sub-rule that should be called by the first one, and its own reference (see page 56).
3. Insert the Reference box of the second sub-rule in the sequence of the first sub-rule.

Example of calling a sub-rule from another sub-rule

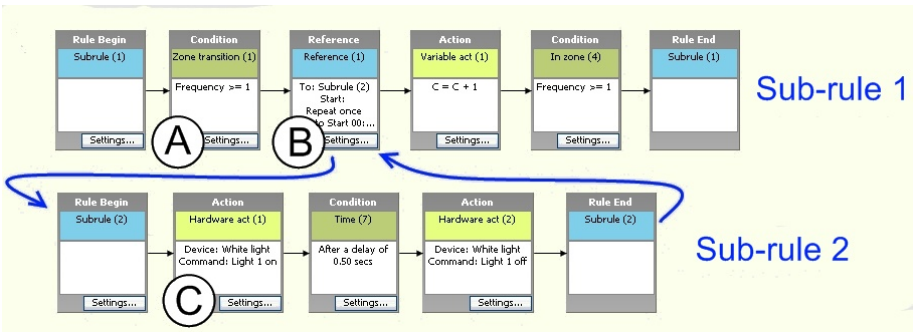


Figure 21 *An example of sub-rule called from another sub-rule. Sub-rule 1 (top) calls Sub-rule 2 (bottom) when the mouse enters the shelter via a particular entry.*
A *- Zone transition condition "Mouse enters the shelter via Entry zone 1" is met (A).*
B *- Reference to Sub-rule 2.* ***C*** *- Action "Light on", followed by a Time condition "0.5 s" and an action "Light off".*

Sub-rule 1 counts the number of times the animal enters the shelter via a particular route. When the animal does so (Condition A in Figure 21), a Reference box (B) calls Sub-rule 2.

Sub-rule 2 is a sub-rule defined to switch a light on for 0.5 seconds (see C for details). As soon as Sub-rule 2 is completed, control is taken back to Sub-rule 1 where the remaining instructions are executed.

Notes

- You can call sub-rules in up to 4 levels of existing sub-rules: for example, you can define the Start-Stop trial rule calling Sub-rule 1, which on its turn calls Sub-rule 2, which on its turn calls Sub-rule 3 etc, but you cannot have Sub-rule 4 calling Sub-rule 5.
  EthoVision XT informs you when you try to create more than 4 levels of sub-rules.
- If a sub-rule includes the reference to another sub-rule, it cannot be completed until the sub-rule that is called within it has been completed.
- You cannot create circular references amongst sub-rules. For example:
  - Insert a reference in the sub-rule that this reference calls.
  - In Sub-rule 1, insert a reference to Sub-rule 2, which on its turn contains a reference to Sub-rule 1.
- EthoVision XT informs you when you try to create a circular reference.

6 Conditions

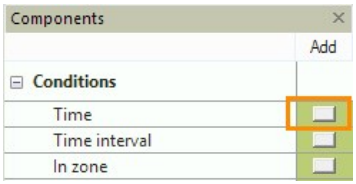
A condition can be based:

- On *Time* (see below) or a *Time interval* (see page 69).
- On a *Trial Control variable* (see page 71).
- On a *Dependent variable* like *Distance moved*, *In zone*, *Mobility*, etc. (see page 72).
- A *combination of dependent variables*, like above a certain velocity in a certain zone (See page 100.
- On a *Manually-scored behavior* (see page 78).
- On a *Hardware device* (see page 79).

CONDITIONS BASED ON TIME

With a Time condition, some time must elapse before the next Trial Control box is evaluated.

1. In the **Components** pane, under **Conditions**, double-click **Time** or click the button next to it.



   The Time condition window opens.

2. Under **Name**, enter a **Condition Name**.
3. Select one of the following options:
   - **After**. To have an action been taken some time after the condition becomes active. For the meaning of condition becoming active, see page 11.
     EXAMPLE Start tracking after two seconds.
   - **After a random time**. To have an action been taken with a delay chosen randomly between two time limits. Enter the lower and higher limits and choose the time unit (seconds, minutes or hours). The random value is counted from the moment the condition becomes active.

Choose this option when programming rewards in a variable interval schedule.
EXAMPLE When a random time between 10 and 30 seconds has elapsed (condition), drop a food item (action).

- **At clock time**. To have an action been taken at an absolute time (hh:mm:ss).
  EXAMPLE At 12:00:00 (condition), start tracking (action).
- **Infinite time (condition never met)**. To wait indefinitely (see the note below).

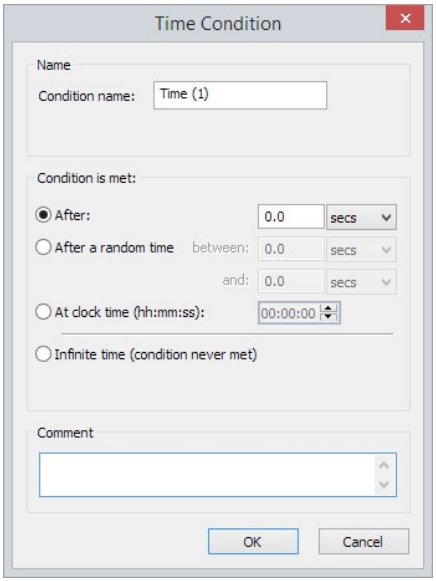


***Figure 22** The* **Time condition** *window.*

4. Insert a **Comment** (optional) and click **OK**.
5. The Condition box appears in the upper-left corner of the Trial Control screen. Insert it in the appropriate position in the Trial Control sequence. For information on how to insert a Trial Control box, see the EthoVision XT Help.

Notes

- If you want to stop a trial after a specific time has been reached, see page 21.
- If you track from video files, see the note **Tracking from video files** under **APPLY TRIAL CONTROL TO YOUR TRIALS** on page 44.
- The format for clock time is hh:mm:ss where: hh = hours, 24-hour format with leading zero, mm = minutes with leading zero, ss = seconds with leading zero.

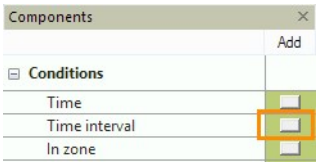
- If you choose clock times, be aware of changeover between wintertime to summertime and vice versa. For example, the time 02:30:00 is encountered twice on the day of onset of wintertime, or never on the day of onset of summertime.
- The **Infinite time** option is handy in the following cases:
  - When you want to stop tracking manually. Place the resulting box immediately to the left of the Stop track box.
  - When two sequences have been placed in parallel, and you want the program to take further actions when Sequence 1 has been completed, independent of Sequence 2. Place a *Time* condition with the **Infinite time** option immediately to the right of Sequence 2. Combine Sequence 1 and the Time box with an Any operator (see page 33).

IMPORTANT If you combine an **Infinite time** condition with an *All* operator, the program does not go further in the Trial Control rule, as it waits forever for the condition to be met!

CONDITIONS BASED ON A TIME INTERVAL

A Time interval condition makes sense when it is combined with another condition. For example: Take an action when the animal is found in Zone A (In zone condition) between 5 and 10 minutes (interval condition).

1. In the **Components** pane, double-click **Time interval** or click the button next to it.



   The **Time interval Condition** window opens (see Figure 23).

2. Under **Name**, enter a **Condition Name**.
3. Condition is met:
   - **Between**. Define an interval between two time points (x and y seconds, minutes or hours). Enter the time values and choose the time units. Note: The time points are counted from the time that the condition box becomes *active*, not from the start of the trial or tracking. For the meaning of *active*, see page 11.
   - **Between clock times**. Define an interval between two absolute time points, e.g., in the previous example, stop the track when the rat is in quadrant A between 12:00:30 and 12:01:00. The time format is hh:mm:ss where hh = hours, 24-hour format with leading zero, mm = minutes with leading zero, ss = seconds with leading zero.
4. Insert a **Comment** (optional) and click **OK**.

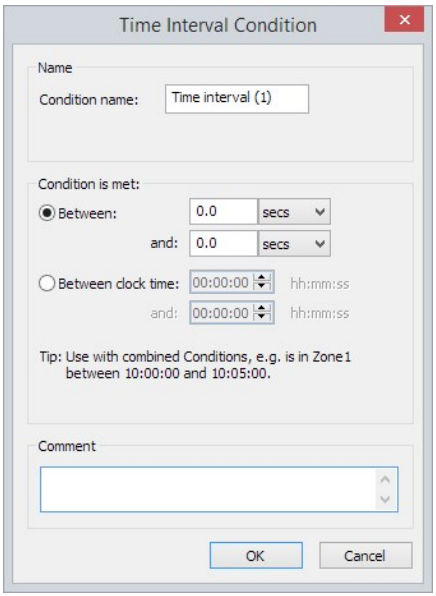


***Figure 23** The* **Time interval condition** *window.*

5. The Condition box appears in the upper-left corner of the Trial Control screen. Insert it in the appropriate position in the Trial Control sequence. For how to do this, see the EthoVision XT Help.

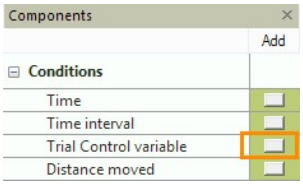
Notes

- **Combining Time interval and Zone conditions**. In a conditioning experiment, the researcher wants to drop a food reward when the animal is found on its shelter some time between 30 s and 60 s from the previous action (for instance, a cue light being switched on). To do this, a Time interval condition must be defined that specifies the interval between 30 s and 60 s. This condition must be met at the same time as the condition "subject on shelter" in order to drop a food pellet. Therefore, the two condition boxes must be placed in parallel with an operator of type All.
- Be aware when using **Time interval** conditions combined with operators or type All. If the animal enters a zone after the upper time limit, the condition is never met, thus Trial Control does not progress.

CONDITIONS BASED ON A TRIAL CONTROL VARIABLE

A condition based on a Trial Control variable is essentially the comparison between a Trial Control variable and a value, another variable or a formula at the time the condition becomes active (for the meaning of becomes active, see page 11).

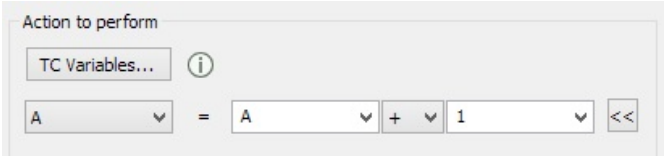
1. In the **Components** pane, under **Conditions**, double-click **Trial Control variable** or click the button next to it.



   TIP If you do not see **Trial Control Variable**, click the **More** button.

   The TC-variable condition window opens.

2. Under **Name**, enter a **Condition Name**.
3. Under **Condition is met when**, select the Trial Control variable from the first list. If the variable has not been defined yet, define it first by clicking **Variables** (see page 28).
4. Select the **comparison operator** (=, >, etc) from the second list.
5. Do one of the following:
   - To compare the Trial Control Variable with another variable, select that variable from the third list.
   - To compare the Trial Control variable with a numerical value, enter this value in the third list.

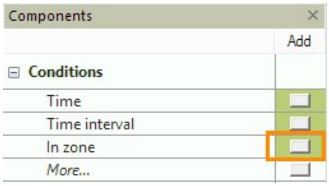


   - To compare a Trial Control variable with a formula, click the double-arrow button and select the operator, then the variable or enter a value to complete the formula.
6. Insert a **Comment** (optional) and click **OK**.
7. Insert the Condition box in the appropriate position in the rule. For information on how to insert a Trial Control box, see the EthoVision XT Help.

IMPORTANT Be careful using the operator "**=**". The comparison only takes place when the condition box becomes active. If the variable is updated in other boxes, then it may be so that this condition box is activated too early or too late. For example, if the condition is met *when A= 4* and an Action box set the value to *A= 5* before the condition box is activated, then the condition is never met. Rather use "**>=**" or "**<=**".

CONDITIONS BASED ON A DEPENDENT VARIABLE

1. In the **Components** pane, under **Conditions**, double-click the name or click the button next to the dependent variable the condition is based on.



   For details about dependent variables, see page 84.

   TIP If you do not see the variable name, click the **More** button.

   NOTE The following variables are not available for creating conditions: Acceleration state, Activity state, behaviors scored with Behavior recognition.

2. In the condition window, under **Name**, enter a **Condition Name**. Under **Comment** (at the bottom) enter a description (optional).

3. Under **Condition is met when**, from the **Statistic** list choose the statistic on which the condition is based. See 'Selecting the Statistic' below.

4. Click the **Settings** button. A window opens with the variable properties.
   - For the corresponding variable, see also page 84.
   - If you track multiple subjects in one arena, see also Selecting the subjects on page 75.

   When finished, click **OK** in the variable window.

5. From the list next to **is:**, choose the comparison **>=**, **=**, or **<=**, to specify whether the statistic must be greater than, equal to, or smaller than a value **x**. Enter this value in the box.

   If you have chosen **Current** as a Statistic (step 3) for a discrete variable, choose between **true** and **false**. *True* means that the current state must be the one chosen in Settings, *False* means that the current state must not be the one chosen in Settings.

6. Click **OK**. Insert the Condition box in the appropriate position in the rule. For information on how to do this, see the EthoVision XT Help.

The Body Points tab in the variable properties window is only available if your experiment is set to **Nose-point, center-point and tail-base detection**.

Select the statistic

- **Current**. The value of the variable in the current sample.

  For example, if a condition based on Velocity is evaluated at sample *n*, Current is the velocity for sample *n*.

  If you rather want to base the condition on the average velocity from the time of activation of the condition to the current time, choose **Mean**.

The remaining statistics below are based on the values of the variable from the time the condition becomes active (see page 11 for what active means).

- **Total**. The total value of the variable (only for continuous variables like Distance or Velocity). This is the sum of all values of the variable up to that time.
- **Mean**. The mean value of the variable (only for continuous variables like Distance or Velocity) up to that time.
- Statistics like **Minimum**, **Maximum**, **Standard error**, **Standard deviation** and **Variance** are normally less obvious to use in a condition. For example, if you select Standard error for Distance moved, the program calculates the standard error of all distance values calculated between consecutive samples of the track up to that time. The condition is met when the standard error is greater than/equal to/less than the value specified.
- **Frequency**. The number of occurrences up to that time. This statistic is available for discrete (state) variables like Movement, and count variables like Rotation and Zone transition.

  Choosing >= or <= for frequency only makes sense when you combine the condition with another condition.
- **Cumulative duration**. The cumulative duration of a state from the start of tracking up to that time. You choose the state when clicking the Settings button. This statistic is available for discrete variables.
- **Current duration**. The duration the animal has been in that state (for example, in a specific zone or *Mobile*) taking only the current (uninterrupted) interval into account. You choose the state when clicking the Settings button. This statistic is available for discrete variables.
- **Latency to first**. The time from the activation of the condition to the first occurrence of the state of the discrete variable (for example *In the zone* becomes true for the variable *In zone*), or count variables like Rotation, or Zone transition. You choose the state when clicking the Settings button.

A condition like *Latency* >= *L* or *Latency* <= *L* does not mean that the condition is met at time L if the first state, or occurrence does not occur within that time. The program first calculates when the first state or occurrence occurs, and then compares this with L.

The following cases are an example of Latency to first *In zone* with L > 0 (see Figure 24, left). Once the condition becomes active, latency can get only one value, which is the time of the first *In zone* state:

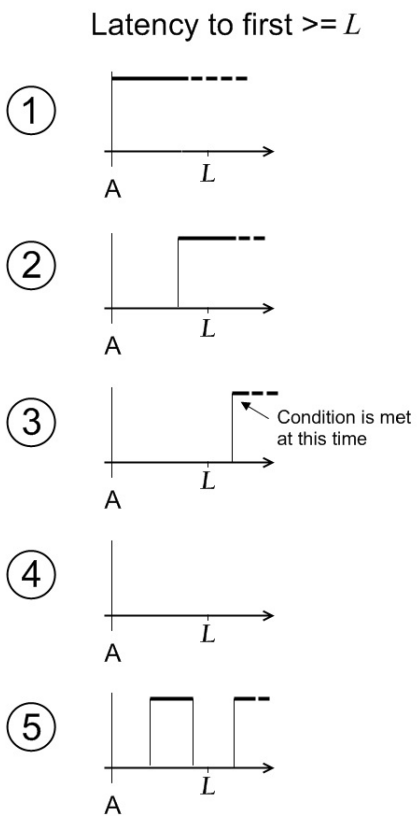


Figure 24 *Five possible cases of outcome of a condition based on Latency. This example refers to a state variable like "In zone", which has two possible states, In zone and Not in zone.*
The condition is met when Latency to first In zone >=L (where L is the value set).
A - Time of activation of the condition (see the picture on page 9 for the concept of activation).
L - Latency set in the condition window.

- **Case 1**. The subject is already in the zone when the condition becomes active. The condition is NEVER met because latency of the first *In zone* is 0, thus smaller than L.
- **Case 2**. The subject enters the zone after the condition becomes active, but at a time < L. The condition is NEVER met because the latency is smaller than L.
- **Case 3**. The subject enters the zone after time L. This means that the latency of the first *In zone* is greater than L. The condition is met at that time.
- **Case 4**. The subject never enters the zone. The condition is NEVER met.

- **Case 5**. The subject enters the zone at a time < L, then exits the zone, then enters again at a time > L. The latency of the first *In zone* state is < L, thus the condition is NEVER met, no matter of how many times the animal enters the zone.

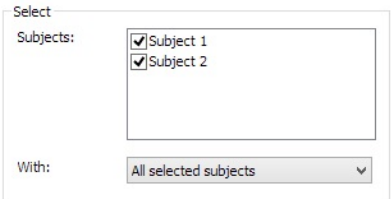
As you can see there are many cases in which the condition is never met, including Case 5 which is due to the fact that only the first instance of *In zone* is taken under consideration.

SUBJECTS IN A CONDITION

Select the subjects

1. In the **Condition** window, click the **Settings** button. In the variables properties window, click the **Actors** tab. In the **Actors** tab, you select the actors of the behavior.
2. Under **Select**, select the subject you want to base your condition on. If your condition is based on social interactions, select the focal subject in the **Actors** tab and the other subjects in the **Receiver** tab. Do so also for dyadic interactions (Subject 1 under Actors, Subject 2 under Receivers),

   For dependent variables with discrete values like In zone, Movement, Mobility, Elongation, Proximity, Body contact and Relative Movement, you can base your condition on the state of multiple subjects at the time the condition is evaluated.



   If you select two or more subjects, select one of the two options from the list immediately below the **Select** box:
   - **All selected Subjects**. The state selected in the dependent variable properties window must be the same for all the actors simultaneously, in order for the condition to be met.
   - **Any selected Subjects**. The state selected in the dependent variable properties window must be true for at least one actor in order for the condition to be met.
3. If the Actors tab also contains the **Body points** options, select one or more body points of the subjects selected above.

4. For the dependent variables of social interaction, a **Receivers** tab is also available. Click this tab and select the subjects that you have not selected under **Actors** (see an example below).

5. Click **OK** to confirm your selection. You return to the condition window (see page 72).

Notes

- Example of choosing **All selected Subjects**. The researcher wants to define a In zone condition that is met when the subjects Subject 1 and Subject 2 have been in a zone simultaneously for at least one minute.

  Solution: In the Condition window, select Cumulative duration as Statistic and enter >= 60 s. Click Settings and select the zone you require, then click Actors and select Subject 1 and Subject 2, finally select All selected Subjects.

- Example of using **Any selected Subjects**. The researcher wants to define a Distance to zone condition that is met when one of the two subjects is at less than 5 cm from a novel object.

  Solution: In the **Condition** window, select **Current** and enter <= *5 cm*. Click **Settings** and select the novel object zone, then click **Actors** and select **Subject 1** and **Subject 2**, finally select **Any selected Subjects**.

- Example with **Actors** and **Receivers**. The researcher wants to define a condition that is met when the nose point of Subject 1 has been close to at least one body point of Subject 2 for more than one minute.

  Solution: Click the button next to **Proximity** and in the **Condition** window, select **Cumulative duration** and enter **f**. Click **Settings**, then:
  - In the **Actors** tab, select **Subject 1** and de-select **Subject 2**. Under **Bodypoints**, select **Nose-point** only.
  - In the **Receivers** tab, select **Subject 2** and de-select **Subject 1**. Under **Bodypoints**, select **all points** and then **Any selected points**, since the condition must be met when Subject 1 is in proximity of at least one of the Subject 2's body points.

WHEN IS A CONDITION MET?

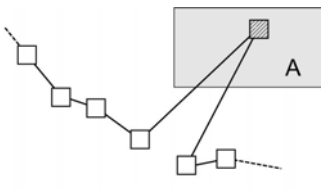
Dependent variables are calculated for each sample. When a condition based on dependent variables is met, depends on a number of factors.

Effect of outliers and track smoothing

Dependent variables used in conditions are not smoothed or filtered using the Lowess filter or the Minimum distance moved as they can be for data analysis (see **Smooth the tracks** in

the EthoVision XT Help). This means that outliers, nose-tail swaps or even subject identity swaps can have a severe effects on whether and when a condition is met.

Consider the following track, where EthoVision detects a reflection within the zone A as the subject. As a result, the track has an outlier.



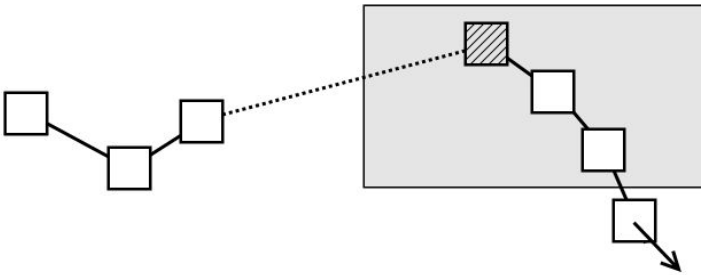
If an In Zone condition is defined that specifies *Frequency of In zone A >=1*, then the condition is met at that time (assuming that the condition box was active). Smoothing would remove the outlier, but that can only be done when analyzing the data, not when Trial Control is active (that is during acquisition).

If such an effect occurs, Track noise reduction may solve the problem. This is applying the Lowess filter during acquisition. In the **Detection settings**, click **Advanced** and under **Smoothing** set **Track noise reduction** to **On**. This smooths the track during acquisition, which means that the track is altered, which cannot be reversed afterwards.

Effect of missing samples

Missing samples can also make Trial Control to react later. For example, an In zone condition is met only when valid samples are found within the specified zone.

Consider the following track, where EthoVision misses the subject some time before it enters the zone A. The dotted line represents a number of missing samples; the hatched square is the first valid (non-missing) sample, found in the zone).



- If an *In zone* condition is defined that specifies *Current of In zone A = true*, then the condition is met later than it would be if there were no missing samples. Interpolating

would remove the missing samples, but that can only be done when analyzing the data, not when Trial Control is active (that is during acquisition).

- If an *In zone* condition states "Cumulative duration of *not in zone* for more than 1 s" (here we assume that a sample rate of 5/s is used, so 5 samples make up 1 second), then the condition does not become true, because there are only 4 samples for which the state "not in zone" was true (see the 4 samples outside the zone).
- Similarly, the condition "Current value of In zone A = false" may become true during the state "not in zone". However, it won't become true in the period of missing samples.

Below you find information on each dependent variable. We assume that the Condition window is open on your screen (step 4 on page 72).

Effect of the statistics used

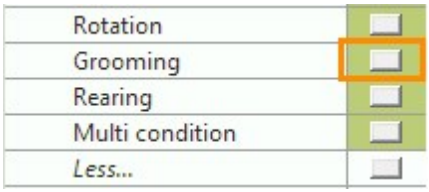
When a condition is evaluated (and therefore may or may not be met) also depends on the statistic chosen.

- For **Current**, the condition is evaluated at the same sample time as when it is activated (for the meaning of "active", see page 11).
- For all other statistics (like **Frequency**, **Current duration**, etc.), it is evaluated at least one sample after the condition is activated. This depends on when the first value available for comparison occurs after the activation of the condition.
  - When using Velocity, and Distance, it takes one sample.
  - When using Heading and In zone, it takes two samples.
  - When using Turn Angle and Angular velocity, it takes three samples.

CONDITIONS BASED ON BEHAVIORS SCORED MANUALLY

Use this kind of conditions to let EthoVision XT carry out an action when the subject performed a behavior you scored manually. For example, in a social interaction test, to stop tracking when the subject attacked three times, or when the duration of *Intimidation* lasted more than 30 seconds.

1. In the **Components** pane, under **Conditions**, double-click the name or click the button next to the manually scored behavior you want to base the condition on.



TIP If you do not see the variable name, click the **More** button.

2. The **Manual Scoring Condition** window opens. Under **Name**, enter a **Condition Name**. Under **Comment** (at the bottom) enter a description (optional).
3. Under **Condition is met when**, click the **Settings** button. If the behavior is of the type Start-Stop or Mutually Exclusive, choose the behavior to base the condition on, for example *Rear* or *Not Rear*.
4. In the **Actors** tab, select the Subjects to which the condition applies. See **SUBJECTS IN A CONDITION** on page 75. If the Actors tab also contains the **Body points** options, select one or more body points of the subjects selected above. Click **OK**.
5. Under **Condition is met when**, select the **Statistic** you want to use.

Common cases of Statistic for *manually scored behaviors*:

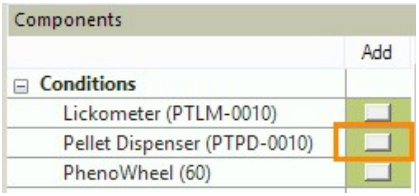
- **Current**. For a condition that is met when the behavior at the current sample is true or not true. This statistic is available for Start-Stop and Mutually exclusive events.
- **Frequency**. For a condition that is met when the behavior occurred a number of times.
- Cumulative duration. For a condition that is met when the animal has shown the behavior for a specific time since the activation of the condition. This statistic is available for Start-Stop and Mutually exclusive events.
- Current duration. For a condition that is met when the animal has shown the behavior continuously for a specific time. This statistic is available for Start-Stop and Mutually exclusive events.
- Latency to first. See page 73 and Figure 24 on page 74.

For more information on *manually scored behaviors*, see the EthoVision XT Help.

CONDITIONS BASED ON HARDWARE DEVICES

Use this kind of conditions to check the status of hardware devices (pellet dispensers, lickometers, etc.) and have EthoVision take actions when the conditions are met.

1. In the **Components** pane, under **Conditions**, click the button next to the hardware device you want to base the condition on.



2. The Hardware condition window appears. Under **Name**, enter a **Condition Name**. Under **Comment** (at the bottom) enter a description (optional).
3. Under **Condition is met when**, select the **Template device** you want to use. A template device represents a group of devices of the same type, one for each arena. For more information, see page 104.
4. Under **Signal to check**, select the criterion that forms the condition.
5. Under **Signal value**, select the value of the criterion in order for the condition to be met.
6. Click **OK**. Insert the Condition box in the appropriate position in the rule. For information on how to do this, see the EthoVision XT Help.

Hardware devices are not defined for each arena separately. If you have a setup with multiple arenas, it is assumed that each arena has the same type of device. The name in the device list is the generic name valid for all arenas. The condition will be evaluated for each arena separately.

If the device type is not listed under **Conditions**, it means that it is not yet recognized by EthoVision XT. See also page 103.

Pellet dispenser

- **Number of drops**. To base your condition on whether more/less than N food items have been delivered since the activation of the condition. Choose >= or <= and enter the value N in the box.
- **In error state**. To base your condition on whether the pellet dispenser is in error state, that is, when it can no longer drop any pellet (this happens when there are no pellets anymore in the feeder). Choose between true (error) or false.

  EXAMPLE Have EthoVision XT give a sound signal when the feeder has run out of pellets.

Lickometer

- **Duration of licks**. To base your condition on the (total) duration of licks since the activation of the condition. Enter this time in the box.
- **Number of licks**. To base your condition on the number of licks since the activation of the condition. You can select >= N, <= N or = N.

Top Unit of PhenoTyper

- Choose the option under **Signal to check** to base your condition on whether a sound or light signal is given since the activation of the condition.
- **For NeuroBsik Top Units: Is Fsel-1/2 on/off, Is Vol-1/2 on/off**. To base your condition on the value of frequency or volume specified by one input. Since the frequency and volume are specified by two inputs, you must define two conditions, one for input 1 and one for input 2. Combine the two conditions with an operator of type All.

  Neuro-Bsik is a consortium of eleven Dutch Neuroscience research groups and two companies including Noldus. The consortium aims to contribute to the battle against brain disorders by developing novel mouse models for brain disorders. For this project, Noldus has developed a special Top Unit of the PhenoTyper cage that can produce ultrasound stimuli. For more information, browse to www.neurobsik.nl.

For more information on PhenoTyper, see the Reference Manual - PhenoTyper.

TTL Port Tester

- **Button 1/2 presses**. To base your condition on the number of presses of button 1 or 2 given since the activation of the condition. You can select either >= N, <= N, or = N. Enter the value N in the box.
- **Is button 1/2 pressed?**. To base your condition on whether the button 1 or 2 is currently pressed. Select true or false from the list below.
- **Is light 1/2 on?**. To base your condition on whether the light 1 or 2 is currently on. Select true or false from the list below.

Beam controller

- **Beam 1/2 breaks**.To base your condition on the number of beam breaks for beam 1 or 2 since the activation of the condition. You can select either **>= N**,**<= N**, or **=N**. Enter the value N in the box.
- **Is Beam 1/2 broken?**. To base your condition on whether beam 1 or 2 is currently broken. Select **True** or **False** from the **Signal value** list.
- **Is cue 1/2 on?**. To base your condition on whether the cue 1 or 2 is currently on. Select true or false from the **Signal value** list.

Activity wheel counter

- **Quarter cycles**.To base your condition on the number of quarter cycles since the activation of the condition. You can select either **>= N**,**<= N**, or **=N**. Enter the value N in the box.

Custom hardware device

This includes the illuminated shelter.

- **Input 1/2 L/H -> H/L count**. To base your condition on how many times there has been a transition from High to Low (or vice versa) in input 1 or 2 during the time t-1 to t, where t is the sample time when the condition is evaluated. Choose the option that applies, select either >= N or <= N. Enter the value N in the box.

  This option let you create a condition based on events (transitions) in the signal, no matter when they happen during the sample time. See the note below.

- **Is input 1/2 High/Low?**. To base your condition on whether the current state of input 1 or 2 is High or Low at the sample time when the condition is evaluated. Select the option that applies, and then either true or false.

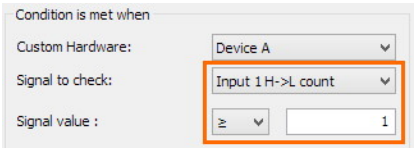
- **Is output 1/2 High?**. To base your condition on whether the current state of output 1 or 2 is High at the sample time when the condition is evaluated. Select the option that applies, and then either true or false.

  This option evaluates the state of the signal (High vs Low) at the sample time when the condition is evaluated. If a quick transition High -> Low -> High occurs within the sample interval, this is not noticed; the state at the sample time is still High. See the note below.

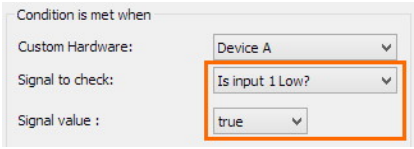
Counts vs. signals: effects on conditions

Consider the two conditions based on the same input (for example Input 1):

- Is the number of transitions **H->L ffh**?



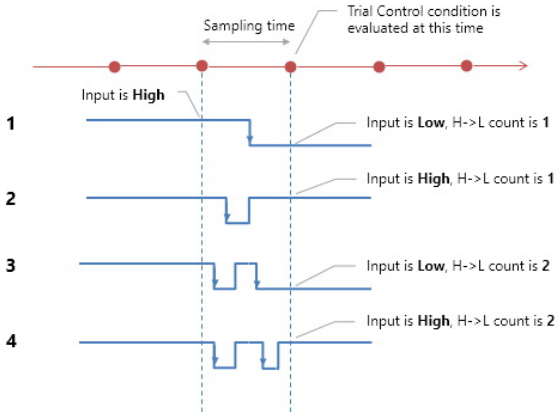
- Is the state of the signal **Low?**



There is a fundamental difference between the two conditions.

- In the first condition, the software counts the number of transitions “Hight” to “Low” *during the sampling interval* (that is, from t-1 to t). The condition may become true or stay false for sample t, depending on that value.

- In the second condition, the signal is evaluated at time t, that is, *at the end of the sampling interval*. If short transitions occur from "High" to "Low" to "High" again, the "Low" signal is not detected by this condition, because the signal is still high at time t. Consider the cases 2 and 4 in the figure below.



	Condition: **Is Input Low?**	Condition: **H>L Count ≥ 1?**
1	True	True
2	False	True
3	True	True
4	False	True

Figure 25 *An example of how two conditions which may seem equivalent give different results. The thick line represents the Input signal from a hardware device (a similar example could be made with Output signals). The red dots represents samples in EthoVision XT. Four cases are presented.* **1**. *The signal changes from High to Low. The two conditions (see text) are both true at the time of evaluation.* **2**. *The signal changes from High to Low to High within the sample time. The instance of "Low" is not detected by the condition based on the signal ("Is Input Low?"), because the signal value at the time of evaluation is High.* **3**. *Similar to case 1.* **4**. *Similar to case 2.*

7 More about conditions based on dependent variables

In this section you find details and examples of the statistics you can use for your condition (step 3 on page 72).

For more information on a dependent variable, see the EthoVision XT Help.

DISTANCE MOVED

Frequently-used statistics

- **Current (default)**. To base your condition on the distance between the previous sample and the current one. This value depends on the chosen sample rate. Use Current velocity instead.
- **Total**. To base your condition on the distance traveled from the moment the condition becomes active up to that point.
- **Mean**. To base your condition on the average distance traveled from one sample to the next calculated from the activation of the condition box up to that time. This depends on the sample rate. Use Mean velocity instead.
- **Effect of hidden zones**. When the animal is in a hidden zone, the distance moved for the first sample in the hidden zone is based on the distance between the last location where the animal was detected and the center of the hidden zone. This is typically a relatively large distance, which can result in the condition becoming true.
- **Effect of outliers (reflections)**. Detection of reflections may result in a too large distance moved and hence the condition being met.

VELOCITY

In the **Condition** window, click the **Settings** button. Choose the body point on which calculation of velocity is based, and the **Averaging interval**. For details, see **Velocity** in the EthoVision XT Help.

Frequently-used statistics

- **Current (default)**. For a condition that is met when the velocity at the current sample is <=/>= a specified value.
- **Total**. This does not have a physical meaning and depends on the sample rate. Choose Mean instead.
- **Mean**. For a condition that is met when the mean velocity since the activation of the condition is <=/>= a specified value.

Notes

- **Effect of hidden zones**. When the animal is in a hidden zone, the Velocity for the first sample in the hidden zone is based on the distance between the last location where the animal was detected and the center of the hidden zone. This is typically a relatively large velocity, which can result in the condition becoming true.
- **Effect of jitter**. When the animal moves slowly, jitter plays a bigger role than at higher speeds. This can make velocity exceed the threshold set in the condition.
- **Effect of outliers (reflections)**. Detection of reflections may result in a too high velocity and hence the condition being met. To prevent this from happening, increase the Averaging interval in the settings.

MOVEMENT

In the **Condition** window, click the **Settings** button. In the **Movement** tab, specify which threshold values of velocity specify the states *Moving* and *Not moving*. Optionally, choose an **Averaging interval** to smooth out random changes in velocity that do not reflect real movement.

Frequently-used statistics

- **Current**. For a condition that is met when the animal's current state is either true (Moving) or false (Not moving).
- **Frequency (default)**. For a condition that is met when the animal has moved/not moved a number of times.
- **Cumulative duration**. For a condition that is met when the animal has moved/not moved for a specific time since the activation of the condition.

  EXAMPLE A Movement condition is defined to stop tracking after 10 minutes of movement. If the animal moves during the first 2 minutes, stops moving for 3 minutes

and then moves again without interruption. The track is stopped 13 minutes after the start.

- **Current duration**. For a condition that is met when the animal has moved/not moved continuously for a specific time. In the same example as above, the condition would be: stop tracking when the current duration of movement is 10 minutes. Tracking would stop when the animal had moved continuously for 10 minutes, that is 15 minutes after the start.
- **Latency to first**. See page 73 and Figure 24 on page 74.

ACCELERATION

In the **Condition** window, click the **Settings** button and choose the options you require.

Frequently-used statistics

- **Current (default)**. To base your condition on the current value of acceleration.
- **Total**. To base your condition on the total acceleration from the moment the condition becomes active up to that point. Note that this value does not have a physical meaning and depends on the sample rate. Choose **Mean** instead.
- **Mean**. To base your condition on the average acceleration. The average is calculated from the activation of the condition box up to that time.

Note that *Acceleration state* is not available as condition criterion.

IN ZONE

In the **Condition** window, click the **Settings** button. Under **In the following zones**, select the zones of your choice.

Under **From following body points**, select the body points of the animal that determine whether or not the animal is considered to be within a zone.

Under Threshold, enter a **Zone exit threshold** to avoid that small movement of the body points across zone borders result in unrealistic scores of zone entries. Such movements are usually caused by body-point jitter, or the behavior of the subject (stretching, curling etc.).

Frequently-used statistics

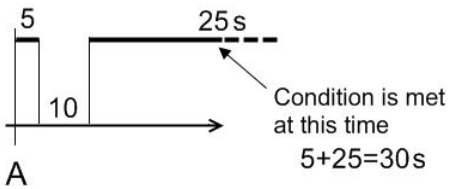
- **Current**. To base your condition on the presence of the animal in a zone at that time: either true (In zone) or false (Not in zone).

- **Frequency (default)**. To base your condition on the number of times the animal has been in the zone.

  IMPORTANT The frequency of In zone becomes high very easily when the animal walks along the edge of a zone. This may result in the condition being met sooner than expected. If this happens, increase the Zone exit threshold. If that does not help, open the **Detection settings** and click **Advanced**, then under **Smoothing** set **Track noise reduction** to **On**. This smooths the track during acquisition, which means that the track is altered, which cannot be reversed afterwards.

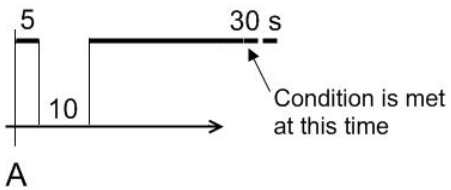
- **Cumulative duration**. To base your condition on the cumulative duration the animal has been in the zone up to that time since the activation of the condition.

  EXAMPLE In a Novel Object Recognition test, stop tracking when the animal has been in the zone with the novel object for 30 seconds. After the activation of the condition, the animal spends the first 5 seconds in the zone with the novel object, then 10 seconds in the zone with the familiar object and 45 seconds in the zone with the novel object, the track is stopped 40 seconds after the activation of the condition (indicated by A).



- **Current duration**. To base your condition on the time the animal has been in the zone without interruption up to the current time.

  If in the example above, the condition had been defined as: stop tracking when the current duration in the zone is 30 seconds, the track would have been stopped 45 seconds after the activation of the condition.



- **Latency to first** - See page 73 and Figure 24 on page 74.

DISTANCE TO ZONE

In the **Condition** window, click the **Settings** button. Under **To the following zones**, select the zones of your choice. Select **Include if in zone** if you want to calculate the distance to the border of a zone, regardless of whether the subject is outside or inside the zone. Under **From the following body points**, select the body point of the animal you want to use to calculate the distance.

Frequently-used statistics

- **Current (default)**. To base your condition on the current distance of the animal's body point to a zone.

  EXAMPLE Switch on a light when the current distance from the entrance of the shelter is less than 5 cm.

- **Total**. This depends on the sample rate. Choose Mean instead.
- **Mean**. To base your condition on the average distance of the animal's body point to a zone measured since the activation of the condition.

  EXAMPLE Stop the track when the average distance from the zone with the novel object is smaller than or equal to 10 cm.

DISTANCE TO POINT

In the **Condition** window, click the **Settings** button:. Under **To the following points**, select the points or center of zones of your choice. If you select two or more points, the condition is based on the shorter distance between those points and the animal's body point. Under **From the following body points**, select the body point of the animal you want to use to calculate the distance.

Frequently-used statistics

- **Current (default)**. To base your condition on the current distance of the subject from the point.
- **Total**. This has little physical significance. Furthermore, its value depends on the sample rate (the higher the sample rate, the greater the cumulative distance). Choose Mean instead.
- **Mean**. To base your condition on the mean distance from the point since the activation of the condition.

MEANDER

In the **Condition** window, click the **Settings** button. In the **Meander** tab, select whether you want the condition to be based on the **Absolute (unsigned)** or **Relative (signed)** meander. Select Head direction meander when you want to base your condition on the angles formed by the Head direction line, not the body points (these will be ignored). In the **Body points** tab, select the body point on which you want to base the definition of meander.

Frequently-used statistics

- **Current (default)**. To base your condition on the current meander value.
- **Total**. To base the condition on the value of Meander cumulated since the activation of the condition.
- **Mean**. To base your condition on the mean value since the activation of the condition.

TARGET VISITS AND ERRORS

Use *Target visits and errors* to control the trial based on the visits to target zones and errors (visits to non-target zones or target zone revisits).

In the **Condition** window, click the **Settings** button. In the **Target visits and errors** tab, select the zones that are targets and non-target. Under **Condition based on**, select which variable you want to use.

Frequently-used statistics

- **Frequency (default)**. To base your condition on the occurrence/number of successes and errors since the activation of the condition.

  EXAMPLE Take an action as soon as the animal has made three errors entering three different non-target zones. From the **Statistics** list, select **Frequency** fff3. Click **Settings**, select the non-target zones and then under **Condition based on**, select **Non-target first visits**.

  Note — When using *first* visits (target or non-target) in the condition, make sure that you enter a frequency less or equal to the total number of corresponding zones selected under **Settings**. For example, if you select **Condition based on Target first visits** and **Frequency** fff3, make sure that you select at least three target zones under **Settings**.
- **Latency to First**. See page 73 and Figure 24 on page 74.

ZONE ALTERNATION

Use *Zone alternation* to create a condition based on alternation between specific zones. For example: stop tracking when the animal makes the ten alternations (A > B > C).

In the **Condition** window, click the **Settings** button. In the **Zone Alternation** tab, select the zones of the alternation (for example A, B, C). Under **Condition based on**, select which variable you want to use. Select whether you want to consider only direct transitions. In the **Body points** tab, select which body points define the transition from one zone to the other.

Frequently-used statistics

- **Frequency (default)**. To base your condition on the occurrence/number of alternations or direct/indirect revisits in zones since the activation of the condition.

  EXAMPLE Take an action as soon as the animal has completed the tenth alternation. Click **Settings**, select the zones and then under **Condition based on**, select **Alternations**. From the **Statistics** list, select **Frequency** ffiio.

- **Latency to First**. See page 73 and Figure 24 on page 74.

ZONE TRANSITION

In the **Condition** window, click the **Settings** button. In the **Zone Transition** tab, click **Add** to define the zone sequence (for example A > B > C). Select whether you want to consider only direct transitions. Enter a **Zone exit threshold** to avoid that small movement of the body points across zone borders result in unrealistic scores of zone transitions. Such movements are usually caused by body-point jitter, or the behavior of the subject (stretching, curling etc.).

In the **Body points** tab, select the body point that moves from one zone to the other.

Frequently-used statistics

- **Frequency (default)**. To base your condition on the occurrence/number of transitions between zones since the activation of the condition.

  EXAMPLE Take an action as soon as the animal has gone from the Middle zone to the Left Arm of a T maze five times (Frequency=5).

  IMPORTANT The frequency of **Zone transition** can become high very easily when the animal walks along the boundary between zones. This may result in the condition being met sooner than expected. If that happens, increase the Zone exit threshold. If this does not help, open the **Detection settings** and click **Advanced**, then under **Smoothing** set **Track noise reduction** to **On**. This smooths the track.

- **Latency to First**.

  EXAMPLE Take an action when the latency of the first transition from Zone A to Zone B is longer than 1 minute.

  See also page 73 and in the figure on page 74 replace the state **In zone** with a zone transition event.

HEADING

Use *Heading* for a condition based on compass-like orientation, provided that the axis orientation in the Arena Settings has some biological meaning.

See also the notes for *Head direction* on page 92.

In the **Condition** window, click the **Settings** button. In the **Body points** tab, select the body point that you want to use to calculate heading.

Frequently-used statistics

- **Current (default)**. To base your condition on the current heading value.
- **Mean**. To base your condition on the mean value since the activation of the condition.

HEADING TO POINT

In the **Condition** window, click the **Settings** button. Under **Select**, select the point (or zone center) that your condition is based on. Under **For the following body points**, select the body point for which heading is calculated.

Frequently-used statistics

- **Current (default)**. To base your condition on the current meander value.
- **Mean**. To base your condition on the mean value since the activation of the condition.

EXAMPLE Take an action when the animal's Heading to the Entry zone is less than 5 degrees. Select **Current** as Statistic, <= *5*°. Click **Settings** and specify **Entry zone (center)**.

To restrict the condition to when the animal is moving, create a condition based on Movement, and combine the two conditions with an operator of type All.

Note that Heading is about movement. If you want to create a condition based on the animal's head orientation, use Head directed to zone (see below).

HEAD DIRECTION

Use *Head direction* for a condition based on compass-like orientation. Since Head direction depends on the orientation of the axes in the Arena Settings, Head direction make sense if the axis orientation has biological significance.

If the animal must point to a zone or point, use Head directed to zone.

Frequently-used statistics

- **Current (default)**. To base your condition on the current value of head direction.
- **Mean**. To base your condition on the average head direction angle up to that time.

We advise you to specify a range of Head direction, using two conditions (one specifying >=, the other <=). Then, combine the conditions with an operator of type All.

EXAMPLE If the x axis points to North, then to make a condition that is met when Head direction is between NE and NW, combine:

- A condition that states Current Head direction <= +90°.
- A condition that states Current Head direction >= -90°.

HEAD DIRECTED TO ZONE

In the **Condition** window, click the **Settings** button. Under **Zone of interest**, select the zone or point that your condition is based on. Under **Calculate when**, specify to calculate the variable only when the animal (or one of its body points) is in a specific zone.

We advise you to define a zone around the focal zone/point, so the variable is calculated only when the animal is near the focal zone.

Frequently-used statistics

- **Frequency (default)**. To base your condition on the number of times the animal's head points to a zone (or point).
- **Current**. To base your condition on whether the animal's head points to a zone (or point).

  EXAMPLE Take an action when the animal's head is directed to the novel object.

  By selecting **Current**, the condition will become true as soon as the animal's head points to a zone for an instant. If you want to make the condition become true when the animal's head points to a zone for some time, use **Current duration** instead.
- **Cumulative duration**. To base your condition on the cumulative time that the animal points to a zone or point since the activation of the condition.

- **Current Duration**. To base your condition on the time that the animal's head has been pointing to the zone without interruption up to the current time. For comparison, see the same for In zone (page 87).

  EXAMPLE Take an action when the animal's head is directed to the novel object for five seconds.

- **Latency to first**. See page 73 and Figure 24 on page 74.

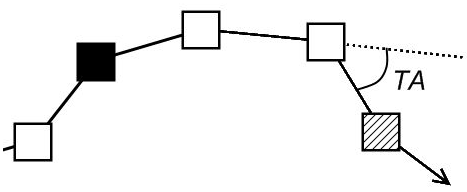
TURN ANGLE

In the **Condition** window, click the **Settings** button. In the **Turn Angle** tab, specify whether the turn is **Absolute (unsigned)** or **Relative (signed)**. Select whether you want to calculate the **Head direction turn angle** (body points will be ignored). In the **Body points** tab, select the body point that you want to use to calculate Turn angle.

Frequently-used statistics

- **Current (default)**. To base your condition on the current value.
- **Total**. To base your condition on the cumulative value (whether absolute or relative) since the activation of the condition.
- **Mean** - To base your condition on the mean value (whether absolute or relative) since the activation of the condition.

Because the way turn angle is defined, the first value available for comparison occurs at the third sample (hatched square) after the condition has become active (black square).



ANGULAR VELOCITY

In the **Condition** window, click the **Settings** button:. In the **Angular velocity** tab, specify whether the turn is **Absolute (unsigned)** or **Relative (signed)**. Select whether you want to calculate the **Head direction angular velocity** (body points will be ignored). In the **Body points** tab, select the body point that you want to use to calculate **Angular velocity**.

Frequently-used statistics

See Turn angle above.

Because Angular velocity is based on Turn angle, the first value available for comparison occurs at the third sample after the activation of the condition (see above).

ACTIVITY

With *Activity* you can create a condition based on the change in pixels in the whole arena (thus not only in the detected body area) between samples. When properly set, this pixel change is supposed to be a measure for the subject's activity (for example, it is low when the subject freezes).

Frequently-used statistics

- **Current** - The percentage pixel change in the arena relative to the previous sample.
- **Total** - The total percentage pixel change in the arena, from the moment the box becomes active (see Figure 3 on page 11) onwards.
- **Mean** - The mean percentage pixel change in the arena, from the moment the box becomes active (see Figure 3 on page 11) onwards.

Set the **Threshold** in the field next to **Is:**. Click the **Settings** tab and specify the **Averaging interval**.

IMPORTANT If you have inserted **Condition** boxes based on **Activity** in your Trial Control rule and then deselect **Activity analysis** in the **Experiment settings**, your rule becomes invalid. The **Condition** boxes based on **Activity** are removed from your sequence and the connecting arrows are removed. Redesign your Trial Control rule and connect the arrows between the boxes.

Note that *Activity state* is not available as condition criterion.

BODY ELONGATION

In the **Condition** window, click **Settings** and specify the **Averaging interval**

Frequently-used statistics

- **Current**. To base your condition on the current value. For example, the condition is true when *Body elongation* < 50%.
- **Total**. This does not have a physical meaning and depends on the sample rate. In most cases you will need the statistic **Current** instead.

- **Mean**. For a condition that is met when the mean Body elongation since the activation of the condition is <=/>= a specified value. Like **Total**, this statistic does not have a "physical" meaning. In most cases you will need the statistic **Current** instead.

BODY ELONGATION STATE

In the **Condition** window, click the **Settings** button. Specify the **Thresholds** and the **Averaging interval** that define the three Elongation states. Under **Condition based on**, select the state you want to base the condition on: **Stretched**, **Normal**, or **Contracted**.

Frequently-used statistics

- **Current**. To base your condition on the current elongation state.

  EXAMPLE Take an action when the animal's state *Contracted* = True.

- **Frequency**. To base your condition on the number of times the selected elongation state occurred.
- **Current duration**. To base your condition on the duration of the current Elongation state.
- **Cumulative duration**. To base your condition on the cumulative duration of the Elongation state.
- **Latency to first**– See also page 73 and Figure 24 on page 74. To base your condition on the time from the moment the condition becomes active to the first occurrence of the chosen Elongation state.

  EXAMPLE Take an action when the latency of *Stretched* is longer than one minute. The condition is met when the animal is stretched, at least one minute from the moment the condition becomes active.

BODY ANGLE

In the **Condition** window, click **Settings** and select **Absolute angle** (0° - 180° for the body bent either clockwise or counterclockwise) or **Relative angle** (0° - 360°).

Frequently-used statistics

- **Current**. To base your condition on the current value. For example, the condition is true when *Body angle* > 20°.
- **Total**. This does not have a physical meaning and depends on the sample rate. In most cases you will need the statistic **Current** instead.

- **Mean**. For a condition that is met when the mean Body angle since the activation of the condition is <=/>= a specified value. Like **Total**, this statistic does not have a "physical" meaning. In most cases you will need the statistic **Current** instead.

BODY ANGLE STATE

In the **Condition** window, click the **Settings** button. Specify the **Threshold** and the **Averaging interval** that define the four **Body angle** states. Under **Condition based on**, select the state you want to base the condition on: **Straight**, **Bent**, **Bent counterclockwise**, or **Bent clockwise.**

Frequently-used statistics

- **Current**. To base your condition on the current body angle state.

  EXAMPLE To take an action when the animal's current state *Bent counterclockwise* = True.

- **Frequency -** To base your condition on the number of times the selected body angle state occurred.
- **Current duration**. To base your condition on the duration of the current body angle state.
- **Cumulative duration** - To base your condition on the cumulative duration of the body angle state up to that time.
- **Latency to first**– See also page 73 and Figure 24 on page 74. To base your condition on the time from the moment the condition becomes active to the first occurrence of the chosen Body angle state.

  EXAMPLE Take an action when the latency of *Bent* is longer than one minute. The condition is met when the animal is bent, at least one minute from the moment the condition becomes active.

MOBILITY

With *Mobility* you can create a condition based on the change in the detected shape between the previous sample and the next one, expressed in percentage.

Frequently-used statistics

- **Current** - The percentage change in the detected body area in comparison with the previous sample.
- **Total** - The total percentage change in the detected body area, from the moment the box becomes active (see Figure 3 on page 11) onwards.

- **Mean** - The mean percentage change in the detected body area, from the moment the box becomes active (see Figure 3 on page 11) onwards.

Set the **Threshold** in the field next to **Is:**. Click the **Settings** tab and specify the **Averaging interval**.

MOBILITY STATE

In the **Condition** window, click the **Settings** button. Specify the **Thresholds** and the **Averaging interval** that define the three mobility states. Under **Calculate nesting for**, select **Highly mobile**, **Mobile**, or **Immobile**.

Frequently-used statistics

- **Current**. To base your condition on the current mobility state.

  EXAMPLE Take an action when the animal's current state *Immobile* = True.

- **Frequency -** To base your condition on the number of times the selected mobility state occurred.
- **Current duration**. To base your condition on the duration of the current mobility state.
- **Cumulative duration** - To base your condition on the cumulative duration of the selected mobility state up to that time.
- **Latency to first**– See also page 73 and Figure 24 on page 74. To base your condition on the time from the moment the condition becomes active to the first occurrence of the chosen Body angle state.

  EXAMPLE Take an action when the latency of *Highly mobile* is longer than one minute. The condition is met when the animal is highly mobile, at least one minute from the moment the condition becomes active.

ROTATION

In the **Condition** window, click the **Settings** button. In the **Rotation** tab, specify the criteria to define a rotation. Select **Body axis rotation** when you want to base the condition on the rotation of the body axis, rather than that of body points. In the **Body points** tab, select the body point you want to use to calculate rotation.

Frequently-used statistics

- **Frequency -** To base your condition on the number of rotations occurred.

- **Latency to first -T**o base your condition on the time from the moment the condition becomes active to the first rotation. See also page 73 and Figure 24 on page 74.

  EXAMPLE Stop tracking when the first rotation event occurs less than one minute from the moment the condition becomes active.

DISTANCE BETWEEN SUBJECTS

In the **Condition** window, click the **Settings** button. In the **Actors** tab, select the subject and its body point that you want to use to calculate the distance to another subject. In the **Receivers** tab, select the other subject and its relevant body points (see also page 75).

EXAMPLE To make an action when ano-genital sniffing takes place, use a condition box with a minimal distance between the nose point of one subject and the tail base of the other. Select **Current** as Statistic, then click **Settings**. Under **Actors**, select the focal subject and **Nose-point**. Under **Receivers**, select the other subject and **Tail-base**.

PROXIMITY

In the **Condition** window, click the **Settings** button. In the **Proximity** tab, specify the criteria to define proximity. In the **Actors** tab, select the focal subjects and their body points that you want to use to calculate proximity to other subjects (Receivers). In the **Receivers** tab, select the other subjects and their relevant body points (see also page 75).

For dyadic interactions, select Subject 1 under **Actors** and Subject 2 under **Receivers**.

Frequently-used statistics

- **Current**. To base your condition on the current proximity state.

  EXAMPLE Take an action when the nose-point of subject 1 is in proximity of tail-base of subject 2.

- **Frequency -** To base your condition on the number of times the subjects were in proximity of each other.
- **Current duration**. To base your condition on the duration of the current occurrence of In proximity.
- **Cumulative duration** - To base your condition on the cumulative duration of the selected proximity state up to that time.
- **Latency to first**– To base your condition on the time from the moment the condition becomes active to the first occurrence of the chosen proximity state. See also page 73 and Figure 24 on page 74.

EXAMPLE Take an action when the latency of *Nose point of subject 1 is in proximity of Tail base of subject 2* is longer than one minute. The condition is met when the nose point of subject 1 is in proximity of the tail base of subject 2, at least one minute from the moment the condition becomes active.

BODY CONTACT

In the **Condition** window, click the **Settings** button. In the Body Contact tab, specify whether the condition is based on body contact or no contact. In the Actors tab, select the subjects that should be in contact (or not in contact) with any other for the condition to become true. From the Statistics list, choose the statistic to be used and the value that make the condition true. For example, if you want to create a condition that is met when body contact last 10 seconds without interruption, choose Current Duration >=10 s.

Frequently-used statistics

See the statistics for *Proximity* on page 98.

NOTE When you select multiple Subjects in the Actors tab, the condition is evaluated separately for each subject and then combined according to what you select from the **With** list. If you select Subject 1 and Subject 2 in the Actors tab, and select **All selected subjects** from the **With** list, the condition is met when both Subject 1 and Subject 2 are in contact with any subject (thus not necessarily in contact with one another). See also **SUBJECTS IN A CONDITION** on page 75.

RELATIVE MOVEMENT

In the **Condition** window, click the **Settings** button. In the **Relative Movement** tab, specify at which maximum distance the subjects should be considered as 'interacting', and the criteria to define relative movement. In the **Actors** tab, select the focal subjects and their body points that you want to use to calculate relative movement to/from other subjects. In the **Receivers** tab, select the other subjects and their relevant body points (see also page 75).

Frequently-used statistics

See the statistics for *Proximity* on page 98.

NET WEIGHTED MOVEMENT

In the **Condition** window, select **Current** as Statistic, then click **Settings**. In the **Net Weighted Movement** tab, specify at which maximum distance the subjects should be considered as

'interacting'. In the **Actors** tab, select the focal subject and its body point that you want to use to calculate net relative movement to/from another subject. In the **Receivers** tab, select the other subject and its relevant body points (see also page 75).

WEIGHTED MOVEMENT FROM

In the **Condition** window, select **Current** as Statistic, then click **Settings**. In the **Weighted Movement From** tab, specify at which maximum distance the subjects should be considered as 'interacting'. In the **Actors** tab, select the focal subject and its body point that you want to use to calculate weighted movement from another subject. In the **Receivers** tab, select the other subject and its relevant body point (see also page 75).

WEIGHTED MOVEMENT TO

In the **Condition** window, select **Current** as Statistic, then click **Settings**. In the **Weighted Movement To** tab, specify at which maximum distance the subjects should be considered as 'interacting'. In the **Actors** tab, select the focal subject and its body point that you want to use to calculate speed of moving to another subject. In the **Receivers** tab, select the other subject and its relevant body points (see also page 75).

CONDITIONS BASED ON MULTIPLE DEPENDENT VARIABLES

Use multiple conditions to let EthoVision carry out an action when the subject performs a combination of behaviors. For example, to stop tracking when the subject's nose point has been in the novel object zone while having its head directed to the object for 20 seconds.

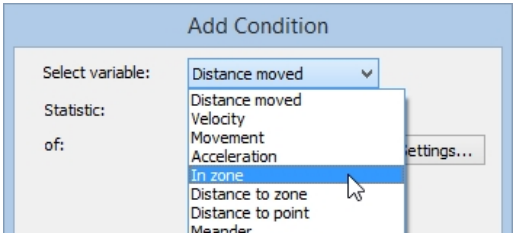
1. In the **Components** pane, under **Conditions**, double-click the name or click the button next to the **Multi condition**.

   TIP If you do not see the variable name, click the **More** button.

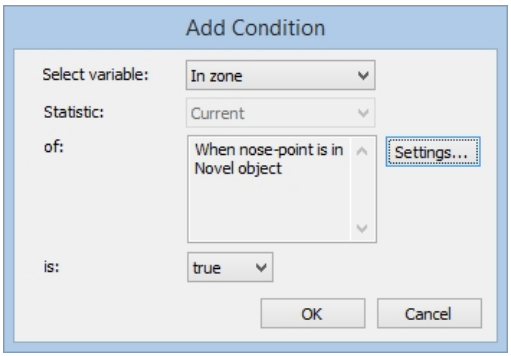
2. The **'Multi Condition' Condition** window opens. Under **Name**, enter a **Condition Name**. Under **Comment** (at the bottom) enter a description (optional).

3. Click the **Settings** button. In the **Actors** tab, select the Subjects to which the conditions apply. See **SUBJECTS IN A CONDITION** on page 75.

4. Click **Add Condition**. Select a dependent variable from the list. Click the **Settings** button and specify the details for this condition. See the paragraph on using this dependent variable as condition for details.

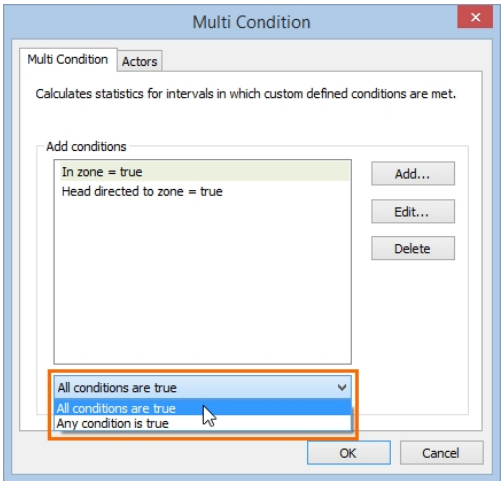


5. In the **Is:** field, specify the criteria, for example *When nose-point is in zone Novel Object = True*.



6. Repeat steps 4 and 5 for other conditions.

7. From the list at the bottom select whether all conditions or one of them should be met. Click **OK**.



8. Under **Condition is met when**, select the **Statistic** you want to use.

Frequently-used statistics

- **Current**. For conditions that are met when the dependent variables are true or not true at the current sample.
- **Frequency**. For conditions that are met when the dependent variable values occurred a number of times.
- **Cumulative duration**. For conditions that are met when the dependent variables values occurred for a specific time since the activation of the condition.
- **Current duration**. For conditions that are met when the dependent variable values occurred continuously for a specific time.
- **Latency to first**. See page 73 and Figure 24 on page 74.

You cannot use conditions based on hardware devices in multi conditions. If you want to use multiple conditions with hardware devices, use multiple condition boxes with an Operator instead. See **OPERATORS** on page 33.

8 Control hardware devices

DEVICES SUPPORTED IN ETHOVISION XT

- Noldus Pellet dispenser.
- Noldus Pellet receptacle.
- Noldus Lickometer.
- Top Unit of PhenoTyper.
- DanioVision Observation Chamber.
- PhenoWheel.
- Activity wheel counter and brake.
- Noldus Backlight unit with white light.
- Beam controller.
- Third-party and custom hardware devices. This includes any device that can be controlled with signal of TTL type. For example:
  - The Illuminated shelter.
  - The Ugo Basile Fear Conditioning System.
  - The Prizmatix optogenetics setup.
  - The Inscopix nVista Calcium imaging system (see page 149).
  - Med Associates devices (see page 107).
- TTL Port tester.

For the fear conditioning system, see the Quick Start Guide - EthoVision XT with the Ugo Basile Fear Conditioning System. For the optogenetics setup, see the Application Manual - EthoVision XT. For the Optogenetics setup in DanioVision, see the Reference Manual - DanioVision DVOC-0041.

To operate hardware devices

- You must have EthoVision XT with the Trial and Hardware Control Module (see page 14).

- You must have one Noldus USB-IO box or Noldus Mini USB-IO box, connected between your PC and the devices. See **The USB-IO box and the Mini USB-IO box** on page 119.

Figure 26 *The Noldus USB-IO Box.*

- All hardware devices must be connected through a Ethernet straight-through cable with RJ45 connectors.

- In EthoVision XT, you must define the devices you want to use in the Experiment Settings (see page 108). Next, assign devices to arenas (page 112), and finally program Trial Control with hardware-based actions and conditions (see page 116).

Time accuracy

- The accuracy of sending or receiving signals from the USB-IO box is 40-120 ms, depending on factors such as the camera frame rate and the Windows priority scheduling.

Terminology

- **Physical device**. A single device identified by a ID number or code. Each physical device must be associated with a specific arena in your hardware configuration (see Figure 27).

  EXAMPLE PD03 for a Pellet dispenser.

- **Device name**. A generic name that indicates the same type of physical device used in each arena. The **Device name** is valid for all arenas defined in your Arena Settings, even if a particular arena has no physical device associated with it.

  EXAMPLES Pellet dispenser, Wheel, Yellow light.

WORK WITH MULTIPLE DEVICES

EthoVision XT supports the use of one or two USB-IO boxes or one Mini USB-IO box at a time. We recommend to test a specific combination of devices before carrying out the actual trials. Whether a combination of devices works also depends on the timing of the commands. Controlling 12 pellet dispensers may, for example, work if the pellets are dropped at different times. Dropping 12 pellets at exactly the same moment will, however, not work.

When working with two or more USB-IO boxes, see **USE MULTIPLE USB-IO BOXES** on page 129.

Tested configurations

The following configurations were tested separately:

- Four PhenoTyper Top Units.
- Four Pellet dispensers.
- Four PhenoWheels.
- Four Lickometers.
- Four IR Beam Controllers (for the Mouse Feeding Monitor).
- Sixteen PhenoTyper Top Units.
- Six TTL devices (e.g. Pellet dispensers) and six SDI devices (Lickometers).

Devices in multiple arenas

If you work with multiple arenas, those arenas are expected to have the same type of devices. Trial Control is applied to each arena independently. For example, if you define an action *Drop a pellet* to be triggered when a condition is met, that action is executed on the pellet dispenser associated with the arena in which the condition is met.

An arena may contain more than one physical devices of the same type. In this case you must define unique Device names that can be associated with the physical devices. For example, if each cage has two pellet dispensers, you must define two Device names, for instance Pellet dispenser Left and Pellet dispenser Right (Figure 27).

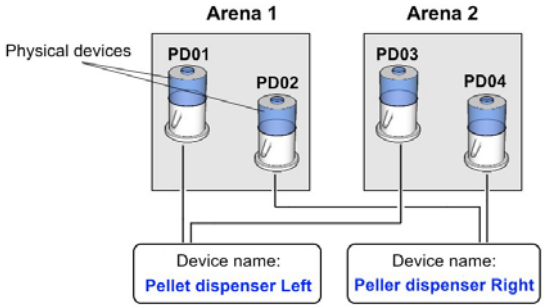


Figure 27 *An example of mapping hardware devices in multiple arenas.*

CONNECT THE HARDWARE DEVICES

Before defining a hardware configuration, close EthoVision XT.

1. Connect the power cord to the 24V in socket on the USB-IO box/Mini USB-IO box rear panel. Connect the other end of the power cord to the mains socket.
2. Connect the USB socket on the rear panel of the USB-IO box/Mini USB-IO box to the EthoVision computer using a USB cable.
3. Connect the hardware device to the first available port of the USB-IO box/Mini USB-IO box using the RJ45 cable. Depending on the device you want to connect, choose the port located on:
   - **TTL Control** (front panel of the USB-IO box). For devices controlled with TTL logic (One/Zero signal type). For example, the Pellet Dispenser, the TTL Port Tester or any Custom Hardware devices working with TTL logic.
   - **SDI Control** (rear panel of the USB-IO box). Special Device Interface, for devices controlled with a serial data stream. For example, the Lickometer, Running wheel and the PhenoTyper's Top Unit Interface (see below).
4. Repeat step 3 to connect other devices.
5. To test the device, follow the next sections Setting the port connections (see below) and Assigning devices to arenas (see page 112).

Connect Phenotyper

To control one or more PhenoTypers, you must have the Top Unit Interface box, which functions as an interface between the USB-IO box and the Top Units (see the figure below).

Connect each of the Top Units to one of the four RJ-45 sockets in the Top Unit interface. To connect a Top Unit to the Top Unit Interface, you must have an adapter between the Top Unit's TTL-in 9-pin Sub-D socket and the Top Unit connector cable.

Connect the Top Unit interface to the **SDI Control 13** port in the USB-IO box.

You can control up to four Top Units from one SDI port using the Top Unit Interface. By using multiple Top Unit Interfaces, you can connect groups of four PhenoTypers to the USB-IO box.

For more information, see the Reference Manual - PhenoTyper.

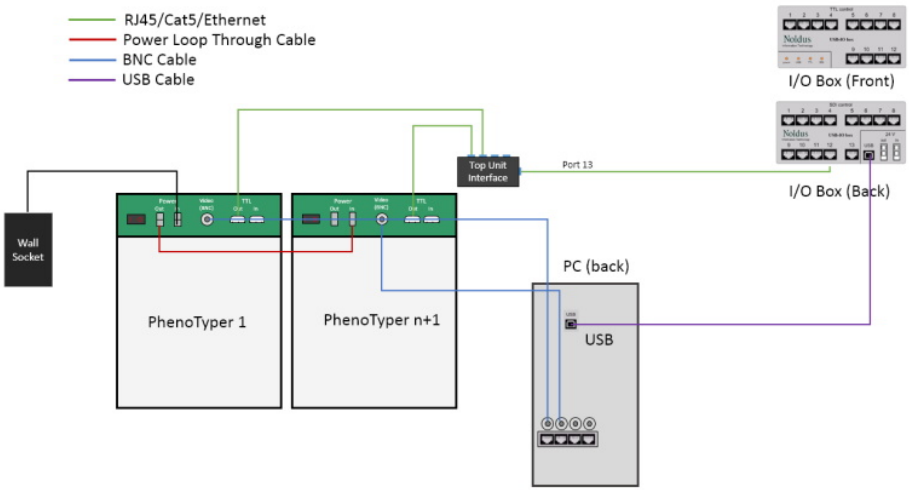


Figure 28 *Connecting the PhenoTypers' Top Unit.*

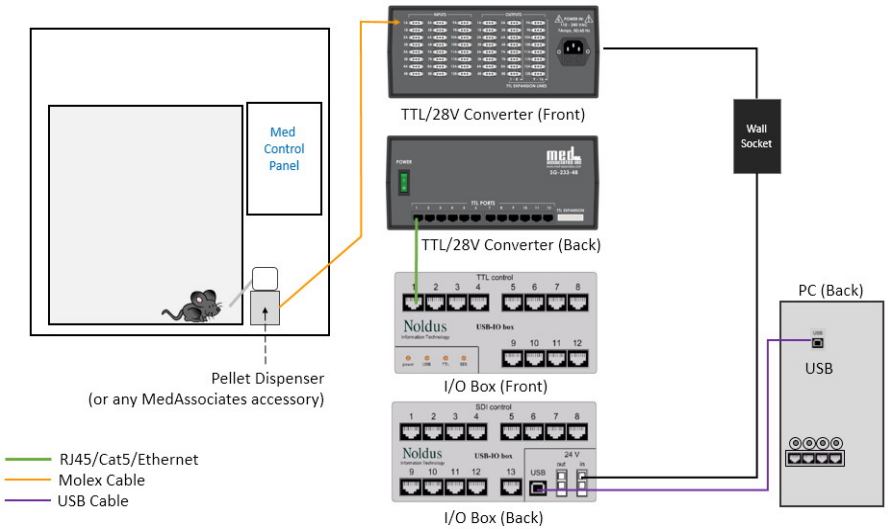
Connect third-party devices

For third-party devices you may need additional cables and adapters between the Noldus USB-IO box / Mini USB-IO box and those devices.

For example:

- For the Ugo Basile Fear Conditioning System, see the Quick Start Guide - EthoVision XT with the Ugo Basile Fear Conditioning System.
- For the Inscopix camera system, you need the Optical Isolated Interface PTISO-00x0 (see page 149).

- For Med Associates devices, see the following figure:



- For Med Associates devices and other systems with 28V inputs, you need the TTL to 28V interface. See the Service Manual - PhenoTyper. You need this converter when you want to send signals to the Med Associated device (for example, a pellet dispenser). For input devices, for example a lever switch, contact Noldus.
- To connect a custom device like a LED lamp or a simple switch, see page 127. Contact Noldus if you need special adapters or custom solutions.

SET THE PORT CONNECTIONS

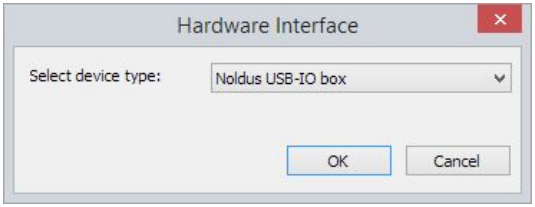
Prerequisites

- Connect all devices to the EthoVision PC, via the USB-IO box or Mini USB-IO box.
- When working with two or more USB-IO boxes, make sure that **all** IO-boxes are connected to the PC.

To set the port connections

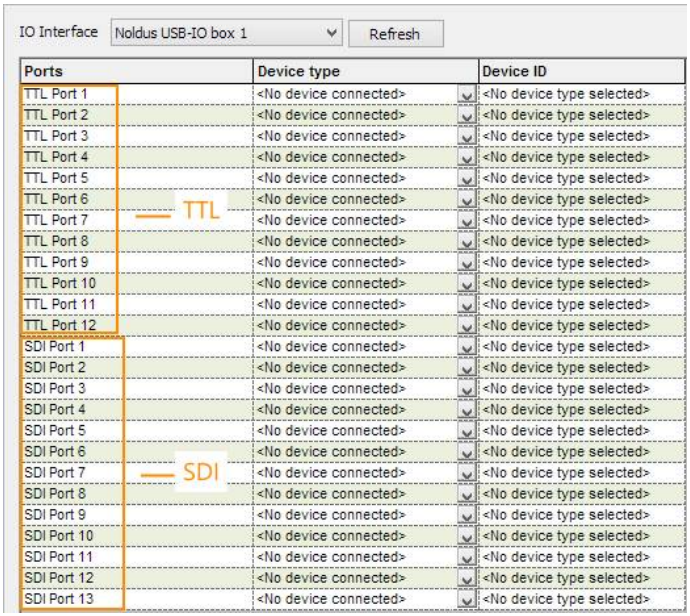
1. Start EthoVision XT, and open your experiment or create a new one.
2. Choose **Setup** > **Experiment Settings**.
3. Under **Trial Control Hardware**, select **Use of Trial Control Hardware**, and click the **Settings** button.

4. Select whether you use the **Noldus USB-IO Box**, the **Noldus Mini USB-IO Box** or a DanioVision Observation Chamber, and click **OK**.

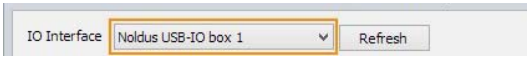


TIP To know what type of USB-IO box you have, check the label at the bottom side of the IO-box: PTIO-002x (for the Noldus USB-IO Box) or PTIO-003x (for Noldus Mini USB-IO Box).

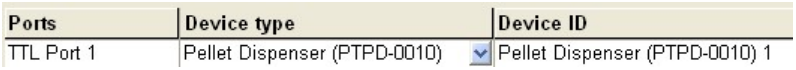
5. The Device Configuration window appears, listing the available ports of the interface device USB-IO box (left-most column): **TTL Port 1**, **2**,... for the TTL ports of the USB-IO box, and **SDI Port 1**, **2**,... for the SDI ports. Each row can be assigned to one physical device (see page 104 for the meaning of physical device).



6. If you have connected multiple USB-IO boxes, select one from the list at the top.

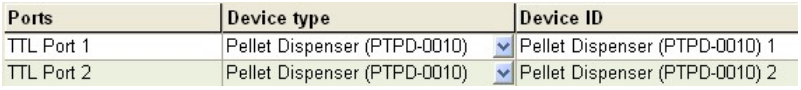


7. In the **Ports** column, locate the first available port of the correct type.
   - Locate a TTL Port and click under **Device type** to view the list of the devices. Select the device that applies.
   - Locate an SDI Port and click under **Device type** to view the list of the devices. Select the device that applies. Use SDI ports to connect the Lickometers and the PhenoTyper's Top Unit Interface. If you select the latter, four additional rows appear below the option. Select the PhenoTypers' Top Units. For details, see ***Set the port connections for Phenotyper*** on page 111.
8. From the **Device type** list, select the type of device that is (or will be) connected to that port.
9. In the **Device ID** field, enter the name of the physical device that should be connected to that port).



Ports	Device type	Device ID
TTL Port 1	Pellet Dispenser (PTPD-0010)	Pellet Dispenser (PTPD-0010) 1

10. Repeat step 5-7 for other devices, until they are all assigned to a port of the IO-box.



Ports	Device type	Device ID
TTL Port 1	Pellet Dispenser (PTPD-0010)	Pellet Dispenser (PTPD-0010) 1
TTL Port 2	Pellet Dispenser (PTPD-0010)	Pellet Dispenser (PTPD-0010) 2

11. Repeat the steps from **6** for the next USB-IO box.
12. When ready, click **OK**. You return to the Experiment Settings screen. Proceed with assigning devices to the arenas (see page 112).

Notes

- The number next to IO-box is the identification number of the IO-box set by the manufacturer. You cannot change this number.
- You must set the port connections only the first time you connect them.
- The port connection settings are at experiment level, and are applied to all your Trial Control Settings. Once you acquire at least one trial, the settings become locked and you can no longer change them. If you need to add devices, create a new experiment or remove the acquired trials.
- If you plan to create several experiments with the same set of devices, you can use an experiment as a template experiment with the hardware settings selected but containing no trials. To create a new experiment from a template experiment: from the **File** menu, select **New template experiment** and then **Use a custom template**. Next, open

the experiment you want to use as a template and use the new experiment to acquire data.

- The Device ID is by default the name of the Device type + a progressive number 1, 2,... We advise you to enter a name that you can recognize easily in your setup.
- For the difference between TTL and SDI ports, see page 106.
- Under **Device type**, select **TTL Port Tester** if you want to test the functionality of a TTL port. See page 142.
- Under **Device type**, select **USB-IO monitor** if you want to test the connection in the USB-IO box. See page 130.
- When you select a device type in step 5 (page 109), that device type is added to the file Hardware Setup.xml, which is stored in one of the folders located in the experiment folder ...\Configuration Files\Add-ins\Hardware. Which file is used depends on whether you use the USB-IO box or the Mini USB-IO box.

  {A8DA8F3E-7EA3-4629-8327-9D21C90FB3ED} for the USB-IO box.

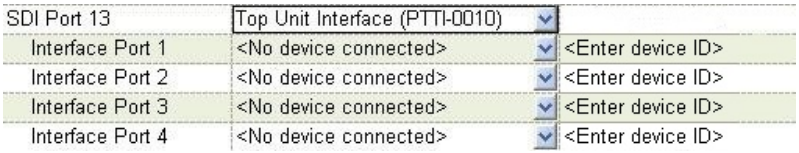
  {0308106B-1EBE-4620-B00D-396ABEBBC219} for the Mini USB-IO box.

Use multiple USB-IO boxes

You can connect multiple Noldus USB-IO Boxes to one EthoVision XT computer, to control more devices. For more information, see **USE MULTIPLE USB-IO BOXES** on page 129:

Set the port connections for Phenotyper

1. Locate the **SDI Port 13**. Under **Device type** select **Top Unit Interface**. This expands the SDI Port to four extra rows:



2. For each of the four rows, under **Device type**, select **Top Unit (Standard)** for standard PhenoTypers or **Top Unit (NeuroBsik)** for PhenoTypers of the Neuro-Bsik type.
3. Under **Device ID** for the rows appended, enter the name of the physical Top Units.

SDI Port 13	Top Unit Interface (PTTI-0010)	
Interface Port 1	Top Unit (Standard)	Top Unit (Standard) 1
Interface Port 2	Top Unit (Standard)	Top Unit (Standard) 2
Interface Port 3	Top Unit (Standard)	Top Unit (Standard) 3
Interface Port 4	Top Unit (Standard)	Top Unit (Standard) 4

4. When ready, click **OK**. Next, assign the Top Units to the arenas (see the next section).

To set the port connections for other groups of PhenoTypers, you must adjust the jumpers for the ports you are going to use. See the Service Manual - PhenoTyper.

ASSIGN DEVICES TO ARENAS

For experiments with one arena only, hardware devices are automatically assigned to the arena. You can skip this paragraph and continue with the next step: testing the hardware devices (see page 114) or programming trial control with devices (see page 116). For multi-arena experiments follow the procedure below.

Assuming that you have defined your arenas in the Arena Settings (see EthoVision XT Help), do the following:

1. In the **Experiment Explorer**, click the **Arena Settings** in which the arenas you want to use are defined.
2. In the **Arena Settings** window, click the **Arena - Hardware mapping** button. The Arena - Hardware mapping window appears.
3. Click **Add device**. A new row is appended to the table.
4. From the **Device type** list, select the type of device you want to assign.

   Select **Custom hardware** if the device type you are using is not listed.
5. Under **Device name**, a default name is displayed. This is the suggested Device name for the devices that you specify in that row; for more information on the meaning of Device names, see page 104.

   We advise you to enter a new name that can be easily recognized, especially if you have more devices of the same type in each arena.

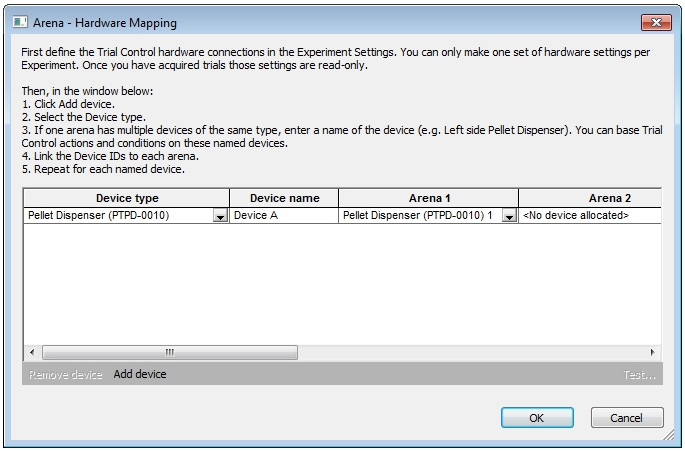


Figure 29 *The Arena - Hardware mapping window.*

EXAMPLE Each arena is provided with two pellet dispensers of the same type. Enter Pellet Dispenser Left under Device name. In the next step (6), select the pellet dispensers to be placed on the left side of each arena. Next, click **Add device** again and enter **Pellet Dispenser Right** under **Device name**, then select the pellet dispensers to be placed on the right side of each arena.

6. Under the **Arena name**, select the physical device that you want to assign to that arena.

Assign the other devices to the remaining arenas.

7. Repeat steps 3 to 6 to add a new set of devices.
8. When finished, click **OK**.

Notes

- To remove a set of devices, select the corresponding row in the table and click the **Remove device** button.
- If your setup includes two or more devices of the same type in one arena, make sure that you select the physical devices that belong to the Device name specified under **Device name**. See page 104 for the meaning of Device name.
- If you cannot select any physical device under the arena names, check that the Device type selected for that row is specified in the Port Device Mapping (see page 108).
- The Arena - Hardware Mapping settings are valid for all Arena Settings in your experiment. They are added automatically to every new Arena Settings.

Assign arenas to the PhenoTypers

1. Click **Add device** in the **Arena - Hardware Mapping** window.
2. Under **Device type**, select **Top Unit**.
3. Under **Device name**, enter a generic name (for example, Top Unit).
4. Under **Arena n**, beginning with **1**, select the Device ID of the Top Unit associated with that arena.
5. Repeat step 4 to assign the Top Units to the remaining arenas.

TEST THE HARDWARE DEVICES

1. Make sure that the hardware devices are connected to the EthoVision computer via the USB-IO box or the Mini USB-IO box (see page 106).
2. In the **Experiment Explorer**, click any **Arena Settings**.
3. In the **Arena Settings** window, click the **Arena - Hardware mapping** button. The Arena - Hardware mapping window appears.
4. Under the **Arena name**, select the physical device you want to test, and click **Test**. A new window appears.
5. Depending on the hardware device:
   - **For any device that can receive a signal from and send it back to the computer**– Examples are the Pellet dispenser, Activity wheel break, or Backlight unit white light. Select the appropriate action, click **Test** and check that the action has been taken. You

can also view the result in the **Test** window, that is, for example the Number of drops has increased by 1.

Signal	Value
Number of drops:	3
In error state?	false

- **For any device that can only send a signal to the computer, not receive any**. Examples are the Lickometer, PhenoWheel, or the Activity wheel counter. Trigger the device manually and check the result in the **Test** window.
- For the **Top Unit**. From the Command list, select the action you want to test, click **Test** and check the result in the PhenoTyper.
- For the **Custom Hardware Device** (for example, the Illuminated shelter). From the **Command** list, select the action you require, click **Test** and check that the device has been activated correctly.
- For the **TTL Port Tester**. Use this device to check the two-way communication for a specific port. Press a button on the device and check the result in the **Test** window. From the **Command** list, select a command and click **Test**. Check the result on the tester box.

6. When ready, click **OK**.

Notes

- Data from the Lickometer and the PhenoWheel are read out in regular time intervals. For the Lickometers currently sold by Noldus IT, the time interval is two seconds, and for the current PhenoWheel it is 60 seconds.
- For **all devices**: The fact that a signal is given correctly in the **Test** window but you do not see the response from the device could mean that the device is not functioning (for example, a lamp is broken).
- For the **Pellet dispenser**: If the **Test** window shows in error state = true, the pellet dispenser is not connected to the IO-box, or the food pellets are no longer available.
- For the **Lickometer**: Number of licks and Duration of licks is the cumulative number and duration of licks since the **Test** button has been clicked, respectively. The device is read out at regular time intervals. At the end of the interval the number and duration of licks is displayed very briefly.

  IMPORTANT If you realize that the values of Number and Duration of licks given by EthoVision XT are much higher than expected, close EthoVision XT, disconnect and re-connect the IO-box to the computer and then restart EthoVision XT.
- For the **Top Unit**, **TTL Port Tester** and **Custom Hardware Device**. You can also view the result of the action to be tested in the **Test** window. Locate the **Signal** you want to test

and verify that **Value** changes from false to true or vice versa. For the TTL Port Tester, you can also press a button and check the counts of presses for that button.

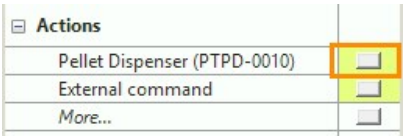
PROGRAM TRIAL CONTROL WITH HARDWARE DEVICES

In the Trial Control Settings, you can define:

- **A condition based on a hardware device**. For example, As soon as the cumulative duration of licks equals 1 minute, stop recording. See page 79.
- **An action on a hardware device**. For example, have the Pellet dispenser drop a pellet, or switch on the PhenoTyper's white light.

To define an action on a hardware device

1. In the **Components** pane, under **Actions**, double-click the button next to the name of the device type you want to control, or click the button next to it.



   The Hardware Action window appears.

2. Under **Name**, enter an **Action name**. Under **Comment** (at the bottom) enter a description (optional).
3. Under **Action to perform**, select the **Device name** you want to use. The names listed there are defined in the in the **Arena-Hardware mapping** window.
4. From the **Action to perform** list, select the action that the device must take.
5. Click the **Test** button to check the hardware action. Click the **Reset** button to reset all hardware.
6. Click **OK**. Insert the **Action** box in the appropriate position in the rule. For information on how to do this, see the EthoVision XT Help.

What you need to control hardware devices

- The Noldus USB-IO box, the Mini USB-IO box, or the DanioVision Observation Chamber. These devices work as physical interfaces between EthoVision XT and the devices.
- The hardware interface software **Noldus HardwareInterface USB-IO box** version 5, which is automatically installed when you install EthoVision XT.

TIP To check what is installed on your EthoVision XT computer, see the Windows **Control Panel** > **Programs and Features**.

Notes

- For general information on programming trial control, see page 23.
- The items listed under **Actions** are the Device names selected in the **Arena-Hardware mapping** window (page 112). If the device name is not listed under **Actions**, check that the port connections are set correctly (page 108) and that the devices are mapped in the arenas (see page 112).
- If you have a setup with multiple arenas, it is assumed that each arena has a physical device under that **Device name**. An action is taken in each arena separately, when the accompanying condition is met in that arena.
- Actions like *Light on* and *Sound on* result in the light/sound device being activated for an indefinite time. Remember to add an extra action that switches that light/sound off. The two actions should be separated by a time, for example Light on --> Time 1 s --> Light off.
- If you define a sequence of multiple actions on the same device, these are probably evaluated at the same sample time. This happens because the computer is fast enough to analyze multiple Trial Control boxes in one sample. To make sure that actions are taken in sequence, add a Time condition between actions so that the program waits for a short time (for example, 0.1 s) before taking the next action.
- You can also activate a hardware device like a Pellet dispenser manually. In the Manual Scoring Settings, define a fictitious behavior, for example, *Pellet*, and select the type **Point event**. In the Trial Control Settings, create a subrule that contains the following conditions and actions: [Rule Begin] > [Condition: Frequency (Pellet)>=1] > [Action: Drop pellet] > [Rule End]. During acquisition, every time you score *Pellet*, the drop pellet command is executed. For details, see **Controlling the Pellet dispenser manually** in the Service Manual - PhenoTyper.
- If the hardware action follows a condition based on **In zone**, or **Zone transition**, the action may be carried out repeatedly if the animal walks along the border of a zone. For example, if the detected body flickers on the border of two zones, and you want to drop a pellet every time the zone border is crossed, many pellets will be dropped. If this is the case, open the **Detection settings** and click **Advanced**, then under **Smoothing** set **Track noise reduction** to **On**. This smooths the track during acquisition, which means that the track is altered. This cannot be reversed afterwards.
- For NeuroBsik Top Units: To produce ultrasound of specific frequency and intensity: Define two actions that, when combined, specify the frequency you require.
  - **4 kHz. Fsel-1 off, Fsel-2 off**.
  - **8 kHz. Fsel-1 off, Fsel-2 on**.

- **16 kHz. Fsel-1 on, Fsel-2 off**.
- **32 kHz. Fsel-1 on, Fsel-2 on**.

Next, define two actions that, when combined, specify the volume you require.

- **25 dBm. Vol-1 off, Vol-2 off**.
- **50 dBm. Vol-1 off, Vol-2 on**.
- **75 dBm. Vol-1 on, Vol-2 off**.
- **100 dBm. Vol-1 on, Vol-2 on**.

Combine the four actions with an operator of type *All*, and then add a Time condition (see the previous note) followed by a *Sound on* action.

9 The USB-IO box and the Mini USB-IO box

THE USB-IO BOX

General information

The Noldus USB-IO box is the device that the EthoVision XT video tracking software must use in order to work together with external devices such as the Noldus Pellet dispenser, the Noldus Lickometer or the light and sound devices of the PhenoTyper Top Unit.

Figure 30 *The USB-IO box.*

With one USB-IO box you can operate:

- Up to 12 Pellet dispensers or other TTL-driven devices.
- Up to 12 Lickometers.
- Four PhenoTyper Top Units (by default), expandable to a maximum of 52. For this special connection, port SDI **13** is reserved.

For information on which third-party devices can be connected to the USB-IO box, see page 127.

Note

The total number of devices that you can connect is limited by the number of ports on the USB-IO box. For example, because the Lickometer uses the same type of port (named SDI) as the PhenoTyper Top Unit, and 13 SDI ports are available on the UBSB-IO box, if you connect 52 Top Units, all the suitable ports of the USB-IO box are occupied. Therefore no lickometer can be connected. When planning your experiment, make sure that you have enough ports on the USB-IO box for the devices you want to use.

A typical setup

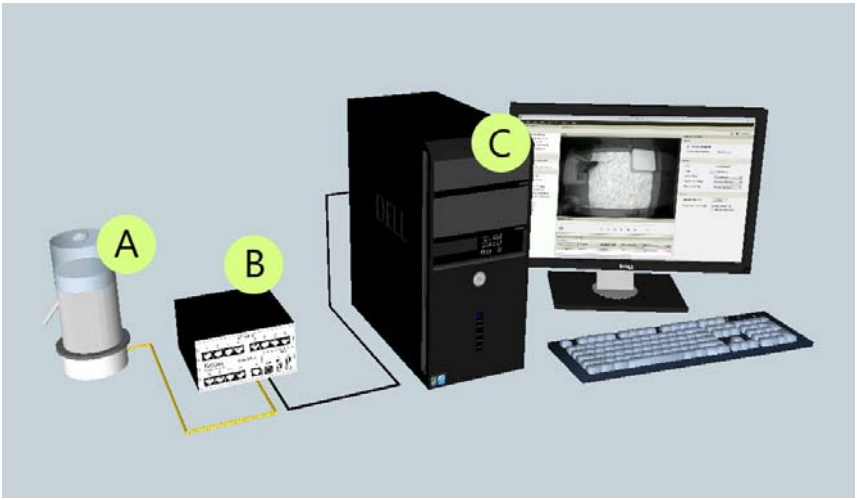
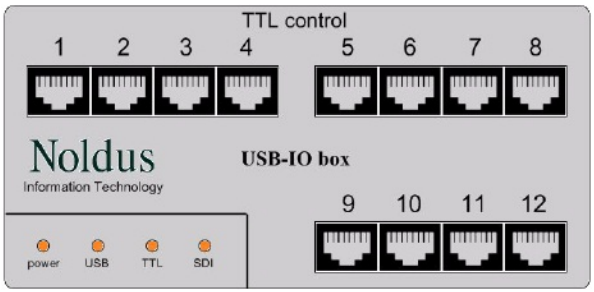


Figure 31 *EthoVision XT setup for controlling a Pellet dispenser.* **A** *– Pellet dispenser,* **B** *– USB-IO box,* **C** *– Computer with EthoVision XT. More devices can be attached to the USB-IO box. More USB-IO boxes can be connected to one computer with EthoVision XT.*

1. Connect the hardware devices to the computer, via the USB-IO box (see page 124).
2. Define the hardware configuration in EthoVision XT (see page 134).
3. With the Trial and Hardware Control function of EthoVision XT, you can define:
   - Actions on the hardware device (for example, *Drop a pellet*).
   - Conditions that must be met in order for Trial Control to progress.
   - Sub-rules for a sequence of actions and conditions to be repeated.
4. Acquire data using the Trial Control settings specified above.

Defining Hardware configuration means that you specify which communication ports of the USB-IO box are connected to which hardware devices, and, in the case you work with multiple arenas, which individual device is assigned to which arena.

Connectors and displays - Front panel



TTL control 1...12

These are input/outputs for devices that are controlled with TTL (Transistor Transistor Logic). Use a TTL port for the following devices:

- Noldus Pellet dispenser (Noldus type number PTPD-001x).
- Noldus Beam controller (Noldus type number PTBC-001x).
- Shock, Tone, Light and Noise in the Ugo Basile Fear Conditioning System.
- White light in the Noldus Backlight unit.
- Noldus Pellet receptacle (Noldus type number PTPR-001x)
- The Activity Wheel Brake (ENV-042).
- Any custom hardware device that works with TTL signals.
- The TTL Port Tester (PTTB-001x; for testing purposes).
- The USB-IO box monitor (virtual device; for testing purposes).

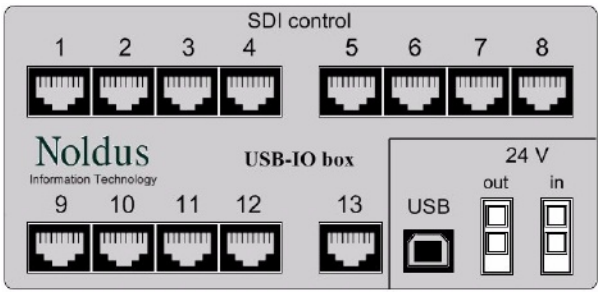
Notes

- Each of the 12 TTL control connectors can provide limited supply power (18 Volt DC, max. current 100 mA) available on pin 1 (ground) and 2 (+).
- You can control up to two physical devices with one TTL port. A typical example of controlling two devices with one TTL port is the Shock and Tone devices in the Fear Conditioning System (see the corresponding manual). For this you need a split cable from the TTL port that ends in the two devices. Please contact Noldus if you need such a cable.
- Ports are organized in groups of four. To optimize communication speed, we advise you to connect TTL devices to ports in the same group. For example, connect four pellet dispensers to ports 1 to 4, not to ports 1, 5, 7 and 12. Use ports 1-4 first, then 5-8 etc.

LEDs

- **Power** – When the light is on, it indicates that the USB-IO box receives power from the mains adapter.
- **USB** – When burning, it indicates that the USB-IO box is connected to a computer's USB port.
- **TTL** – When burning, it indicates that communication to a TTL device is in progress.
- **SDI** – When burning, it indicates that communication to a SDI device (see Rear panel below) is in progress.

Connectors - Rear panel



SDI control 1...13

SDI stands for Special Device Interface. SDI control ports are inputs/outputs for devices that are controlled with a serial data stream. You can connect one physical device to a SDI port.

Use these ports for the following devices:

- The Noldus Lickometer (Noldus type number PTLM-001x).
- The PhenoTyper Top Unit Interface (Noldus type number PTTI-001x).
- The Noldus PhenoWheel.
- The Activity Wheel Counter (ENV-042).

Notes

- Each Top Unit Interface is connected to one port of the USB-IO box, and can control up to 4 PhenoTyper Top Units. For more information, see the Reference Manual - PhenoTyper.
- SDI control **13** is set by default to connect the Top Unit Interface. If you want to connect two or more Top Unit Interfaces, you must alter the jumpers for the ports other than 13 that you use to connect the Top Unit Interfaces. See page 131.

- Each of the 13 SDI control connectors can provide limited supply power (12 Volt DC, max. current 100 mA) available on pin 1 (ground) and 2 (+).
- All SDI devices mentioned above get their full power supply by the 12 Volts provided by the USB-IO box.

USB

Connects the USB-IO box to the PC using a USB cable.

24 V In

Connects the USB-IO box to the Power supply via the 24V adapter.

24 V Out

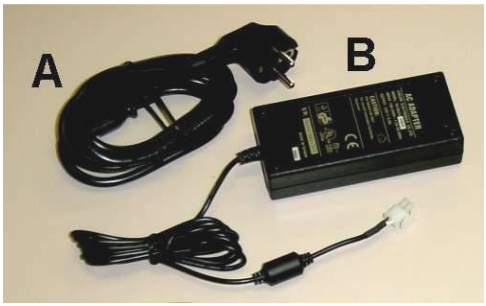
Connects the USB-IO box to other devices/power consumers with a power feed-through cable. For example, a PhenoTyper Top Unit.

IMPORTANT When you connect devices to the **24 V Out** power supply, do not exceed the maximum power rating of your mains supply.

INSTALL THE USB-IO BOX

The USB-IO box comes with the following cables:

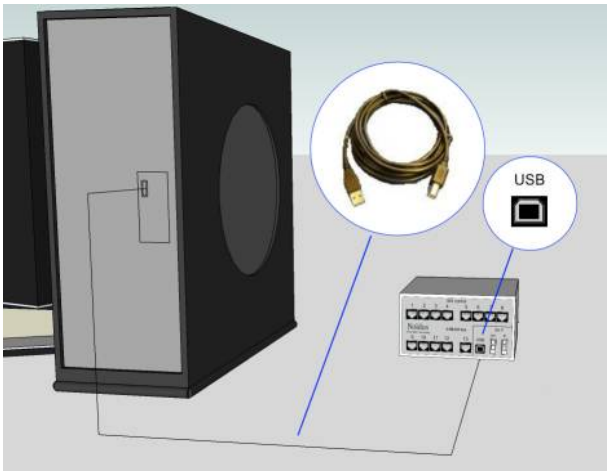
- Power supply 24 V 3A DC (**A**) with mains cable (**B**)



- USB cable with two different connectors.

Connect the USB-IO box to the computer

1. Make sure that EthoVision XT is not running.
2. Connect the USB port on the USB-IO box to a USB port on the EthoVision XT computer using the USB cable.



Notes

- Use a direct USB port on your PC, not a USB hub or a USB port on your PC's monitor. Try to minimize the number of USB devices connected to your computer.
- You can in principle connect multiple USB-IO boxes to the EthoVision XT computer; however, we cannot guarantee that all possible combinations of devices will work. For more information, see page 129.
- If you disconnect the USB-IO box while EthoVision XT is open, a message appears on top. Re-connect the USB-IO box and in EthoVision XT click **Try again**. Note that It can take some time before the software recognizes the USB-IO box after you re-connect it.

Connect the USB-IO box with the Noldus USB extender cable

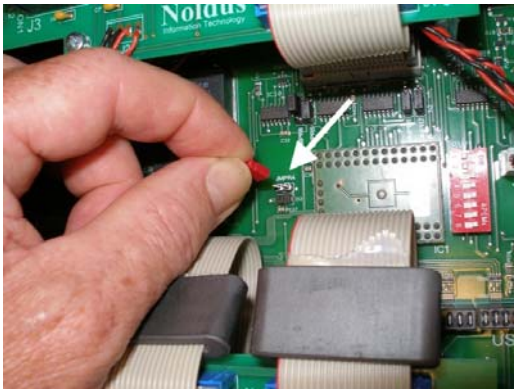
The standard USB cable length is limited to 3 meters. When you need a longer distance between the computer and the PhenoTyper setup, we recommend to use the Noldus active USB extender cable of 20 meters because not all standard USB extenders work fine with the USB-IO box.

The Noldus USB extender cable comes with a small jumper that must be set inside the USB-IO box in order for the USB extender cable to function correctly.

When you use the USB extender cable in combination with the mini USB-IO box (PTIO-003x) you do not need to set the jumper. When you purchased your USB-IO box together with the USB extender cable, the jumper already has been set by Noldus.

To set the jumper, do the following:

1. Open the USB-IO box by unscrewing 2 screws at the bottom, and remove the top part (see page 132).
2. Set the jumper on position **JMPR4** (indicated by the arrow in the figure below).



3. Re-assemble the USB-IO box.
4. Now connect the USB-IO box to the computer (page 124).

The USB-IO box device drivers

For EthoVision XT 16, the driver software for both the USB-IO box and the Mini USB-IO box is Noldus HardwareInterface USB-IO box 5.

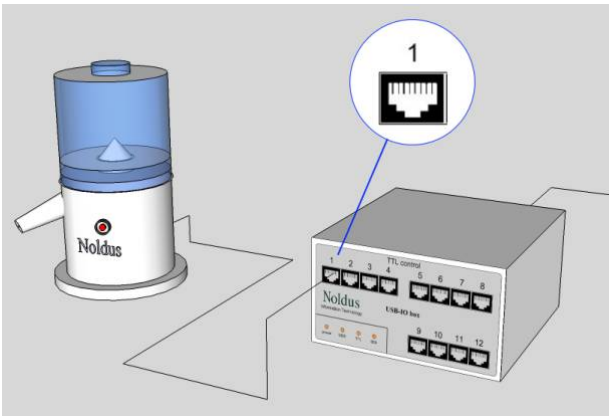
For EthoVision XT 11 and older versions, the USB-IO box and the Mini USB-IO box had separate drivers.

When you install the EthoVision XT software, the driver software is installed automatically. It is not necessary to uninstall the drivers of previous versions of the Noldus USB-IO box or the Noldus Mini USB-IO box.

To uninstall the drivers, open the **Control Panel** and go to P**rograms and Features**. Select **Noldus HardwareInterface USB-IO box 5** and click **Uninstall**.

CONNECT THE USB-IO BOX TO DEVICES

Connect one device



1. Plug one of the ends of the modular network cable in the hardware device.

   Although an RJ45 cable is used in computer networks, the signal from and to the hardware device is not a network signal. The device cannot be connected via a network hub or similar.

2. Plug the other end of the network cable in one of the ports available on the USB-IO box.
   - TTL (front panel) for the Pellet dispenser and other devices that communicate via TTL.
   - SDI (rear panel) for the Lickometer and the Top Unit Interface.

Note:

- The network cable comes with the hardware device, not the USB-IO box.

- To connect the PhenoTyper's Top Unit, connect the network cable to the Top Unit Interface, then connect the Top Unit Interface to each Top Unit. Connect the Top Unit Interface to port SDI 13. For more information, see the Reference Manual - PhenoTyper.

Connect multiple devices

Connect each individual device to a TTL or SDI port of the USB-IO box.

If you use PhenoTypers with devices like the Noldus Lickometer or the Noldus Pellet dispenser, connect the devices of the same type to the same group of connectors (e.g. four Pellet dispensers to TTL 1-4, or TTL 5-8).

EXAMPLES

- Four Lickometers: SDI ports 1 to 4
- Four Pellet dispensers: TTL ports 1 to 4
- Top Unit Interface: SDI port 13

Speed of TTL communication is optimized if devices are connected to the same group of connectors.

IMPORTANT We always recommend to test a specific combination of devices before carrying out the actual trials. Whether a combination of devices works also depends on the timing of the commands. Controlling 12 pellet dispensers may, for example, work if the pellets are dropped at different times. Dropping 12 pellets at exactly the same moment will, however, not work.

Connect a third-party device: LED lamp

You can connect a third-party device that can be controlled by TTL. Below you find two examples, one with a LED lamp and one with two switches.

EXAMPLE Connect a LED lamp

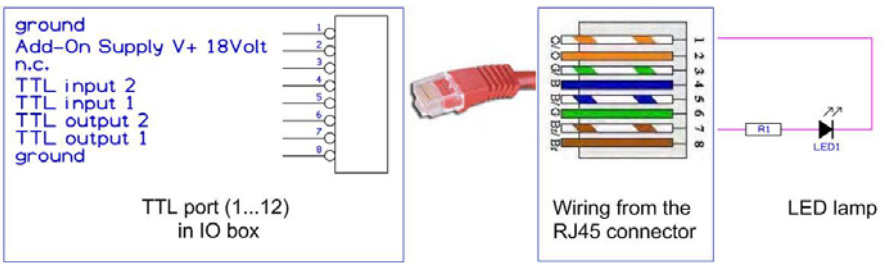


Figure 32 *Wiring scheme for connecting a LED lamp.*

- The LED lamp must be connected between TTL output1 (color: brown/white) and ground (color: orange/white). Do not forget to add a series resistor (approximate value 560 ohm) to limit current through the LED.
- To control the LED lamp with EthoVision XT, in the Port device mapping window select the TTL port that is connected to the LED and under **Device type** select **TTL Port Tester**. To define an action on this device, use the **Light 1 on/off** option.

The resistor can be skipped when you connect a real TTL device like a shocker input or a maze door. Check the documentation of the device to be connected.

Connect a third-party device: two-switch device

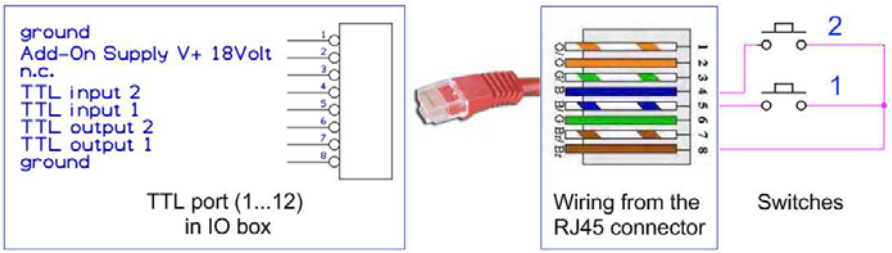


Figure 33 *Wiring scheme for connecting a two-switch device.*

- Connect switch 1 between TTL input1 (color: blue/white) and ground (color: brown).
- Connect switch 2 between TTL input2 (color: blue) and ground (color: brown).
- To read the status of each switch with EthoVision XT, in the Port device mapping window select the TTL port that is connected to the switch device and under **Device type** select **TTL Port Tester**. To define a condition based on this device, use the **Is button 1/2 pressed** option (to check that a switch is on/off) or the **Button 1/2 presses** option (to check the number of presses).

NOTE When a switch is open, the TTL value of the corresponding input is High. When a switch is closed, the TTL value of the corresponding input is Low (0).

Connect a third-party device: simple lever

When the lever is a simple "switch" that makes contact from signal to ground, without a circuit board in it, you can connect the device directly to a **TTL input** line of the USB-IO (or mini USB-IO) box.

- When the lever is not activated, the input is "high" (logic 1).

- When the lever is pressed, input will go to “low” (logic 0).

Connect pin 1 and/or pin 8 for ground and either pin 5 or 4 for TTL input 1 or 2 respectively).

NOTE Inside the (mini) USB-IO box, all TTL input lines are “forced high” to 5Volt TTL level by an internal resistor. So whenever an input line is not connected (open), it is interpreted as logical “high” or 1. If this line is connected to ground it will be seen as logical “low” or 0.

NOTE These instructions are valid if the lever is based on a (micro) switch. Other retractable levers may need a different connection!

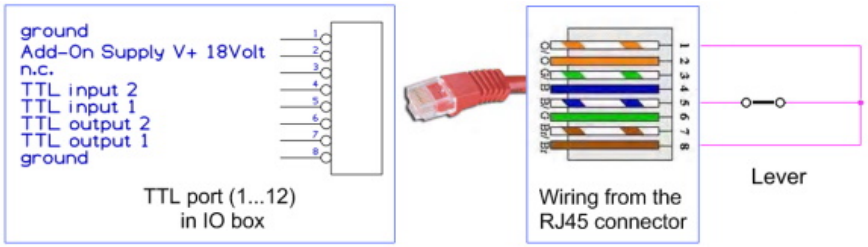


Figure 34 *Wiring scheme for connecting a lever device.*

USE MULTIPLE USB-IO BOXES

It is in principle possible to connect multiple USB-IO boxes to the EthoVision XT computer, provided that each box has a unique **ID**.

Tested configuration

We have tested the following combination and found no issues regarding data reliability: Two USB-IO boxes, each connected to 6 Noldus Pellet dispensers (TTL devices) and 6 Noldus Lickometers (SDI devices). Tests consisted of repeated trials of half an hour each.

In practice, there may be limitations due to the number of devices that can be attached and controlled simultaneously with EthoVision XT. We always recommend to test a specific combinations of USB-IO boxes and devices before carrying out the actual trials. See also the important note in **Connect multiple devices** on page 127.

Procedure

1. Make sure that each USB-IO box as a unique ID. To do so, adjust the DIP switches in one of the USB-IO boxes. See **Alter the DIP switches** page 133.
2. Connect each USB-IO box to its own power supply; do not use power feed-through cables.

3. Connect each USB-IO box to a separate USB port of your PC before configuring EthoVision XT.

4. In the Experiment Settings, click the **Settings** button next to **Use of Trial Control Hardware**. In the window that appears, first select one USB-IO box from the drop-down list and select the devices connected to it (see steps 7 -9 on page 110). Next, select the second USB-IO box and select the devices connected to it.

TEST THE USB-IO BOX

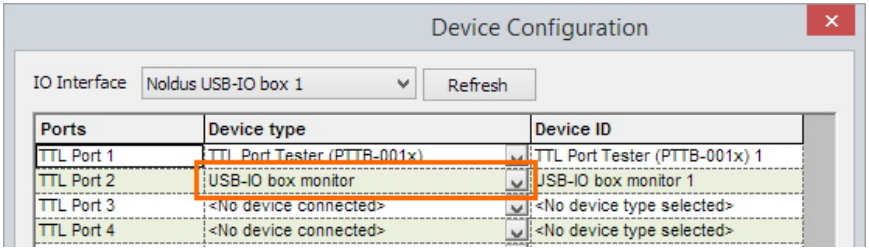
To test the functionality of TTL ports

See page 142.

To test connection in USB-IO box

When you carry out long trials, it may be handy to check that the USB-IO box works properly for the entire duration of the trial. For this purpose, use the **USB-IO box monitor** function.

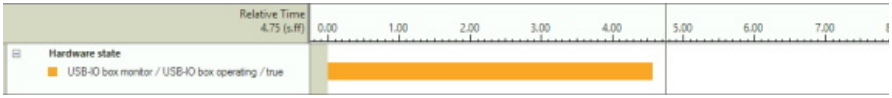
1. In the Device Configuration window (Experiment Settings), locate a free TTL Port and under **Device type**, choose **USB-IO box monitor**.



2. In the Arena-Hardware Mapping window, add a device and, and for that device select **USB-IO box monitor**.

3. After the trial has been acquired, in the Analysis profile click next to **Hardware state** and select:
   - Device type: **USB-IO box monitor**.
   - Value: **true**.

4. In the Integrated Visualization you should see the hardware state **USB-IO box monitor** as a colored bar that goes from the beginning to the end of the trial, if everything went well during the trial. If the USB cable between the PC and the USB-IO box was accidentally disconnected, or some error occurred inside the USB-IO box chip. the colored bar should end at that time in the trial.



Note

- You can use the USB-IO monitor function to check whether the USB cable is still connected or if the communication chip is working fine. However, this function cannot help when:
  - A specific port other than that used for USB-IO box monitor is defective.
  - The power cable of the USB-IO box is disconnected during the trial.

  In both cases the USB-IO box monitor state remains unchanged till the end of the trial.
- Do not use the port reserved for **USB-IO box monitor** for connecting other devices.

JUMPER AND DIP SWITCH SETTINGS

The USB-IO box contains jumpers inside the box next to the SDI ports. Jumpers are short lengths of wire that complete a circuit. By placing the jumper over a pair of available pins, you activate that line. On the USB-IO box board, jumpers can be placed in either position 1 or 2.

The default jumper positions are set by Noldus Information Technology. You do not have to change them unless you intend to connect multiple Top Unit Interfaces to work with multiple groups of PhenoTypers (see **ALTERING THE JUMPERS** below).

Default jumper settings

- **JMPR5** (for SDI ports 1-4) — Set to 1
- **JMPR6** (for SDI ports 5-8) — Set to 1
- **JMPR7** (for SDI ports 9-12) — Set to 1
- **JMPR8** (for SDI port 13) — Set to 2

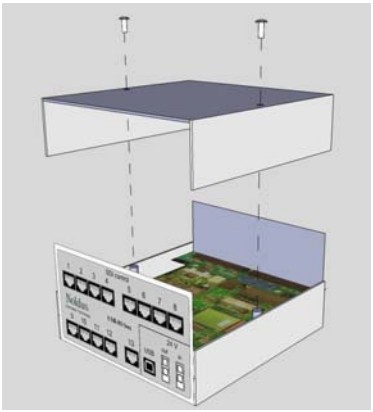
Position 1 enables use of the Lickometer. Position 2 enables communication between the USB-IO box and multiple PhenoTyper Top Units.

Alter the jumpers

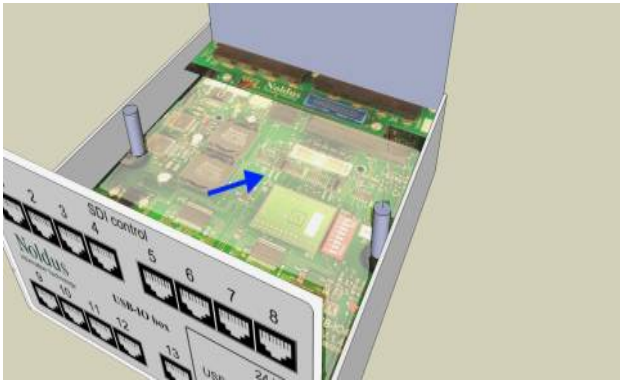
Follow this procedure only if you want to use SDI ports other than 13 to connect the Top Unit Interface. For example, when you want to connect 8 PhenoTypers to your PC and therefore SDI port 13 is not sufficient as it can work with up to four Top Units.

Note that if you alter a jumper other than JMPR8, this is done for all the four ports it corresponds to.

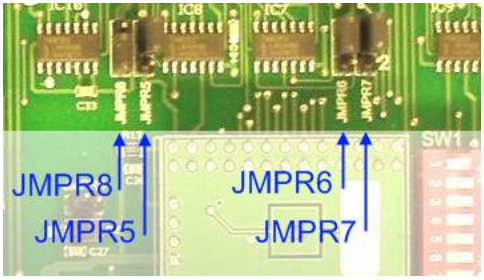
1. Unscrew the cover of the USB-IO box.



2. Locate the jumpers on the main board marked by JMPR5 to JMPR8.



3. Put the jumper to position 2 for the port that you require.



JMPR5 (for SDI ports 1-4)

JMPR6 (for SDI ports 5-8)

JMPR7 (for SDI ports 9-12)

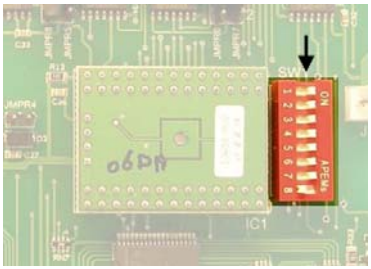
The jumper position 1/2 is marked right of JMPR7. The jumper JMPR8 is set by default to 2. If you want to use SDI port 13 to connect a Top Unit Interface, leave it in that position.

If you want to use port 13 to connect a Lickometer, set it to 1.

4. Close the cover.

Default DIP switch settings

Open the USB-IO box cover (see the previous page). The DIP switches are located in a red block on the main board.



The default settings are (from 1 to 8): **1 Off**, **2-8 On**. This sequence Off-On-On.... corresponds to oooooooı, that is 1 in binary language).

Alter the DIP switches

Follow this procedure if you want to connect multiple USB-IO boxes to the EthoVision XT computer.

DIP switches determine the ID of the USB-IO box. Multiple boxes must have different ID (and therefore DIP switch settings) in order to work properly with EthoVision XT.

1. For the first USB-IO box, leave the DIP switch settings as default (**1 Off**, **2-8 On**; red squares represent switch positions).

2. For the second USB-IO box, use a pen or a small screwdriver to move the DIP switch **1** to **On** and the DIP switch **2** to **Off** (this corresponds to 00000010, that is 2 in binary language).

3. For more USB-IO boxes, move the DIP switches to create unique numbers.

CONFIGURE ETHOVISION XT

Prerequisite

Make sure that the USB-IO box is connected to the EthoVision XT computer, that all devices are connected to the USB-IO box, and that the USB-IO box is connected to a power supply. If you use multiple USB-IO boxes, connect them all to the EthoVision XT computer.

Procedure

See **Control hardware devices** on page 103.

THE MINI USB-IO BOX

Overview

The Noldus Mini USB-IO box (type number PTIO-003x) is an interface device used to control external devices such as an Atlantis platform, a shocker, etc., from the EthoVision XT video tracking software. It is a smaller and simplified version of the USB-IO box (type number PTIO-002x). It is also embedded in the DanioVision system for tracking of zebrafish tracking.

Figure 35 *The Mini USB-IO box.*

Ports

The Mini USB-IO box is provided with (see the top panel of the box):

- **USB** — Connects the USB-IO box to the PC using a USB cable.
- Four **TTL control** connectors, TTL control 1 to 4 (see page 136).

- One **Multi TTL output** connector (see page 136).
- The **SDI control** connector is currently used in the DanioVision system, and will be used in future applications of the stand-alone Mini USB-IO box.
- **24V in** — Connects the USB-IO box to the Power supply via the 24V adapter.

  Each of the 4 TTL control connectors can provide limited power supply (18Volt DC, max. current 100mA) available on pin 1 (ground) and 2 (+).

LED indicators

- **24V On** — Switches on when the Mini USB-IO box is connected to a power source.
- **TTL control busy** — Blinks whenever the Mini USB-IO box communicates with a TTL-controlled device.

- **SDI control busy** — Blinks whenever the Mini USB-IO box communicates with a SDI-controlled device.

TTL control

With a TTL port you can control the following devices (one per port):

- The Noldus Backlight Units (Noldus codes XIRWB-xxxx)
- The Noldus Pellet dispenser (Noldus code PTPD-xxxx; maximally two).

  To use the Pellet dispenser with the Mini USB-IO box, you must replace a device file in EthoVision XT. See the instructions **CONNECT THE PELLET DISPENSER** in the chapter **The Pellet Dispenser and the Pellet Receptacle** in the Service manual - PhenoTyper. You can open this manual from **Apps** > **Noldus** > **EthoVision XT 16 Other Documentation** or on the EthoVision XT installation USB stick.
- Any (custom) hardware device that works with TTL logic (up to two devices per port; see page 138).

To control a Noldus PhenoTyper setup, you must use the Noldus USB-IO box PTIO-002x for full PhenoTyper add-on hardware support.

If you want to control the doors of 8-arm radial maze, you can use the TTL ports. Since the Mini USB-IO box has 4 TTL ports, you have in total 8 output lines, which can control all eight doors. Please contact Noldus for more information.

Multi TTL control

With the Multi TTL output, you can control up to 16 devices independently. This output is suitable for controlling maze doors in a
8-arm radial maze, lights or any other devices with a TTL control input.

The connector is a 20 pin IDC.

Beside these 16 outputs, also some supply voltages are provided: +5Volt, +12Volt and common ground.

There are no TTL input lines available on this connector. Because of this, you cannot use Multi TTL to control the Noldus Pellet dispenser and any other device which requires input channels.

To install the drivers

When you install the EthoVision XT software, the drivers of the Noldus Mini USB-IO box are automatically installed. To check that the driver is installed, open the **Control Panel** and go to **Programs and Features**. The driver name is **Noldus HardwareInterface USB-IO box 5**.

To uninstall the drivers

It is not necessary to uninstall the drivers of previous versions of the Noldus USB-IO box or the Noldus Mini USB-IO box.

However, if for any reason you want to uninstall the drivers of the latest version, open the **Control Panel** and go to **Programs and Features**. Select **Noldus HardwareInterface USB-IO box 5** and click **Uninstall**.

To connect the Mini USB-IO box

NOTE If you want to control the Pellet dispenser with the Mini USB-IO box, first follow the instructions **CONNECT THE PELLET DISPENSER** in the chapter **The Pellet Dispenser and the Pellet Receptacle** in the Service manual - PhenoTyper. You can open this manual from **Apps** > **Noldus** > **EthoVision XT 16 Other Documentation** or on the EthoVision XT installation USB stick.

1. Connect the Mini USB-IO box to a USB port on the EthoVision XT computer, using the USB cable that comes with the Mini USB-IO box.
2. A message may appear as you plug the USB cable to the computer USB port. Please wait that the driver software is installed.
3. Connect the Mini USB-IO box to the power supply using the appropriate cable and the mains adapter. The LED **24V On** switches on.
4. Plug one of the ends of the modular network cable in the hardware device.

5. Plug the other end of the cable in one of the ports available on the Mini USB-IO box.

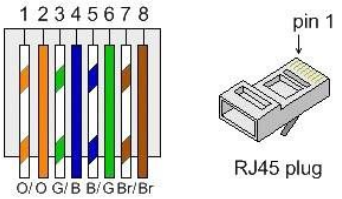
Note:

- If you disconnect the Mini USB-IO box while EthoVision XT is open, reconnect it. Note that It can take some time before the Mini USB-IO box is recognized after you reconnect it.
- Although you use modular network cables to connect devices to the USB-IO box, the signal from and to the device is not a network signal. The device cannot be connected via a network hub or similar.
- The modular network cable comes with the hardware device, not the Mini USB-IO box.

Connect custom hardware

Whenever you want to connect your own device to a **TTL control** connector of the Mini USB-IO box, do so according the information below.

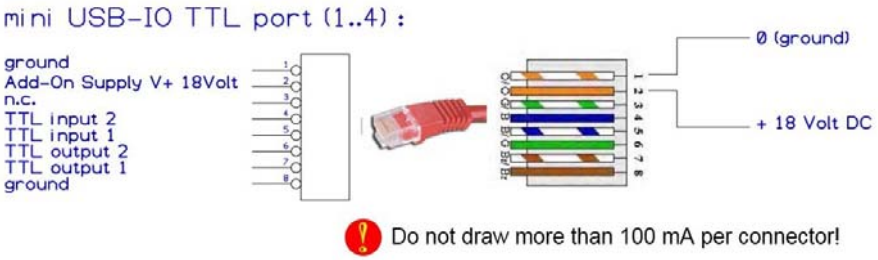
The TTL connector will provide supply (18VDC) and TTL output and input to your device. It is a standard RJ45 modular type. Pinning and cable wire colors for the mating patch cable are shown in the figure below.



To power a device, a +18 Volt power supply can be provided via pin 2 (orange). Common ground for both power and TTL on pin **1** and **8** (orange/white-brown).

IMPORTANT Max. overall current for all TTL ports must not exceed 0.5 A!

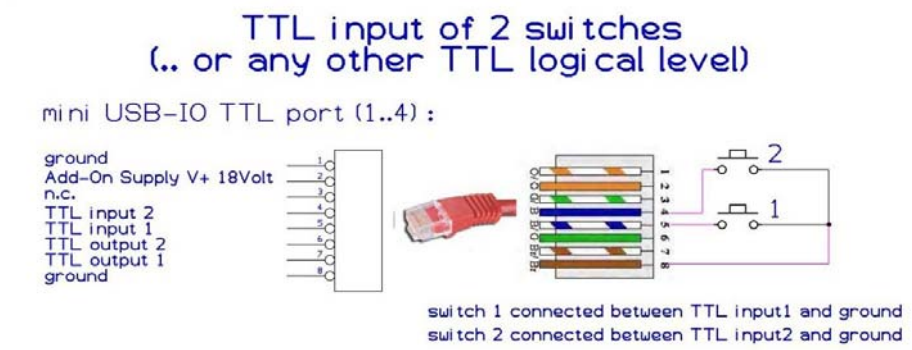
Whenever the device to be connected needs some other (lower) supply voltage, you must use some regulator in between or connect a separate power adapter to it.



Input of a TTL device

Two input lines are available for each TTL-connector, TTL-1 and TTL-2, connected to line 5 (white/blue) and **4** (blue) respectively.

In the example below, two switches send inputs to EthoVision XT via a TTL port.



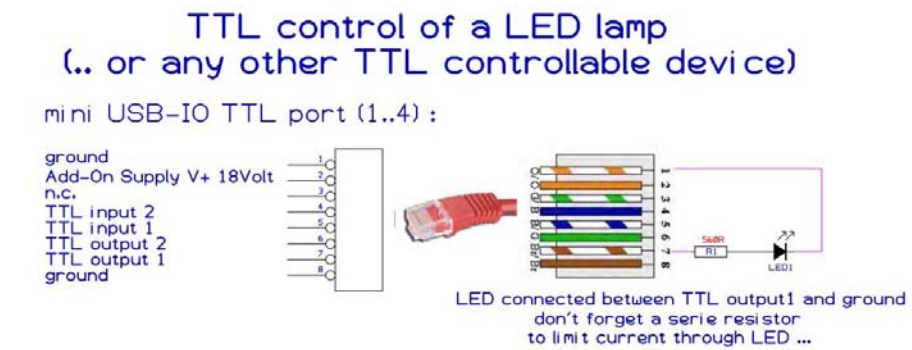
You can use the TTL input to monitor the status of devices connected to those input connections.

Inside the Mini USB-IO box, all TTL input lines are forced high to the +5V TTL logic level by a pull-up resistor. This enables open collector interfacing from your device into the Mini USB-IO box or you can just connect a simple switch between it and the ground. However, you can also connect any TTL logic level signal (0 Volt/5 Volt) to the input.

To prevent unwanted behavior of input lines caused by noise or signal spikes, inputs are protected by debounce/ESD protection circuits.

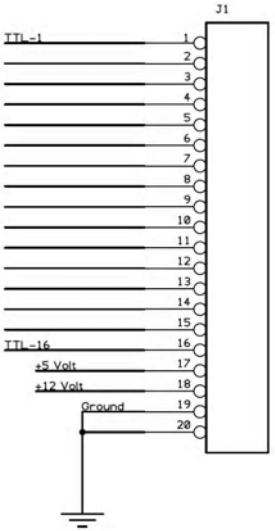
Output of a TTL device

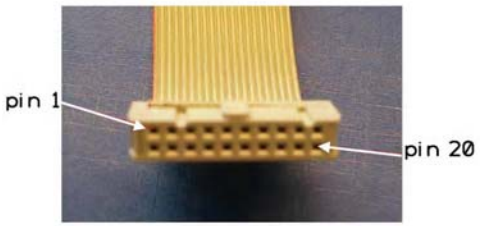
You can use TTL output to control any external device by simple 5V TTL logic. Each of the 4 TTL ports on the Mini USB-IO box is equipped with 2 TTL output lines. Every output line is protected with a current limiting resistor of 100 ohm. Maximum load current of the output is 10mA.



Multi TTL output connector

The Multi TTL output connector contains 16 independent output lines.





Advised connector: Assmann AWP20-7240-T-R.

Pin number:

- **1-16** — 16 x TTL compatible output lines (**TTL-1** to **TTL-16**).
- **17** — +5 Volt reference (max load 10 mA).
- **18** — +12 Volt reference (max load 10 mA).
- **19, 20** — Ground.

Note:

- No TTL input lines are available on this connector.
- To control non-TTL hardware, some kind of interfacing may be needed.

The Mini USB-IO box and EthoVision XT

Defining Hardware configuration means that you specify which communication ports of the Mini USB-IO box are connected to which hardware devices, and, in the case you work with multiple arenas, which individual device is assigned to which arena.

1. Connect the hardware devices to the computer through the Mini USB-IO box (see page 137), then start EthoVision XT.

2. Create a new experiment or open an existing one.
3. Under **Setup**, click **Experiment Settings**. Define the main characteristics of the experiment.
4. Select **Use of Trial Control Hardware** and click **Settings**.
5. In the window that opens, select **Noldus Mini USB-IO Box**.
6. In the **Device Configuration** window, select the devices you require. For more information on this step, see **SET THE PORT CONNECTIONS** on page 108.
7. Under **Setup**, select **Arena Settings**. Define the arenas.

   For more information on this step, see Arena Settings in the EthoVision XT Help.

   If you have only one arena, you can skip the steps below. The hardware is automatically assigned to the arena. If you have more than one arena, proceed with step 8.
8. Click the **Arena - hardware mapping** button in the **Arena Settings** window.
9. Click **Add device**. Under **Device type**, select the device and assign it to one of the arenas. Repeat this step for all the individual devices in your setup. Make sure you assign each device to one arena. For more information on this step, see **ASSIGN DEVICES TO ARENAS** on page 112.
10. Choose **Setup** > **Trial Control Settings**. Define the Trial Control protocol (see page 23 in this manual).

NOTE To use the Pellet dispenser with the Mini USB-IO box, you must replace a device file in EthoVision XT. See the instructions **CONNECT THE PELLET DISPENSER** in the chapter **The Pellet Dispenser and the Pellet Receptacle** in the Service manual - PhenoTyper.

NOTE If you created a DanioVision experiment, or if you used a DanioVision predefined template, select the DanioVision version that you have. For more information on setting up an experiment with the DanioVision Observation Chamber, see the Reference Manual - DanioVision DVOC-0041.

THE TTL PORT TESTER

Use the TTL Port tester to test or demonstrate the TTL control functionality of the USB-IO box. You can also use it to simulate a TTL device (for example a pellet dispenser), trigger an action (for instance, switch on a light) or as a manual input device.

Your TTL Port tester package includes a TTL Port tester PTTB-001x (Figure 36) with a green RJ45 modular cable connected (1 meter long).

Figure 36 *The TTL Port tester.*

Technical specifications

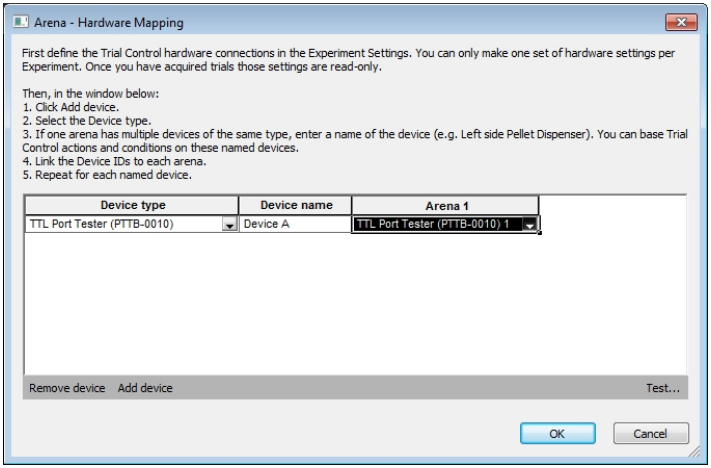
- Noldus device number: PTTB-001x.
- Power supply: 5 V DC, supplied by the USB-IO box.
- Current consumption: 30 mA (when one of the LEDs is on).
- Connector: RJ45 8 pin modular.
- Dimensions: 60 x 40 x 20 mm (l x w x h).
- Weight: 50 grams.
- CE compliant in accordance with EMC directive 2004/108/EC.

Test the USB-IO box TTL functionality

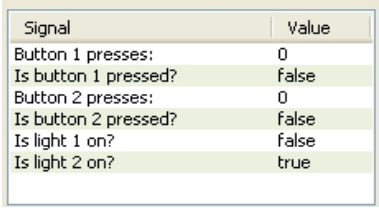
We assume that you have set the port connection in the Experiment Settings and assigned the TTL Port tester to the arena (see **CONFIGURE ETHOVISION XT** on page 134).

1. Make sure that the USB-IO box is connected to (A) your EthoVision XT computer and (B) a power supply.

2. Connect the TTL Port tester to the TTL port on your USB-IO box which you want to test, using the green RJ45 modular network cable. When both the USB-IO box and the TTL Port tester are properly connected, the TTL power LED on the TTL Port tester is on.
3. Start EthoVision XT and open your experiment. In the Experiment Explorer, click **Arena Settings**.
4. In the **Arena Settings** window, click the **Arena - Hardware mapping** button.
5. In the **Arena - Hardware mapping** window, under the arena name, select **TTL Port Tester (PTTB-001x)** and click the **Test** button.



6. A new window appears. In the **Command** drop-down list, select either **Light 1 on**, **Light 1 off**, **Light 2 on** or **Light 2 off** and click **Test**. Check the LEDs on the TTL Port tester (**Light 1** corresponds to **OUT-1**, and **Light 2** to **OUT-2**) and the information in the window.



NOTE The signal from and to the TTL Port tester is not a network signal. The TTL Port tester cannot be connected via a network hub or similar.

Manual input

You can also manually supply TTL input events to a TTL port. Follow steps **1-5** in the procedure above. Press either button **IN-1** or **IN-2** on the TTL Port tester and check the result in the window.

Signal	Value
Button 1 presses:	3
Is button 1 pressed?	false
Button 2 presses:	6
Is button 2 pressed?	false
Is light 1 on?	false
Is light 2 on?	false

Simulate a TTL device

You can connect the TTL Port tester to a TTL port that is defined for another TTL device, for instance, the Noldus Pellet dispenser. In this way, you can simulate the working of the device. You will see that the **OUT-1** LED on the TTL Port tester is turned on whenever EthoVision XT decides to have the dispenser drop a pellet. Because it is not the pellet dispenser connected to the port, there will be no "pellet dropped" reply, but you can simulate this by pressing the **IN-1** button on the TTL Port tester. The **IN-2** button on the TTL Port tester is connected to the same input as the "error" line of the pellet dispenser. With a pellet dispenser connected, this line while become active whenever the dispenser fails to drop a pellet. By pressing the **IN-2** button, you can simulate this occurrence.

A	B	C	D	E
Time	**Device**	**Command/Signal**	**Name**	**Value**
5.12	Pellet Dispenser (PTPD-0010) 1	command	Drop pellet	
6.76	Pellet Dispenser (PTPD-0010) 1	signal	Number of drops	1
14.12	Pellet Dispenser (PTPD-0010) 1	command	Drop pellet	
16.08	Pellet Dispenser (PTPD-0010) 1	signal	Number of drops	1
50.56	Pellet Dispenser (PTPD-0010) 1	signal	In error state	1

Figure 37 *Hardware log when the TTL Port tester is used to simulate a pellet dispenser. EthoVision XT sent two times the command to drop a pellet. With each command the* **OUT-1** *LED on the TTL Port tester was turned on. Each time the* **OUT-1** *LED was on, the* **IN-1** *button on the TTL Port tester was pressed. At t = 50.56* **IN-2** *was pressed to simulate the error state.*

Trigger an action with the TTL Port tester

You can use the manual input buttons on the TTL Port tester to trigger an action, for instance, switch on a light. Define a Trial Control *condition* based on the TTL Port tester and define that the light is switched on when, for instance, **IN-1** is pressed.

1. Open your Trial Control Settings and in the **Components** pane click the button next to **TTL Port Tester** (under **Conditions** - **Hardware**).
2. In the **Signal to check** drop-down list select:

   Is button 1 pressed? or **Is button 2 pressed?** and in the **Signal value** drop-down list select **True**. Then click **OK**. The light will be on as long as the button is pressed.

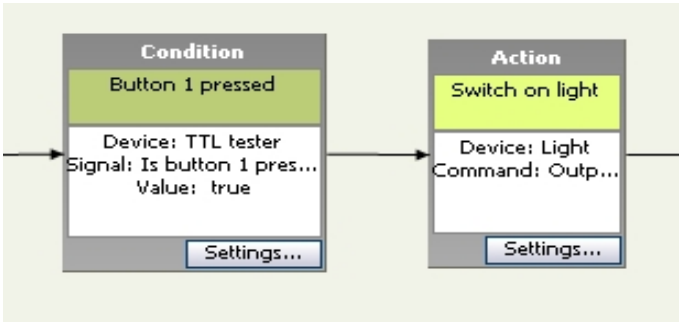


Figure 38 *Using the TTL Port tester to trigger an action (switch on a light).*

Button 1 presses or **Button 2 presses** and in the **Signal value** drop-down list, select **=** and enter **1** as the value. Then click **OK**.

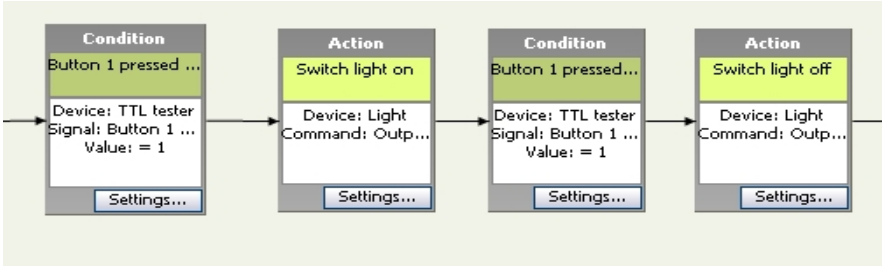


Figure 39 *Using the TTL Port tester to trigger an action (switch on a light). Every time button* **1** *is pressed, the light switches on. When button* **1** *is pressed again the light switches off.*

To define the appropriate action:

1. In the **Components** pane under **Actions**, click the button next to the hardware you want to control.
2. In the **Action to perform** drop-down list, select which action should follow the condition you defined above. Then click **OK**.
3. Insert the **Condition** and **Action** box in the appropriate position in the Trial Control rule.

Use the TTL Port tester as a manual event recorder

Although you can score behaviors manually with EthoVision XT, you can also do so with the TTL Port tester. For example, in a resident-intruder test, during acquisition press the **IN-1** button when the resident mouse is aggressive and the **IN-2** button when the intruder mouse is aggressive. For more complex coding schemes, use the Manual Scoring Settings in EthoVision XT.

If you use the TTL Port tester as a manual event recorder, you can view the number of key presses in a hardware log. In EthoVision XT from the **Export** menu, choose **Track Data** and select the **Hardware log** check box.

A	B	C	D	E
Time	**Device**	**Command/Signal**	**Name**	**Value**
6.28	TTL Port Tester (PTTB-0010) 1	signal	Button 1 presses	1
6.28	TTL Port Tester (PTTB-0010) 1	signal	Is button 1 pressed	1
7.56	TTL Port Tester (PTTB-0010) 1	signal	Is button 1 pressed	0
9.88	TTL Port Tester (PTTB-0010) 1	signal	Button 2 presses	1
9.88	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	1
10.24	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	0
11.56	TTL Port Tester (PTTB-0010) 1	signal	Button 2 presses	1
11.56	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	1
12.48	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	0
14.92	TTL Port Tester (PTTB-0010) 1	signal	Button 2 presses	1
14.92	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	1
15.56	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	0
17.64	TTL Port Tester (PTTB-0010) 1	signal	Button 2 presses	1
17.64	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	1
18.16	TTL Port Tester (PTTB-0010) 1	signal	Is button 2 pressed	0
20.52	TTL Port Tester (PTTB-0010) 1	signal	Button 1 presses	1
20.52	TTL Port Tester (PTTB-0010) 1	signal	Is button 1 pressed	1

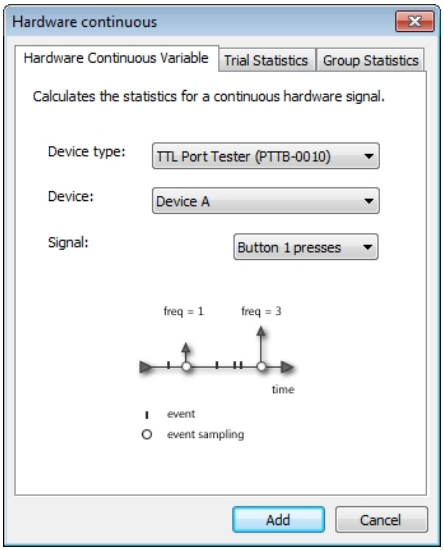
Figure 40 *Hardware log with the number of key presses.*

Calculate the statistics for the ttl port tester

For testing purposes, you can calculate the number of times the buttons were pressed, or visualize when the lights were switched on. To do so, in the Analysis profile:

- To analyze and visualize button presses, under **Hardware** click the button next to **Hardware continuous**. In other cases, skip this step.

  In the window that appears, next to **Device type**, select **TTL Port Tester (PTTB-001x)**. Next to **Device**, select the correct device if you have more than one device connected. Next to **Signal**, select **Button 1 pressed**, or **Button 2 pressed**.



- To analyze or visualize the time when the lights were switched on, under **Hardware** select **Hardware Command**, or **Hardware State**. Choose **Command** to view the time that the command was given. Choose **Hardware state** to visualize the status of the light (feedback from the TTL Port tester).

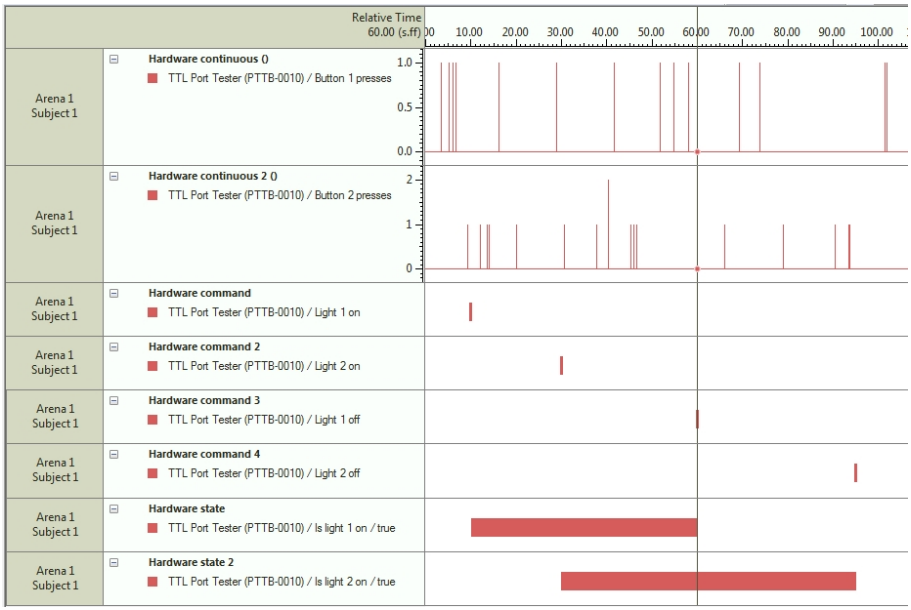
In the **Trial Statistics** tab, of the variable properties window, select the statistic **Total**, then click **Add**.

You can now calculate the number of times the button was pressed, or the light was switched on and off, by running an analysis. Choose **Analysis** > **Results** > **Statistics & Charts**. Make sure that the correct Analysis profile is selected on the toolbar. Next, click **Calculate**.

Trial Statistics | Group Statistics & Charts

		Hardware continuous	Hardware continuous 2
		TTL Port Tester (PTTB-0010) / Button 1 presses	TTL Port Tester (PTTB-0010) / Button 2 presses
		Total	Total
Result 1	Trial 1	0.0000	0.0000
	Trial 2	8.0000	6.0000
	Trial 3	0.0000	0.0000
	Trial 4	14.0000	17.0000

You can also visualize the button presses and the light being switched on and off in the Integrated Visualization. Choose **Analysis** > **Results** > **Integrated Visualization**. You now see in the Time Event Plot when the buttons were pressed, or when the lights were switched on and off.



THE OPTICAL ISOLATED TTL-IO INTERFACE

Aim

- To enable isolation between the Noldus USB-IO box or the Mini USB-IO box and a third party device. For example the Inscopix nVista camera system.
- To connect the Noldus USB-IO box or the Mini USB-IO box to a third party device with BNC connectors

IMPORTANT This isolator is not intended for medical applications!

Features

- It supports TTL-output from and TTL-input to EthoVision XT (two lines each).
- Output/input voltages 0-5 V.
- Isolation protects the device from the so-called “ground loops”.
- Minimizes noise and prevents damage to the USB-IO box in the case the device connected does not function properly.

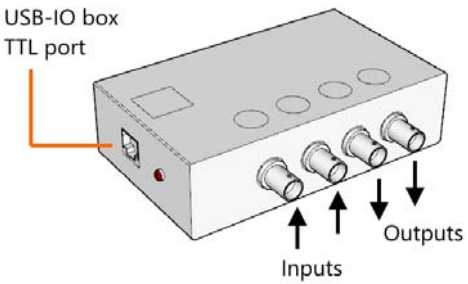
Package contents

- PTISO-00x0 TTL-IO Isolator.
- 1x network cable with RJ45 connectors.
- 2x input/output double cables with BNC connectors.

Connect and use

1. Connect a TTL port of the Noldus USB-IO box / Mini USB-IO box to the **TTL-I/O** port of the PTISO-00x0 Isolator.

2. Connect one or more of the **In**/**Out** BNC connectors of the PTISO-00x0 Isolator depending on your setup. For example, to trigger a device, use **Out 1** or **Out 2**.
3. When setting the port connections (page 108), specify the TTL port and the line used.



Technical specifications

- Type name: PTISO-0010 TTL-IO Isolator.
- Power requirements: 18-24 V DC; 0.02 A from USB-IO box.
- Isolation value: Supply 2kV (1 sec); TTL IO 5.3 kV (1 sec).
- Connections: 1x RJ45 type from USB-IO; 4x BNC type, optical isolated TTL input/output.
- Input/Output: TTL level signal (0-5.5 V DC).
- Dimensions: 31 x 113 x 67 mm; (1 $^{7}/_{32}$ x 4 $^{29}/_{64}$ x 2 $^{41}/_{64}$ inches).

TECHNICAL SPECIFICATIONS

USB-IO box version 2

Noldus model number	PTIO-002x
Power supply	24 V DC
Current consumption	Max 2.0 A (Varies depending on the number and type of devices connected) See also ***TTL control 1...12*** on page 121
Connections	12 TTL control connections (24 TTL Input lines and 24 TTL output lines) with RJ45 ports 13 Special Device Interface connections with RJ 45 ports
Cables	Power supply 24V 3A DC with mains adapter USB cable with connectors of type A and B
Operating temperature	0 to +40 °C (+32 to +104 °F)
Storage temperature	-20 to +70 °C (-4 to +158 °F)
Operating humidity	Up to 80%, no condensation
Dimensions	160 x 160 x 90 mm / 6.3 x 6.3 x 3.5 inches (L x W x H)

Mini USB-IO box version 1

Noldus model number	PTIO-003x
Power supply	24 V DC
Current consumption	1.0 A*
	IMPORTANT Max. overall current for all TTL ports must not exceed 0.5 A!
	See also ***Multi TTL output connector*** page 140
Interface to PC	USB-2
I/O connections	4 TTL control (representing 8 TTL Input lines and 8 TTL output lines) with RJ45 modular type connectors
	1 Multi TTL (representing 16 TTL output lines and external device supply) with 20 way IDC connector
	1 Special Device Interface with RJ45 modular type connector
Cables	Power supply 24V type 70 A24 with mains adapter
	USB cable with connectors of type A and B, 3m type SB2403
Operating temperature	0 to +40 °C (+32 to +104 °F)
Storage temperature	-20 to +70 °C (-4 to +158 °F)
Operating humidity	Up to 80%, no condensation
Dimensions	175 x 81 x 30 mm / 6.9 x 3.2 x 1.2 inches (L x W x H)

*) Varies depending on the number and type of devices connected.

10 User-defined hardware devices

USER-DEFINED DEVICE TYPES

Device types like the PhenoTyper's Top Unit Interface, the Lickometer and the Pellet dispenser are pre-defined in EthoVision XT as default device types, so when you use such devices you do not have to define their properties. In the case you want to use a new device type or a device with parameters different from those set in Noldus devices, you can create a **User-defined device type**.

For example:

- You want to sample the Lickometer at a rate different from the default one, that is one sample every two seconds. To do so, create a new Lickometer device type, where the only difference between the existing Lickometer and the new device type is the value for the sampling interval.
- Add a "Trapdoor" device type by duplicating the existing Custom Device, and by replacing the various command- and signal texts. For instance: replace **Output 1 High** with **Door 1 open**.

This section instructs how to make simple changes to an existing device. For more complex changes, the Noldus HardwareInterface USB-IO box software and maybe even EthoVision XT itself may need to be changed. Contact Noldus Technical support for wishes and ideas.

In order to carry out the procedure below, you need some experience with computers and xml language.

IN A NUTSHELL

When you install EthoVision XT, the following file: **DeviceTypesNoldus.xml** is copied to the following folders (henceforth named **ProgramData** folder):

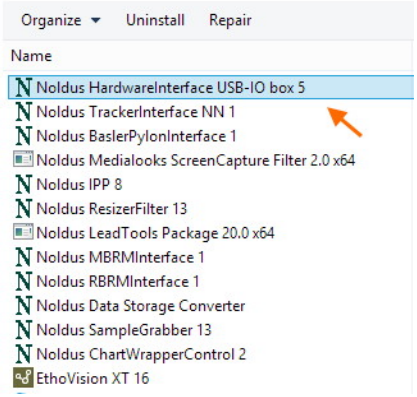
- C:\ProgramData\Noldus\Components\Ethovision\Noldus HardwareInterface **Iobox**\{version number} and
- C:\ProgramData\Noldus\Components\Ethovision\Noldus HardwareInterface **MiniIobox**\{version number}

Two files are present in this folder: **DeviceTypesNoldusChCh.xml** and **DeviceTypesNoldusEnUs.xml**. The first is used with the Chinese version of EthoVision XT, the second with the English version.

The XML file contains all the details of the types of hardware devices that you can control with EthoVision XT with the USB-IO box and the Mini USB-IO box. You can edit this file for small changes in the hardware devices, for example if you want to change the frequency a PhenoWheel counter is read out. You can also define custom devices and create a file **DeviceTypesUser.xml**. If you do so, make sure the file **DeviceTypesUser.xml** and **DeviceTypesNoldus[language version].xml** do not contain settings for the same device.

When you create an experiment, EthoVision XT copies these files to your experiment folder, under **Configuration Files\Add-ins\Hardware\{dll serial number}**. The dll serial number depends on the type of USB-IO box and its version. See in Table 3.1 which serial number belongs to which device and version.

To know which version is the hardware driver installed on your computer, see the Programs and Features list on the Control Panel.



Every time that you open the experiment, EthoVision XT reads the files in the experiment folder, not the ones in the **ProgramData** folder, and does not change them. Therefore:

- If you edit the files in the **ProgramData** folder, the new device types will be available to all your new experiments.
- If you edit the files in a specific experiment folder, the new device types will only be available to that experiment.

When you make a backup of the experiment, the device type files are also copied, so the new devices can be recognized on other computers where you restore the experiment from the backup.

IMPORTANT Always make a backup of the **ProgramData** folder before you edit the file **DeviceTypesNoldus[language version].xml**.

Table 3.1 *The serial numbers for the different versions of the USB-IO box and the Mini USB-IO box.*

Serial Number	USB-IO Box	Mini USB-IO Box
{A8DA8F3E-7EA3-4629-8327-9D21C90FB3ED}	5	
{03081068-1EBE-4620-B00D-396ABEBBC219}		5
{33DF79E0-53C2-44C6-BA2F-A24C4C702615}	4	
{F9AAD805-B910-4FEC-AC2F-31DED85324B2}		4
{C183DC50-0CAB-4E67-905E-9D45D8213BD2}	3	
{B1336ED5-DE91-4C40-A59D-6A2D131C4F9B}		3
{DF396575-4883-47C4-9124-8DE30AE1E4C6}	2	
{2EDFF151-DF50-486E-887F-55AD41457A5B}		2
{83BF6F4E-D35B-489A-9F5E-703C4EE9453B}	1	
{93C9527C-8B2F-4530-9370-7CA330856D15}		1

How to undo changes

If you want to undo the changes made and return to the original situation, proceed as follows:

1. Close EthoVision XT.
2. Remove or rename the file **DeviceTypesUser.xml** in the ProgramData folder.
3. Copy the files from the backup of the **ProgramData** folder to the **ProgramData** folder.
4. (Optional) Re-insert the EthoVision XT installation USB stick and select **Repair**.

Any experiments that you have created which refer to the user devices can still be analyzed and continued. To remove every trace of your user devices, you must also delete these experiments (and if you made experiment backups, delete these too) by removing them from the **Experiments** folder.

DEFINE A NEW DEVICE TYPE

IMPORTANT Before making changes to files in the "ProgramData" folder, make a backup of this folder, so you can recover the files if anything goes wrong. The "ProgramData" folder contains other information important to EthoVision XT. If the files in this folder get corrupted or deleted, you can restore EthoVision XT by re-inserting the installation USB stick and selecting **Repair**.

To define a new device type

1. Make the necessary changes to the **DeviceTypesUser.xml** file in the "ProgramData" folder as described below. Use the program XML Notepad that you can download from www.microsoft.com/downloads. to edit a device type file. Other editors could add undesired characters that may not be read in EthoVision XT.
   - For changes to standard Noldus device types, edit file **DeviceTypesUser.xml**. Note: You can in fact make the changes to the file **DeviceTypesNoldus[language version].xml** but these changes will be overwritten when EthoVision XT is subsequently re-installed, repaired or a new version installed.
   - For the other types, also edit file **DeviceTypesUser.xml**.
2. Test the devices in a new experiment.
3. If EthoVision XT crashes, or does not behave as expected:
   a. Delete the created (failed) experiments.
   b. Make corrections to the file **DeviceTypesUser.xml** in the "ProgramData" folder.
   c. Go to step 2.
4. When everything works fine, create a new experiment and use the changed or new device types.

The change to the customers installation is not recorded in the GLP log (GPL Module only).

SAMPLE FILE

On your installation USB stick you can find two sample files. Open the folder **Drivers and tools/IoBox MiniIoBox Devices**. Unzip the file **User defined device types.zip** and save the files on your computer. The file **DeviceTypeUser-Example.xml** specifies several devices.

```
<?xml version="1.0" encoding="UTF-8" standalone="true"?>
<!DOCTYPE boost_serialization>
- <boost_serialization version="5" signature="serialization::archive">
  - <DeviceTypesUser version="0" tracking_level="0" class_id="0">
    - <m_vecConnectorType version="0" tracking_level="0" class_id="1">
        <count>1</count>
        <item_version>0</item_version>
      - <item version="0" tracking_level="0" class_id="2">
          <m_iId>10000</m_iId>
          <m_strName>User Top Unit Interface</m_strName>
          <m_ePortType>1</m_ePortType>
          <m_iNrPorts>4</m_iNrPorts>
          <m_iOffsetPin>7</m_iOffsetPin>
        </item>
      </m_vecConnectorType>
    - <m_vecDeviceType version="0" tracking_level="0" class_id="3">
        <count>7</count>
        <item_version>0</item_version>
      - <item version="0" tracking_level="0" class_id="4">
          <m_iDeviceTypeId>10000</m_iDeviceTypeId>
          <m_strDeviceTypeName>User Top Unit (Standard)</m_strDeviceTypeName>
          <m_dMaxFrequency>0</m_dMaxFrequency>
          <m_ePortType>1</m_ePortType>
          <m_iConnectorTypeId>0</m_iConnectorTypeId>
        - <m_vecCommand version="0" tracking_level="0" class_id="5">
            <count>6</count>
            <item_version>0</item_version>
          - <item version="0" tracking_level="0" class_id="6">
              <m_iId>1</m_iId>
              <m_strName>White spot on</m_strName>
              <m_bIsDefaultCommand>0</m_bIsDefaultCommand>
              <m_iPin>1</m_iPin>
              <m_bHigh>1</m_bHigh>
              <m_iDuration>0</m_iDuration>
            </item>
          - <item>
              <m_iId>0</m_iId>
              <m_strName>White spot off</m_strName>
              <m_bIsDefaultCommand>1</m_bIsDefaultCommand>
              <m_iPin>1</m_iPin>
              <m_bHigh>0</m_bHigh>
              <m_iDuration>0</m_iDuration>
```

Figure 41 *Part of the sample file* ***DeviceTypeUser-Example.xml****, which you can find on your installation USB stick. The top part specifies the PhenoTyper White spot commands.*

```
- <item>
      <m_iDeviceTypeId>10006</m_iDeviceTypeId>
      <m_strDeviceTypeName>User RunningWheel (60)</m_strDeviceTypeName>
      <m_dMaxFrequency>60</m_dMaxFrequency>
      <m_ePortType>1</m_ePortType>
      <m_iConnectorTypeId>-1</m_iConnectorTypeId>
    - <m_vecCommand>
        <count>0</count>
        <item_version>0</item_version>
      </m_vecCommand>
    - <m_vecSignal>
        <count>1</count>
        <item_version>0</item_version>
      - <item>
          <m_iId>0</m_iId>
          <m_strName>Number of cycles</m_strName>
          <m_eSignalSource>0</m_eSignalSource>
          <m_eSignalHwType>1</m_eSignalHwType>
          <m_iPin>-1</m_iPin>
          <m_iNrBytes>4</m_iNrBytes>
          <m_iIndexFirstByte>0</m_iIndexFirstByte>
          <m_iIndexLastByte>1</m_iIndexLastByte>
          <m_eStateEventType>-1</m_eStateEventType>
          <m_bHighInit>1</m_bHighInit>
          <m_bTotalsSinceLastCall>1</m_bTotalsSinceLastCall>
          <m_eValueType>1</m_eValueType>
          <m_bHasValueDefault>1</m_bHasValueDefault>
          <m_eTypeValueDefault>1</m_eTypeValueDefault>
          <m_bValueDefault>0</m_bValueDefault>
          <m_lValueDefault>0</m_lValueDefault>
          <m_dbValueDefault>0</m_dbValueDefault>
        </item>
      </m_vecSignal>
    </item>
  </m_vecDeviceType>
</DeviceTypesUser>
</boost_serialization>
```

Figure 42 *Part of the sample file* ***DeviceTypeUser-Example.xml****. The bottom part specifies the counter of the RunningWheel.*

For a running wheel, with reading interval of 30 s instead of 60 s as in the standard Noldus running wheel, we would have a file like this:

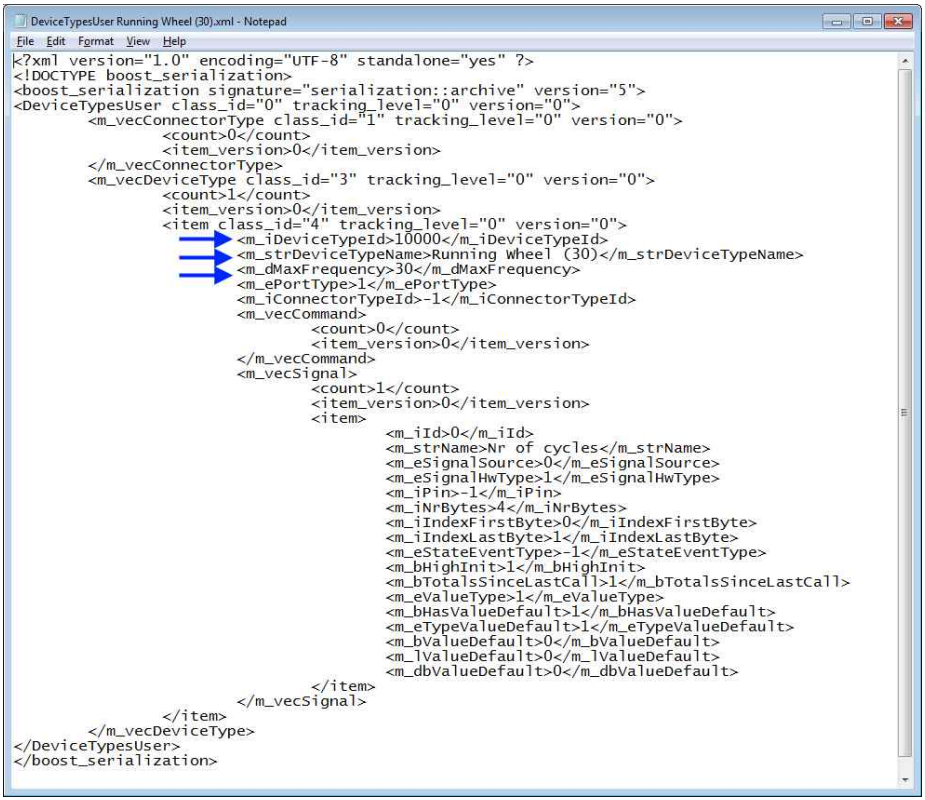


Figure 43 *An XML file that specifies a device type for a running wheel with reading interval of 30 s instead of 60. Arrow indicate the device type ID (10000), name (Running Wheel (30) and frequency (30).*

The new device types are then shown in various elements of EthoVision XT, for example in the Arena-Hardware Mapping window that you access in the Arena Settings.

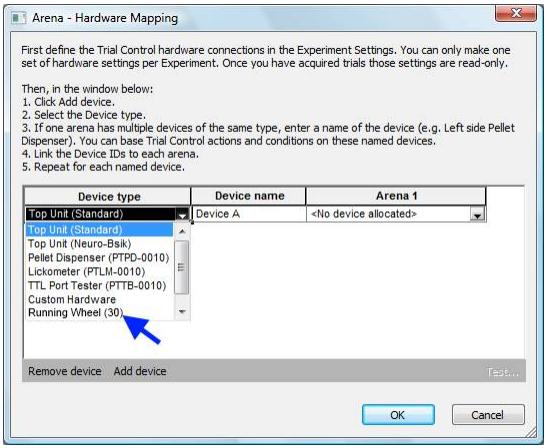


Figure 44 *Arena-Hardware Mapping window showing the new device type in the first column of the table.*

PREDEFINED IDS

For both devices and connectors, IDs must be unique. IDs from 0 to 9999 are for Noldus device (and connector) types. IDs above 10000 are reserved for device (and connector) types that you may want to add.

If you create a device type based on a Noldus device type (for example a Lickometer), always use an ID above 10000.

Device type ID	Name
0	Top Unit (Standard)
1	Top Unit (Special)
2	Pellet Dispenser (PTPD-001x)
3	Lickometer (PTLM-001x)
4	TTL Port Tester (PTTB-001x)
5	Custom Hardware
6	PhenoWheel

7	USB-IO box monitor
8	Beam Controller (PTBC-0010)
9	Activity Wheel Counter (ENV-042)
10	Activity Wheel Brake (ENV-042)
11	Backlight Unit White Light
12	Top Unit Tester (PTTB-002x)
13	Pellet Receptacle (PTPR-001x)

Connector type ID	**Connector type name**
0	Top Unit Interface (PTTI-001x). Note that the Top Unit Standard and Special both use this type of connector.

XML FILE ENTRIES

Not all of the entries below always apply to each signal, command, etc. If you make edits in a device type file, beware that besides editing the data below you might have to change some numbers that the boost::serialize normally creates itself (vector counts, for example).

NCHilobConnectorTypes

Devices can be connected to the USB IO box (or mini IO box) via a connector. There can be more than one connector type in the setup. Following are the properties of the collection of connectors (**m_vecConnectorType**):

Count	Indicates the number of connectors
Item_version	Indicates the version of the connectors

Following are properties of each connector:

NCHilobConnectorType	
Hilob::ConnectorTypeId m_iId	Every connector type must have a unique id. g_ConnectorTypeIdInvalid(-1l): invalid value
Item_version	Indicates the version of the connectors
nstring m_strName	name of the connector

Hilob::ePortTypes m_ePortType	ePrtUndefined(-1): undefined ePrtSD(0): simple device port ePrtCD(1): complex device port
int m_iNrPorts	number of ports used by the connector
int m_iOffsetPin	pin offset for the device connected via the connector

NCHilobDeviceTypes

Devices can be connected to the USB-IO box directly (e.g., not via a connector). Following are the properties of the collection of device types (**m_vecDeviceType**):

Count	Indicates the number of device types
Item_version	Indicates the version of the device types

Following are properties of each device type:

NCHilobDeviceType

Hilob::DeviceTypeId m_iDeviceTypeId	Every device type must have a unique id. g_DeviceTypeIdInvalid(-1l): invalid value
nstring m_strDeviceTypeName	name of device type, showing up in the UI of port-device mapping dialog (hwdll) and various places in EthoVision XT (for example: ATC, test dialog, etc).
int m_MaxFrequency	Most device types are read every read cycle and have m_MaxFrequency = 0. So called "slow device types" (e.g., Lickometer) are not read every read cycle but with the slower frequency MaxFrequency (in seconds). Use integer values only.
Hilob::ePortTypes m_ePortType	ePrtUndefined(-1): undefined ePrtSD(0): simple device port ePrtCD(1): complex device port
Hilob::ConnectorTypeId m_iConnectorTypeId	Every connector type must have a unique id. Most device types do not use a connector and have value. g_ConnectorTypeIdInvalid(-11): invalid value Currently the only connector type is the top unit connector, used with the top unit.

NCHilobSignal

Each device type can have more than one signals. Following are the properties of the collection of signals (m_vecSignal):

Count	Indicates the number of signals
Item_version	Indicates the version of the device type signals

Following are properties of each signal:

Hilob::SignalId m_iId	Every signal must have a unique (within device type) id. g_SignalIdInvalid(-11): invalid value
nstring m_strName	Name of signal, showing up in the interface of Device configuration window and other places, for example: The test window in Trial Control).
Hilob::eSignalSource m_eSignalSource	eSrcUndefined(-1): undefined eSrcInput(0): signal comes from the hardware eSrcOutput(1): signal is created in the software and is related to an output state. E.g.: there are outputs "White spot off" and "White spot on", for these outputs "Is white spot on" is a output state that is treated as an (artificial, because not coming from the hardware) input.
Hilob::eSignalHwType m_eSignalHwType	eShtUndefined(-1): undefined eShtPin(0): uses pins (most device types) eShtBytes(1): uses direct bytes reading (e.g.: lickometer)
long m_iPin	the pin used by the signal. Only relevant if eSignalHwType = eShtPin.
int m_iNrBytes	the number of bytes used for reading the signal. Only relevant if both m_eSignalSource = eSrcInput and eSignalHwType = eShtBytes.
int m_iIndexFirstByte	least significant byte. Only relevant if both m_eSignalSource = eSrcInput and eSignalHwType = eShtBytes.
int m_iIndexLastByte	most significant byte. Only relevant if both m_eSignalSource = eSrcInput and eSignalHwType = eShtBytes.

HiIob::eStateEventType m_eStateEventType	eSetUndefined(-1): undefined eSetL(0): signal is based on Low state eSetH(1): signal is based on High state eSetL2H(2): signal is based on transitions from Low to High eSetH2L(3): signal is based on transitions from High to Low Only relevant if eSignalHwType = eShtPin
bool m_bHighInit	initial value for the signal. True if High, false if Low. Only relevant if m_eSignalSource = eSrcInput and eSignalHwType = eShtPin.
bool m_bTotalsSinceLastCall	true if signal returns a value (via the hwdll COM interface) that represents a total since the last call.
EAdinsIdtHiValueType m_eValueType	The type of the value the signal returns (via the hwdll COM interface) One of the values: eAdinsIdtHiVtUndefined(-1) eAdinsIdtHiVtBool(0) eAdinsIdtHiVtLongLong(1)eAdinsIdtHiVtDouble(2) eAdinsIdtHiVtDuration(3) This value is used by EthoVision XT: Trial Control and logging
bool m_bHasValueDefault	true if the signal has a default value. Indicates how the value of the signal has to be treated by the EXPORTED hardware log in VTS: If true: only the values not equal to the default value are logged If false: only changes in the values are logged (default value is not used)
NCHiIobValue m_valueDefault	Default value for the signal. Only relevant if m_bHasValueDefault = true;
HiIob::eValueType m_eTypeValueDefault	Default value type for the signal (see m_eValueType) for the values.

bool m_bValueDefault	Default value for the signal if m_eValueType = eAdinsIdtHiVtBool.
LONGLONG m_lValueDefault	Default value for the signal if m_eValueType = eAdinsIdtHiVtLongLong.
double m_dbValueDefault	Default value for the signal if m_eValueType = eAdinsIdtHiVtDouble.

NCHilobCommand

Each device type can have more than one commands. Following are the properties of the collection of commands (m_vecCommand):

Count	Indicates the number of commands
Item_version	Indicates the version of the device type commands

Following are properties of each command:

HiIob::CommandId m_iId	Every command must have a unique (within device type) id. g_CommandIdInvalid(-11): invalid value
nstring m_strName	name of command, showing up in the UI of port-device mapping dialog (hwdll) and various places in EthoVision XT (for example: Trial Control boxes, test dialog, etc).
bool m_bIsDefaultCommand	true if command is default command. Default commands are called before start sampler (start of a trial) and after stop sampler (end of a trial).
long m_iPin	the pin used by the command (if any).
bool m_bHigh	true if command corresponds to a High bit value, true if command corresponds to a Low bit value
int m_iDuration	duration in milliseconds. After writing this command a new command is scheduled to be written the given duration time later (only if duration > 0). If the original command was High, the scheduled command is Low and vice versa. Used for the pellet feeder to reset.

11 Analysis of Trial Control data

INTRODUCTION

You can analyze the events that occur during a trial by means of statistics or time plots. You can answer questions like:

- **Simple events**. How many times did the subject press the lever? ➔ See page 167
- **Intervals**. How much time elapsed from the moment the cue light switched on to when the subject entered the reward zone? ➔ See page 169

  Analyze learning behavior. Did the time between the event *Light on* and the event *Subject in reward zone* decrease during the test? ➔ See page 173
- **Data within an interval**. How many times did the subject press the lever from the moment the reward was delivered to when it was eaten? ➔ See page 174
- **Trial Control variables**. For example, what is the maximum value of the variable Counter that I defined in the Trial Control Settings? ➔ See page 175
- **Hardware command**. How many times did EthoVision send a hardware command to, for example, a pellet dispenser to drop a pellet? ➔ See page 175
- **Hardware variables**. How many times did your mouse drink from its water bottle?

  ➔ See page 176 and page 179

Trial Control data are analyzed as *Dependent variables* defined in Analysis Profiles. For more information, see **Dependent variables** in the EthoVision XT Help.

Notes

- To visualize Trial Control data, from the **Analyze** menu, select **Results** and then **Plot Integrated Data** (see the EthoVision XT Help). You find an example of visualization of Trial Control data on page 41.
- To export Trial Control data, see page 182.
- Trial statistics of Duration and Latency can only be a multiple of the sample interval (=1/sample rate). For example, a condition like *Subject in zone A for >= 3 s* is met when the time elapsed from its activation exceeds 3 s. If the sample rate is 12.5 frames/s (thus the sample interval is 1/12.5= 0.08 s), the condition is met at the first multiple of 0.08 greater than 3 s, that is 3.04 s. For this reason, the latency of the event *Condition becomes true* is 3.04 s.

SIMPLE EVENTS

If you are interested in the rate of events like the number of times the lever was pressed, or the number of pellets dropped, or the latency of an action, use the Trial Control event function in the Analysis profile.

1. In the **Experiment Explorer**, right-click **Analysis Profiles** and select **New**, or click an existing profile.
2. In the **Dependent Variables** pane, under **Trial Control**, double-click **Trial Control event** or drag it to the middle of the screen, or click the button next to it.
3. The Trial Control event window appears. Under **Event**, from the **Element** list select the element to be analyzed. For example, if you want to analyze the hardware-base action "Drop pellet" locate this among **Action: Drop pellet** and select it.
4. From the **Event** list, select the event for the element selected. The options available depend on what you have chosen as Element (see Possible states of Trial Control elements on page 168).
5. Click the **Trial Statistics** tab and select the statistic you want to analyze.
6. Click the **Group Statistics** tab and select additional statistics if you want summarized statistics over all trials, or the groups of trials defined in your Data Profile.
7. Click **Add**. The new Trial Control event appears in the list of Selected Dependent Variables.
8. Choose the option you require:
   - To calculate statistics of the selected events, choose **Analyze** > **Results** > **Statistics & Charts**. (see **Calculate Statistics** in the EthoVision XT Help).
   - To visualize the events, choose **Analysis > Results** > **Plot Integrated Data** (see **Visualize Data** in the EthoVision XT Help).

Notes

- If one or more other instances of Trial Control event are listed under **Selected Dependent Variables** in the main window, the Trial Control event name is followed by a progressive number (1, 2, 3...).
- The Element list contains the Trial Control elements defined in all your Trial Control Settings. For each element, the list shows the name of the corresponding Trial Control box.
- Choose **Latency to first** to analyze the time of the first instance of the event defined since the start of tracking. Choose **Latency to last** to analyze the time from the start of data recording to the last instance of the event defined.

- For calculating frequency and latency of the subject's behavior, choose the appropriate Dependent Variable (see **Dependent variables in detail** in the EthoVision XT Help).

Possible events of Trial Control elements

- **Becomes active** (for Start-stop trial rule begin, sub-rule begin, conditions, actions, references and operators). Marks the time that the trial starts (for the Start-stop trial rule begin) or the element becomes active (in all other cases). This is the time when control passes from the previous box in the Trial Control sequence. See the Notes below for the difference between **Becomes active** and **Becomes true**.
- **Becomes true** (for conditions and operators). Marks the time that a condition is met. Choose this option for example to analyze the instances when the condition *In zone A* was met.
- **Becomes false** (for conditions and operators). Marks the time that a condition is not met. Choose this option for example to analyze the instances when the condition *In zone A* was not met.
- **Becomes inactive** (for Start-stop trial rule end, sub-rule end, conditions, actions, references and operators). Marks the time that the element becomes inactive, that is, when control passes to the next box in the Trial Control sequence. In practice, this is the time a condition becomes true or an action is taken. For the Start-Stop trial rule end, this is the time that the trial stops.
- **Makes sub-rule active** (for sub-rule references). Marks the time that a reference to a sub-rule triggers the sub-rule. This is the time that one of the Start conditions set in the Reference box (see page 59) becomes true.
- **Makes sub-rule inactive** (for sub-rule references). Marks the time that the last repeat of a sub-rule planned for a specific Start condition is completed.

Notes

- For a condition like *Is the subject in zone A?*, choose **Becomes true** to consider the moment when the condition is met. Choose **Becomes inactive** when you want to consider the time that a condition could be met (no matter whether or not the subject is in zone A).
- Becomes active/inactive are especially used when analyzing intervals (see below).

TRIAL CONTROL STATES

If you are interested in the time interval between two events, for example from the Action "*cue light on*" to the Condition "*subject on shelter*" being met, use the Trial Control state function in the Analysis profile.

1. In the **Experiment Explorer**, right-click **Analysis Profiles** and select **New**, or click an existing profile.
2. In the **Dependent Variables** pane, under **Trial Control**, click **Trial Control state** or drag it to the middle of the screen, or click the button next to it.

   The Trial Control State window appears (see below).
3. Next to **From**, from the **Element** list select the Trial Control element that makes the criterion for the start of the interval. From the **Event** list, select the event that makes the start of the interval (see the previous page for information on the events available for a specific element).
4. Next to **To**, from the **Element** list select the Trial Control element that makes the criterion for the end of the interval. From the **Event** list, select the event that makes the end of the interval.

   The event that marks the end of the interval may occur multiple times. Therefore, choose which **occurrence** (from **1th** to **9th**) should be used. **1th** is the first occurrence of the event selected under **To** available after each occurrence of the event selected under **From**. See an example in Figure 46.

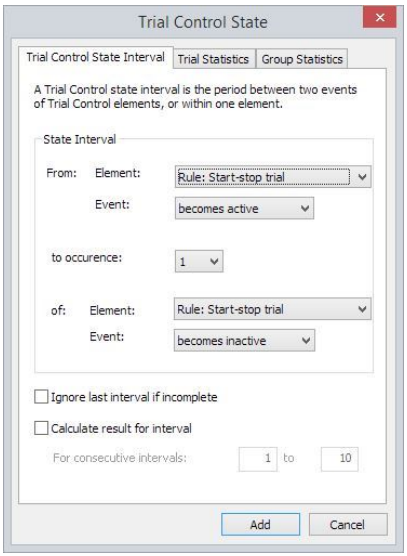


Figure 45 *The Trial Control State window.*

5. Select **Ignore last interval if incomplete** to ignore the interval when the Trial Control event that defines the end of the state is not found. If you do not select this option, and the end criterion is not met, EthoVision XT defines an interval up to the end of the track.

6. An interval may occur several times in a trial. If your want to have statistics for each occurrence, select the **Calculate statistics per interval** option. Next to For consecutive intervals, choose the range of occurrences of the intervals you want to have in the results.

   EXAMPLE If the interval from *Light On* (cue) to *Mouse on Shelter* (response) occurs 10 times and you want to have the first four occurrences displayed in your table, select 1 to 4. If you want to have all occurrences displayed, select 1 to 10.

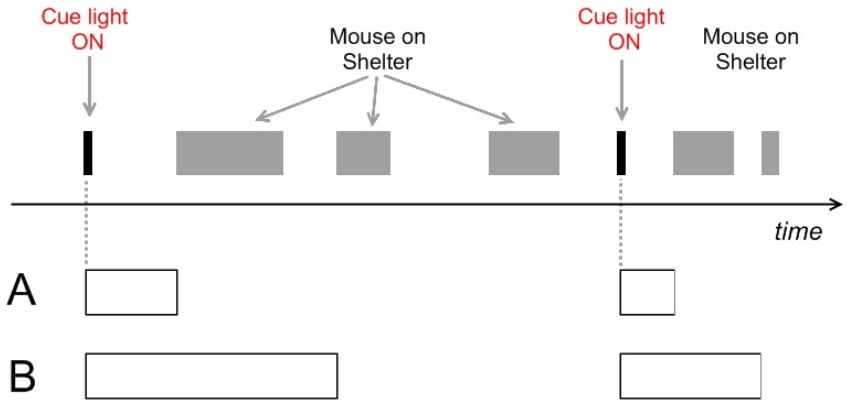


Figure 46 *An example of time intervals defined with in Trial Control data. In a conditioning test, it is required to teach the subject to sit on the shelter after a cue stimulus is given, in order to get a reward. An interval is defined from when the cue light switches on to when the subject is found on top of the shelter. Solid bars represent the time that the subject is on the shelter (condition met).* ***A*** *- Interval from "Cue light On" to 1st occurrence of "subject on shelter",* ***B*** *- Interval from "Cue light On" to 2nd occurrence of "subject on shelter".*

If you do not know exactly how many instances of the state interval the tracks contain, enter a number reasonably high (for example 50 or 100) so you make sure analysis is carried out on all instances.

NOTE If you do not select this option, the multiple occurrences are considered as one interval. Click the **Trial Statistics** tab and select the statistic you want to analyze. Click the **Group Statistics** tab and select additional statistics if you want summarized statistics over all trials, or the groups of trials defined in your Data Profile. Click **Add**. The new Trial Control state appears in the list of Selected Dependent Variables.

7. Choose the option you require:
   - To calculate statistics of the selected intervals, from the **Analysis** menu, select **Results** and then **Statistics & Charts**. The analysis window that appears has the tab **Trial Statistics** for the analysis results per trial and **Group Statistics** for the statistics and charts from the summarized results over different trials. If you have different filters, data profiles, or analysis profiles make your selection on the toolbar. Click **Calculate**. (see **Calculate Statistics** in the EthoVision XT Help).
   - To visualize the intervals, choose **Analyze** > **Results** > **Plot Integrated Data** (see **Plot integrated data** in the EthoVision XT Help).

Notes

- If one or more other instances of Trial Control state are listed under **Selected Dependent Variables** in the main window, the Trial Control state name is followed by a progressive number (1, 2, 3...). We advise you to rename the trial control state (right-click the name and select **Rename**).
- The Element list contains the Trial Control elements defined in all your Trial Control Settings. For each element, the list shows the Name of the corresponding Trial Control box.
- Choose **Becomes active** instead of **Becomes true** when you want the interval to be based on a condition from the moment it could have been met, no matter when it was met actually. See example 1 on page 173.
- If you define an interval by choosing the same event under **From** and **To**, the instance of the event marking the end of the interval is not used as a starting point for the next occurrence of the interval.

  For example, you have a sequence of four actions of the same type: A1, ..., A2, ..., A3, ..., A4. Selecting a trial control state **From A To A** results in analyzing the time between A1 and A2, and between A3 and A4.
- For Trial Control states based on *Conditions*:

  The sample time at which a Trial Control state starts (or ends) also depends on the statistic used in the condition in the Trial Control rule.
  - If you use **Current** to define the condition (for example, "when Current *In zone*= true"), the Trial Control state starts (or stops) at the expected sample time. For example, when the animal actually enters the zone (and thus **Current**= true for that sample).
  - If you use any other statistic to define the condition (for example, "when **Frequency** of *In zone* >= 1):
    - When the condition becomes true *after* the condition box is *activated* (see Figure 3 on page 11 for an explanation of the terms true and active), the Trial Control State starts (or stops) at the expected sample time (see **A** in Figure 47).

- When the condition is already true when the condition box is *activated* (for example, a condition "Frequency of *In zone* =1" is activated when the animal is already in the zone), such statistic is only evaluated at the next sample (or in the second next sample, in the case of Heading). In that case, the Trial Control state starts (or stops) one sample (or two) later than expected from the condition (see **B** in Figure 47). However, for the consecutive frequencies of *In zone* =2, 3, etc, the condition is already active by definition; therefore the Trial Control State starts at the expected time, when the condition becomes *true*.

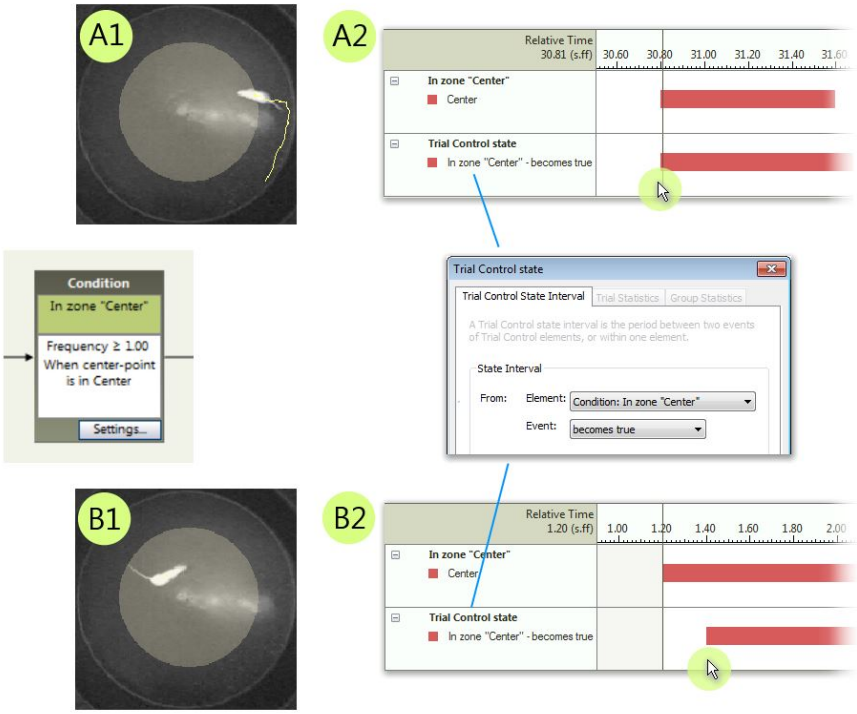


Figure 47 *In this example, a Trial Control condition has been defined in the Trial Control Settings, which checks that the Frequency of In zone is >=1 for the Center of the open field. A Trial Control state has been defined in the Analysis profile, which starts when the condition "Frequency of In zone >=1" is* **true**.
A1 — *The animal enters the Center zone (and therefore the condition becomes* **true***) after the condition is activated. The* Frequency *statistic is evaluated at the same sample time.* **A2** — *Plot of the variables "In zone" for the Center and the Trial Control state. The Trial Control state starts at the expected sample time, that is, when the animal actually enters the zone.*
B1 — *The animal is already in the zone when the condition is activated. Therefore, the condition becomes* **true** *at the same time that it is activated, but the* Frequency *statistic is evaluated at the next sample (here, sample rate = 5/s, thus after 0.2 s).* **B2** — *Same plot as A2; Here, the Trial Control state starts 0.2 s after the In zone state.*

- If you de-select **Calculate statistics per interval**, the Cumulative duration statistic is the total duration of the intervals in that trial.
- If you want to know how many occurrences of the intervals a specific trial contains, follow the procedure above making sure that **Calculate statistics per interval** is **not** selected, and **Frequency** is selected under **Trial Statistics**. Click the **Group Statistics** tab and select additional statistics if you want summarized statistics over all trials, or the groups of trials defined in your Data Profile. Next, run the analysis. The table shows the number of intervals under Frequency for that dependent variable. The occurrences are counted if the **From** condition is met. If the trial ends before the **To** condition is met, the interval is still counted in the Frequency.

Examples of Trial Control states

EXAMPLE 1 In a conditioning test, it is required that the subject must go to zone A in order to receive a reward. The researcher wants to know the time from the moment the subject could have responded (Condition *Subject in zone A* becomes active) to the moment the subject eats the reward (Condition *Subject in feeder zone* becomes true).

The Trial Control sequence is as follows: Rule Begin ➔ Condition - In zone A ➔ Action - Drop a food item ➔ Condition - In Feeder zone ➔ Rule End.

In the Analysis profile, click **Trial Control state** and select:

- **From - Element Condition**: In Zone A, Event= becomes active,
- **To - 1st occurrence of Element Condition**: In Feeder zone, Event= becomes true.

In the **Trial Statistics** tab, make sure that **Cumulative duration** is selected.

EXAMPLE 2 In a variant of the experiment above, the researcher wants to know the time that it takes the mouse to get the pellet after being in the zone A. Here we assume that if the animal enters zone A, then gets out and subsequently gets back in it before collecting the food item, one wants to analyze from the first instance that the mouse went in zone A.

In the **Analysis Profile**, click **Trial Control state** and select:

- **From - Element Condition**: In Shelter zone, Event= becomes true (because one wants to analyze the time from when the condition is met).
- **To - 1st occurrence of Element Condition**: In Feeder zone, Event= becomes true.

In the **Trial Statistics** tab, make sure that **Cumulative duration** is selected.

Analyze learning behavior

When a sequence like Cue stimulus ➔ Subject's action ➔ Reward is repeated a number of times, you may want to know whether there is a trend of decreasing/increasing duration of

intervals from one component of the sequence to the next. For example, is the time from the cue stimulus to the subject's lever press decreasing during a 30-minutes test?

To analyze learning behavior, follow the procedure on page 169, and make sure that:

- In the **Trial Control State Interval** tab, select the **Calculate statistics per interval** option.
- Next to **For consecutive intervals**, select which occurrences of the interval you want to analyze.
- In the **Trial Statistics** tab, make sure that **Cumulative duration** is selected. Click the **Group Statistics** tab and select additional statistics if you want summarized statistics over all trials, or the groups of trials defined in your Data Profile.

Calculate the statistics, and export the results (see **Calculate Statistics** in the EthoVision XT Help).

DATA WITHIN TRIAL CONTROL STATES

If you want to calculate statistics on or visualize data *within* intervals defined by Trial Control events, then you must first define those intervals as Nesting intervals in the Data Profile.

1. In the **Experiment Explorer**, right-click **Data Profiles** and select **New**, or click one of the existing profiles.
2. In the **Components** pane, under **Nesting**, double-click **Trial Control state** or drag it to the middle of the screen, or click the button next to it.
3. The Trial Control state window appears. Define the interval as described in steps 3 to 5 of the procedure on page 169 (the options are the same, only the Statistics tab is not present).
4. Click **OK**. The Nest box appears on the screen. Insert this box between the **Start** and the **Result** box.
5. In the **Experiment Explorer**, right-click **Analysis Profiles** and select **New**, or click one of the existing profiles.
6. Define the dependent variables you want to analyze and run analysis or visualize the data.

Notes

- If the interval specified in step 3 above occurs in multiple instances, the program considers the total time line included in those intervals to calculate durations, frequencies etc.
- If your experiment includes multiple subjects per arena, the **Nesting over Subjects** group of options is also available. Select **Trial Control state** under **Nesting over Subjects** if you

want to define an interval based on what one or more of the subjects do during the trial. For example, to define an interval that goes from the action "*Cue light on*" when activated, to the condition "*Subject 1 in zone A*" when it becomes true. Click the **Actors** tab and select the subject that the condition refers to.

Analysis will be done for all the subjects, in the interval defined by Subject 1. Note that conditions can be met by specific subjects, while actions cannot be triggered by specific subjects. However, they can be triggered indirectly, when an action follows a condition met by that subject.

- For information on Nesting, see **Analyze Track Segments** in the EthoVision XT Help.

TRIAL CONTROL VARIABLES

You can analyze and visualize the values of the Trial Control variables defined in the Trial Control Settings. Use this function also for testing purposes. For example, to check that a Trial Control variable receives the value expected at a specific time.

1. In the **Experiment Explorer**, right-click **Analysis Profiles** and select **New**, or click an existing profile.
2. Click the **Add** button next to **Trial Control variable** and choose a variable from the list.
3. Calculate the statistics or plot the variable with the Integrated Visualization.

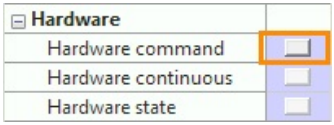
For more information, see the EthoVision XT Help.

HARDWARE COMMAND

A Hardware command is a command given by the EthoVision software to your hardware device. You can define when and what hardware commands are sent as Actions in your Trial Control settings. For instance, you can have EthoVision send a command to your pellet dispenser to drop a pellet after the mouse has been detected in the Trigger zone. For more information about programming Trial Control see Chapter 3.

How to add a Hardware command to an Analysis Profile

1. In the **Experiment Explorer**, right-click **Analysis Profiles** and select **New**, or click an existing profile.
2. Click the **Add** button next to **Hardware command**.



The **Hardware command** window opens.

3. From the **Device type** list, select the hardware device you want to calculate statistics for. For example, if you want to analyze how many *Drop pellet* commands EthoVision gave to your pellet dispenser, select **Pellet Dispenser (PTPD-001x)**.
4. In the **Device** list, the name of the hardware device will appear as you defined it when you assigned the device to the arena (see page 112).
5. From the **Command** list, select which command you want to analyze. For instance, for the Phenotyper's Top Unit, you can select *White spot on*, *White spot off*, *Yellow light on*, *Yellow light off*, *Sound on* or *Sound off*. For other hardware devices (Pellet Dispenser, Lickometer) there is only one option.
6. Click the **Trial Statistics** tab to choose the statistics for the dependent variable. Next, click **Add**. Click the **Group Statistics** tab and select additional statistics if you want summarized statistics over all trials, or the groups of trials defined in your Data Profile. See **Calculate Statistics** in the EthoVision XT Help details.

Application

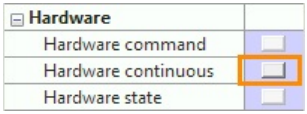
By selecting Hardware command as Dependent Variable, you can calculate the frequency of the commands the EthoVision software gives to your hardware device. In addition, you can calculate the latency to the first command and the latency to the last. You can also visualize Hardware commands. This enables you to check whether your Trial Control settings do what you expect them to do. For example, visualize the Hardware command *Drop pellet* for your pellet dispenser and plot it in the integrated visualization together with the video to check how often the command was given and when. Hardware commands appear as Point events in your plot, that is, as events with no duration (see the upper panel in Figure 48 for an example).

HARDWARE CONTINUOUS VARIABLE

After EthoVision XT has started tracking, connected hardware devices start sending signals to the EthoVision computer. You can analyze these signals by adding Hardware continuous variables to your analysis profile. You can calculate/ visualize, for instance, the number of pellets dropped by your pellet dispenser (see Figure 48 for an example) or the duration of licks at the lickometer.

How to add a Hardware continuous variable to an Analysis Profile

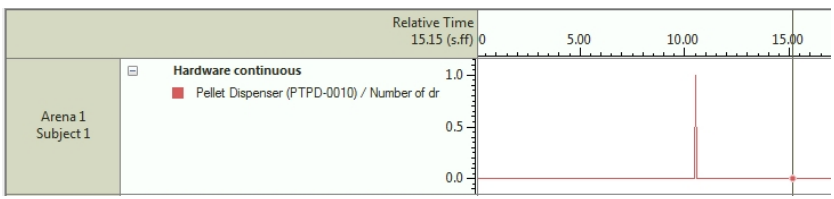
1. In the **Experiment Explorer**, right-click **Analysis Profiles** and select **New**, or click an existing profile.
2. Click the **Add** button next to **Hardware continuous**.



3. The Hardware continuous variable window opens. From the **Device type** list, select the hardware device you want to calculate statistics for. For example, if you want to analyze how many pellets your pellet dispenser dropped, select **Pellet Dispenser (PTPD-001x)**.
4. In the **Device** list the name of the hardware device will appear as you defined it when you assigned the device to the arena (see page 112).
5. From the **Signal** list, select which signal you want to analyze. For instance, for the pellet dispenser you can select *Number of drops*.
6. Click the **Trial Statistics** tab to choose the statistics for the dependent variable. If you want summarized statistics over all trials, or the groups of trials defined in your Data Profile, click the **Group Statistics** tab and select additional statistics. Next, click **Add**.

   For details, see **Calculate Statistics** in the EthoVision XT Help.

An example of a visualization of a hardware continuous variable is shown below.



Application

By selecting Hardware continuous variable as Dependent Variable, you can calculate, for instance, how many pellets your pellet dispenser dropped or how long your mouse has been drinking from its water bottle. You can also calculate the frequency drinking from the bottle took place. But often many of these drinking occurrences together form a single drinking bout. Analyzing such bouts is not possible in the current version of EthoVision XT. This means that to calculate how often such bouts occurred, you have to export the data to a Hardware log and analyze this in an external program like Excel.

Note

Devices such as the Lickometer and PhenoWheel send data to EthoVision XT at fixed times, every two seconds for the Lickometer and every 60 seconds for PhenoWheel. At each sample time, EthoVision XT reads the data since the last reading event:

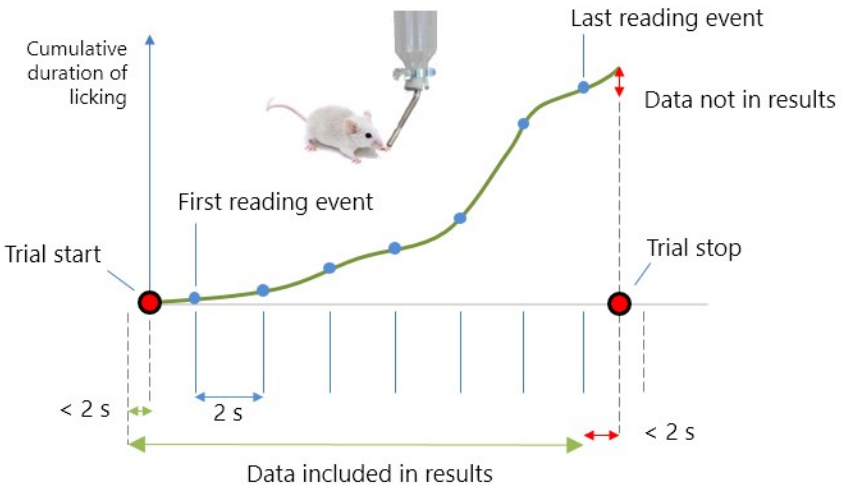
- For the Lickometer, number and duration of licks.
- For PhenoWheel: number of rotations.

The reading events are not in sync with the trial start and stop. This means that (see the figure below for an example with the Lickometer):

- The data of the first reading event *after* the trial start also include some time *before* the trial start (< 2 s for Lickometer, < 60 s for PhenoWheel). This time is included in the results (figure below, left).
- The data between the last reading event and the stop of the trial are **not** included in the results (figure below, right). This time is again < 2 s for Lickometer, < 60 s for PhenoWheel, depending on when the trial stopped.

In most cases the two discrepancies have no effect on your data when the trial lasts at least a few minutes or hours, like in typical PhenoTyper tests.

TIP If you want the devices to have another sampling rate, contact Noldus.

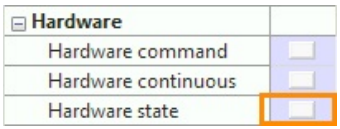


HARDWARE STATE VARIABLE

A variable based on a Hardware continuous variable. You can, for example, define a Hardware state variable *Number of dropped pellets is equal to or greater than 1* and calculate, for instance, the duration of this interval.

How to add a Hardware state variable to an Analysis Profile

1. In the **Experiment Explorer**, right-click **Analysis Profiles** and select **New**, or click an existing profile.
2. Click the **Add** button next to **Hardware state**.



3. The Hardware state variable window opens. From the **Device type** list, select the hardware device you want to calculate statistics for. For example, if you want to analyze the signal from your pellet dispenser, select **Pellet Dispenser (PTPD-001x)**.
4. In the **Device** list the name of the hardware device will appear as you defined it when you assigned the device to the arena (see page 112).
5. From the **Signal** list, select which signal you want to analyze. For instance, for the pellet dispenser you can select *Number of drops* or *In error state*.
6. Set the appropriate **Value** (threshold) or select the value you are interested in. For instance, for *Number of drops* set a threshold of *equal to or greater than 1*, for *In error state* select *true* of *false*.

   Cumulative value. Select this option to calculate/visualize the cumulative value of the signal. See Figure 48 for an example of the effect of selecting this option.
7. Click the **Trial Statistics** tab to choose the statistics for the dependent variable. Click the **Group Statistics** tab and select additional statistics if you want summarized statistics over all trials, or the groups of trials defined in your Data Profile. Next, click **Add**. For details, see **Calculate Statistics** in the EthoVision XT Help.

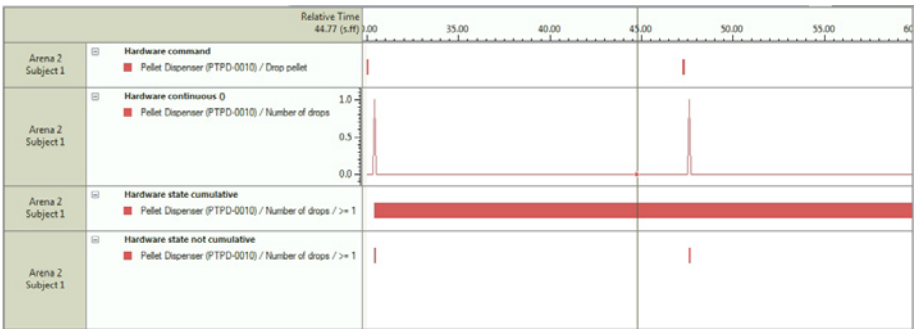


Figure 48 *From top to bottom: Integrated visualization of four Dependent Variables 'Hardware command', 'Hardware continuous variable', 'Hardware state variable' with the option 'cumulative value' selected and 'Hardware state variable' with the option 'cumulative value' not selected. In this learning experiment a rat received a pellet when it climbed on top of its shelter. The rat did this two times and EthoVision two times gave a command to the pellet dispenser to drop a pellet (see the plot at the top). The pellet dispenser dropped a pellet after each command and sent a signal to the EthoVision software (two times) (second plot). For plots 3 and 4, the state variable 'Number of drops is equal to or greater than 1' is visualized. In plot 3 the option 'cumulative value' was selected, in panel 4 this option was not selected. The duration of one occurrence of the state variable in panel 4 is one sample (0.04 seconds if the sample rate is 25 frames/second).*

Application

You can use Hardware state variables to analyze, for instance, how long a light was on. You can also use Hardware state variables to analyze learning behavior. For instance, when training an animal you may want to know how long it takes before it completes a task (and receives a food pellet). For this purpose, you can calculate the statistic 'Latency to first' for the Hardware state variable 'Number of drops greater than or equal to 1'. For naive animals the latency will be high at the start of the training and will decrease as the animals learn the task in subsequent trials.

Please note that the following question requires a different procedure: What was the average time between "*light on*" and "*lever press*" in a learning experiment? It is not possible to define a variable based on two separate hardware events. To answer this question, define a Free interval using hardware events. For more information, see **Free interval** in the EthoVision XT Help.

FREE INTERVALS BASED ON TRIAL CONTROL AND HARDWARE DATA

Free intervals are the most flexible solution to analyze slices of time which go from an event, or time, to another event or time. You can use them in the Analysis profile and in the Data profile.

EXAMPLE In a DanioVision experiment, analyze the speed of the subjects in the 5-s time interval before the onset of the light stimulus; and in a second interval, in the 5-s time interval after the onset of the light stimulus. Because you want to analyze the speed *within* the intervals, define those intervals Free intervals in the Data profile.

How to add a Free interval to an Analysis profile

In the Analysis profile, under **Custom Variables**, click the **Add** button next to **Free interval**.

For more information, see **Dependent variables in Detail** > **Custom Variables** in the EthoVision XT Help.

How to add a Free interval to a Data profile

In the Data profile, under **Nesting**, click the **Add** button next to **Free interval**.

For more information, see **Analyze Track Segments** > **Nesting over a free interval** in the EthoVision XT Help.

THE ANALYSIS RESULTS

To calculate the statistics

1. Click **Statistics & Charts** in the Project Explorer. The analysis window that appears has the tab **Trial Statistics** for the analysis results per trial and **Group Statistics & Charts** with statistics and charts from the summarized results over different trials.
2. If you have different filters, data profiles, or analysis profiles make your selection on the toolbar.
3. Click **Calculate**.
4. **OPTIONAL** Click the **Layout** button to modify the layout of the results table.

Batch analysis

To run different analyses with different combinations of these filters, do the following:

1. Click **Batch.**
2. Select the profiles from the lists and click **Add.**

3. Repeat step 2 for other combinations.
4. Click **Calculate**.
5. Select the combination of profiles from the lists on the toolbar to view the results.

EXPORT TRIAL CONTROL DATA

You can export Trial Control data just like the other dependent variables.

1. Choose **Analysis** > **Export** > **Raw Data**.
2. Select the following:
   - **Track & dependent variables**. To export events and intervals defined in the Analysis profile. For example, actions and conditions and intervals in between (see page 166). This option also includes hardware-based actions and conditions, not the actual communication signals.
   - **Hardware log**. To export the communication signals between EthoVision and the hardware device (see below).
3. Click **Start Export**.

Track data file

In the track data file, each Trial Control event or Trial Control state defined in the Analysis Profile is represented by a column.

Time	Trial Control event "Drop pellet"	Trial Control state From "Light On" to "Drop pellet"
0	0	0
0.04	0	0
0.08	0	0
0.12	0	0
0.16	0	1
0.2	0	1
0.24	0	1
0.28	0	1
0.32	0	1
0.36	1	1
0.4	0	0

Figure 49 *An example of Track data export file that includes a Trial Control event "Drop pellet" and a Trial Control state "From Light On to Drop Pellet". The arrows mark the event and the start/stop of the state. To export such data, define them first in the Analysis profile. For clarity, other columns have been hidden.*

- A Trial Control event is exported as "1" in the row corresponding to the time when the event occurs. In the other rows, it is exported as "0".

- A Trial Control state interval is exported as "1" in the rows corresponding to the time included in the interval. This can be multiple segments of "1" if the interval occurs in multiple instances in the trial.

For more information about exporting track data, see **Export tracks** in the EthoVision XT Help.

Hardware log file

When selecting Hardware log in the export screen, hardware events are exported to a separate file. The data are organized in columns:

- **Time** is the time when the signal value is read or the action is taken. Note: This is the time elapsed since the start of the trial, not the start of tracking (see a note below). For lickometers, values are updated about every 2 s.
- **Device ID** is the name of the physical device that the event refers to.
- **Command/Signal** is the type of data (Command, or Signal).
- **Name** is the name of the command or signal
- **Value** is the value of the signal at that time.
- Each row of the file contains one of the two:
  - Rows marked with **Signal**. The value of the signal since the last time the device was read (for example, the number of licks or the duration of licks since the last reading). Duration of licks is expressed in milliseconds.
  - Rows marked with **Command**. The command (for example, *Drop pellet*).
- The hardware log file is one per trial, no matter how many arenas and devices are used.
- If you re-do a trial, the log files recorded with the previous instance of the trial are deleted.
- In order to be logged, hardware devices must be connected to the computer and configured in EthoVision XT (see page 108).
- Notice that the **Time** in the Hardware log file is the time since the start of the trial, while the time in the Track data file is the time since the start of tracking (see page 48).

  EXAMPLE If you have defined a command like *Drop pellet* as a Trial control event (see page 166), to find a specific *Drop pellet* event in the data file, take note of the Time in the Hardware log file for that event and locate that time in the Time trial column in the Track data file.
- For Pellet dispensers. *Number of drops* is the feedback signal sent by the device to EthoVision. Usually it is recorded 0.04 s after the actual Drop pellet command.

- Choose **Hardware log** if you want to export communication data between EthoVision and the hardware device, even when the device was not used during the trial.
- If you select both **Track data** and **Hardware log**, data are exported in separate files when choosing the Text format, and in one file when choosing the Excel format. In the Excel file, Track data and Hardware log data are in stored in separate sheets.

Time	Device	Command/Signal		Value
0	Pellet Dispenser 1	Signal	Number of drops	1
0	Pellet Dispenser 2	Signal	Number of drops	1
10.08	Pellet Dispenser 1	Command	Drop pellet	
10.48	Pellet Dispenser 1	Signal	Number of drops	1
14.36	Pellet Dispenser 1	Command	Drop pellet	
14.68	Pellet Dispenser 1	Signal	Number of drops	1
19.48	Pellet Dispenser 1	Command	Drop pellet	
19.88	Pellet Dispenser 1	Signal	Number of drops	1
19.88	Pellet Dispenser 2	Command	Drop pellet	
20.24	Pellet Dispenser 2	Signal	Number of drops	1

Figure 50 *An example of Hardware log export file for an experiment with two pellet dispensers.*

12 Apply different protocols to different arenas

INTRODUCTION

This document is intended for EthoVision users who record data in with multiple arenas simultaneously, and want to apply different Trial Control protocols to different arenas.

EXAMPLE The camera points at two separate arenas, each with one animal. In arena 1, provide a foot shock 30 seconds from the start of the trial. In arena 2, provide a foot shock at a randomly chosen time between 0 and 90 seconds from the start.

Multiple arenas in your EthoVision experiment

In EthoVision XT, the Trial Control function allows you to define a protocol of your trials, from simple (for example, stopping the trial when the rat has reached the platform in a water maze) to complex (for example, a learning experiment where the animal must carry out a task in order to receive a reward, and the sequence task-reward is repeated a number of times during a trial).

If you work with multiple arenas, each arena is treated as an independent replica of your experiment. In most cases, you want to apply the same protocol to all arenas.

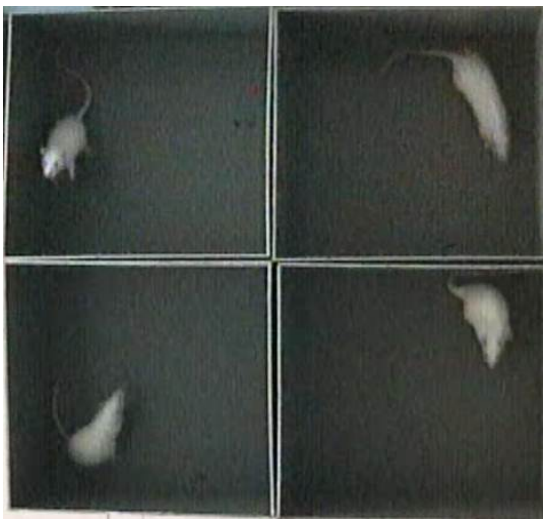


Figure 51 *Four arenas in the same camera view. Each arena is an independent replicate. The protocol defined in the Trial Control Settings is applied to each arena independently.*

Same protocol, but independent processes

When you use Trial Control, the Trial Control procedure is applied to each arena independently. Suppose that the protocol is "After starting the trial, switch on a light when the animal enters the zone named Food zone". A light device has been installed on top of both arenas, and a zone named "Food zone" has been defined in the Arena Settings for both arenas.

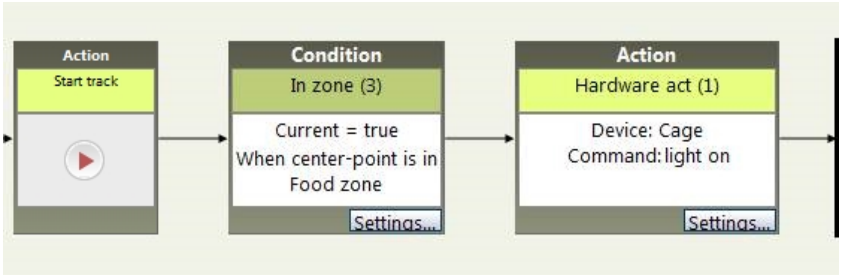


Figure 52 *A simple trial control protocol. When the center point of the subject is detected in the "Food zone", EthoVision sends a command to switch on a light in that arena.*

If the animal in Arena 1 enters its Food zone, the corresponding light will be switched on. However, the light on top of Arena 2 won't be activated, unless the animal in Arena 2 enters its own Food zone too (see Figure 53). Although the protocol is the same, it is applied in two copies, one for each arena.

This can be represented on a time line:

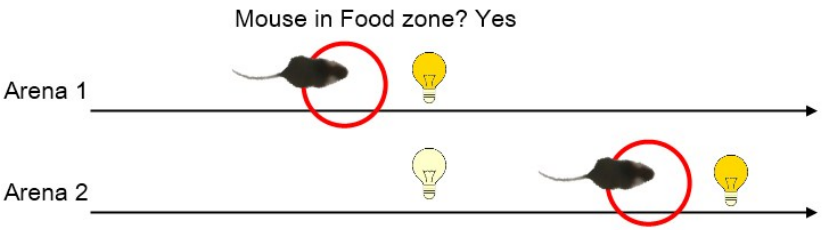


Figure 53 *Time line of a trial with the same trial control protocol active in two arenas. The circle represents the Food zone.*

To implement this, select the different physical devices (Light 1 for Arena 1, Light 2 for Arena 2 etc.) on the same line in the **Arena - Hardware Mapping** window (Arena Settings). This way

different arenas refer to the same generic **Device name** (e.g. “Light”). This “generic” name is then used in the instructions of the Trial Control protocol.

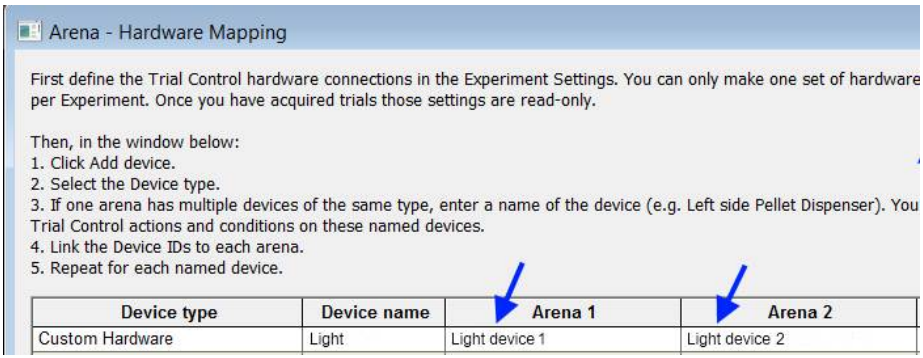


Figure 54 *In order for the same protocol to be applied to different arenas, the different physical devices specified under* ***Arena 1*** *and* ***Arena 2*** *(see the arrows) must refer to the same* ***Device name*** *(e.g. Light).*

Another typical example is the PhenoTyper setup, where the same protocol is applied to a group of 4 or more cages. Conditions and actions are processed independently for each cage.

But what if Arena 2 needs a completely different protocol than Arena 1? See the next section.

Different protocols in different arenas

In some cases two or more arenas may need different protocols. For example, your camera view includes three arenas. In Arena 1, a light (Light 1) is switched on at a specific time, for example at one minute after start. In Arena 2, the light (Light 2) is switched on at a moment randomly chosen between 30 s and 2 minutes (because this event is randomly based, it will differ among the animals tested in Arena 2). Arena 3 is then used as a control, with no light stimuli presented. For all arenas, the trial lasts 3 minutes.

If we based the Trial Control procedure on an **Arena - Hardware Mapping** like the one in Figure 54, the two light devices Light 1 and Light 2 would be represented by the same **Device name**. When using this name in a Trial Control instruction, for example “switch a light using the **Device name = Light**), that instruction would be executed for both arenas. As a result, the light would be switched on in both arenas twice, one at 1 minute, and a second at a random time between 30 s and 2 minutes. Also if the action is depending on the animal’s behavior (for example, “Switch on the light when the animal is in the Food zone”), this command would be executed in both arenas, producing obviously false results.

The solution to this problem is to map devices in a different way, by specifying one physical device in each row in the **Arena - Hardware Mapping** window.

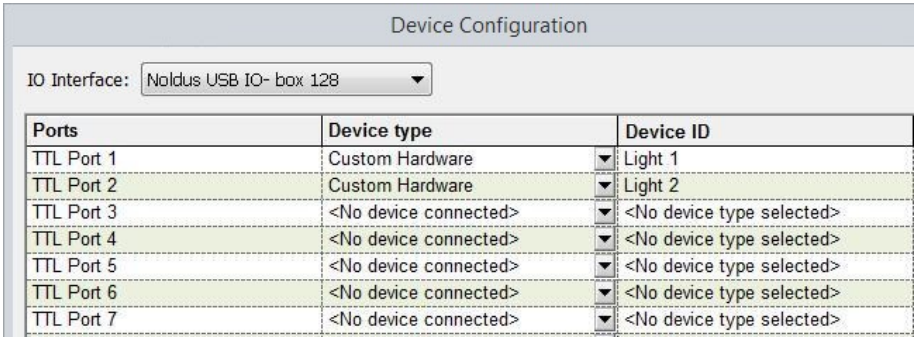
SIMPLE CASE: TWO ARENAS

To illustrate the concept, we assume that we work with two arenas, and one animal in each arena. The animals are subject to two different treatment protocols.

Figure 55 *Schematic representation of the treatments in two arenas.*

Experiment Settings

1. Connect all hardware devices to the EthoVision XT computer, via the appropriate port in the Noldus USB-IO box.
2. In the **Experiment Settings**, under **Trial Control Hardware** click **Settings**.
3. Choose **Noldus USB-IO box**.
4. In the Device Configuration window, assign a hardware device to a port of the USB IO box (or Mini USB IO box), as you normally do. Here **Device ID** is the name of each physical device. For example, **Light 1** will be installed in Arena 1, **Light 2** in Arena 2, etc.



Ports	Device type	Device ID
TTL Port 1	Custom Hardware	Light 1
TTL Port 2	Custom Hardware	Light 2
TTL Port 3	<No device connected>	<No device type selected>
TTL Port 4	<No device connected>	<No device type selected>
TTL Port 5	<No device connected>	<No device type selected>
TTL Port 6	<No device connected>	<No device type selected>
TTL Port 7	<No device connected>	<No device type selected>

Arena Settings

1. In the **Arena Settings**, define and calibrate the arenas as usual. Next, click the **Arena - Hardware Mapping** button in the **Arena Settings** window.
2. In the **Arena - Hardware Mapping** window, click **Add device** two or more times to create multiple rows, one row for each physical device that has to work independently.

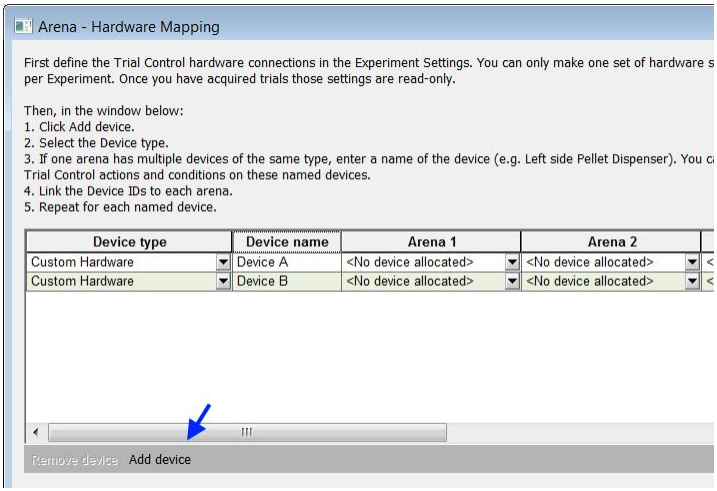


Figure 56 *Adding devices to the* ***Arena - Hardware Mapping*** *window,*

3. For each arena, assign a device in a different row. Make sure that the other cells of the row show **<No device allocated>** .

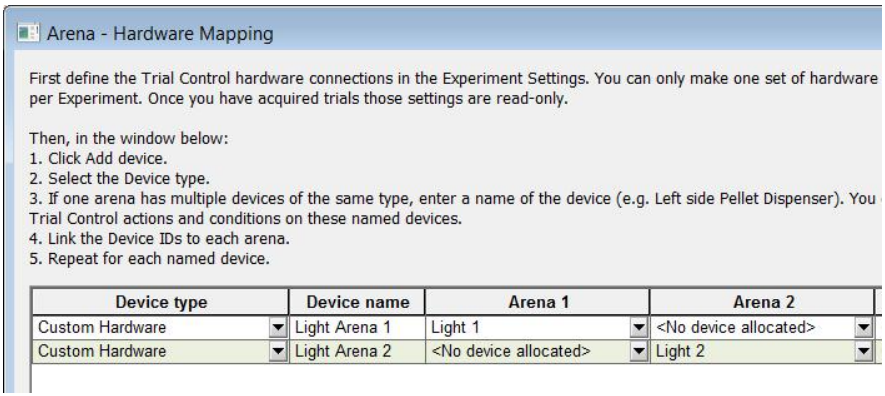


Figure 57 *First row: The physical device Light 1 is assigned to Arena 1 with the generic name "Light Arena 1". Second row: The physical device Light 2 is assigned to Arena 2 with the generic name "Light Arena 2".*

Define the protocols

Try to make a scheme of the events in each arena. Draw a time line for each arena. On the time line, write down the events that should occur, and their duration. This will help creating the Trial Control protocols.

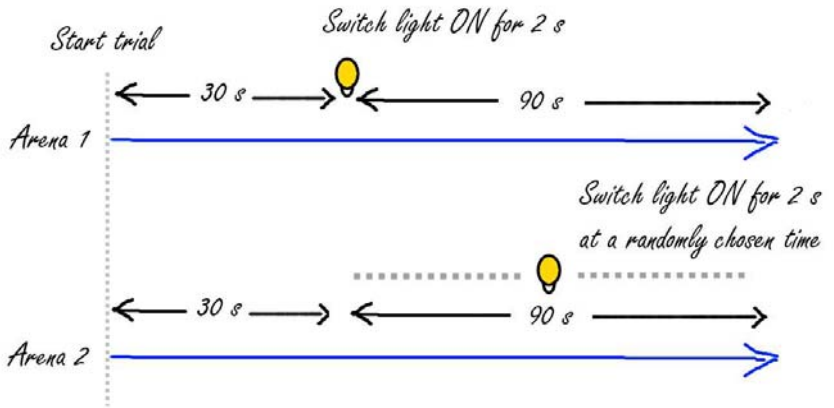


Figure 58 *Make a sketch of your protocols on different time lines.*

Trial Control Settings

- There may be parts of the protocol shared between arenas. In the example of Figure 59, the first 30 seconds after the start of the trial are the same for the two arenas. Therefore, one condition box after the **Start track** box will suffice.

Figure 59 *A condition box that waits 30 seconds for both arenas.*

- The sequence of instructions for the two treatments must originate from the same point (so they will start at the same time) and proceed in parallel.

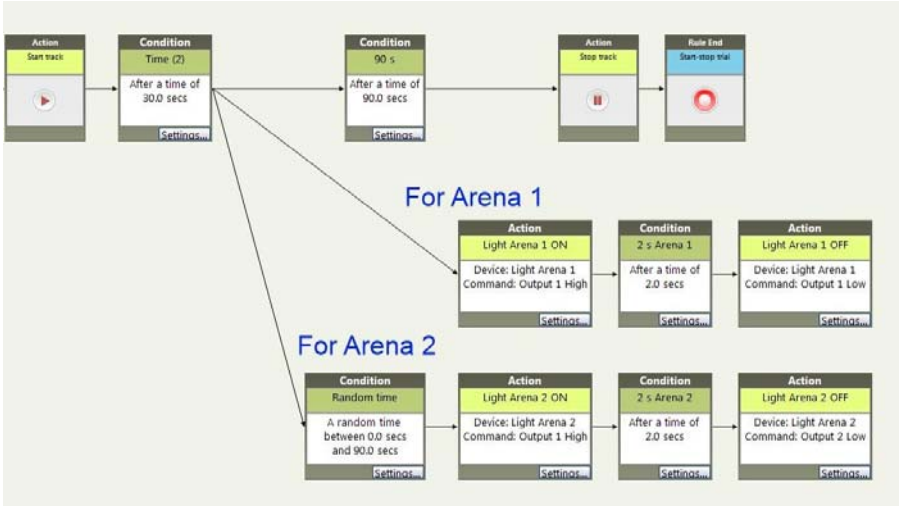


Figure 60 *After a 30-s period, two procedures are activated, one for each arena.*

- Note that the two branches do not have to end back into the main start-stop sequence. However, you must make sure that the trial does not stop before the two branches have been processed. In this example, we have set the trial to stop after 2 minutes. Therefore, a Time box has been added to the Start-stop sequence that waits 90 s to complete the trial (30 s of the first box + 90s = 120 s)
- **Sequence for Arena 1** — According to the protocol, for Arena 1 the light must be switched on immediately after the 30 s period, then 2 s later it must be switched off. This can be programmed with two hardware actions (ON and OFF) and a Time condition in between. The actions must specify the device **Light Arena 1**.

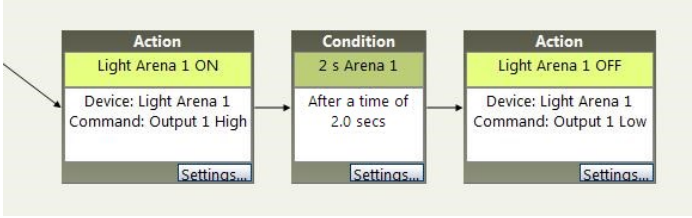


Figure 61 *Instruction sequence for switching on a light in Arena 1 for two seconds.*

- **Sequence for Arena 2** — According to the protocol, for Arena 2 the light must be switched on at a time randomly chosen between 0 and 90 s. This can be programmed with a time condition that determines the random time, followed by a sequence similar to that for Arena 1. The actions must specify the device **Light Arena 2**.

Figure 62 *Instruction sequence for switching on a light in Arena 2 for two seconds. The first box defines a random time chosen between 0 and 90 s.*

The **Random time** condition defines a time between 0 and 88 seconds, to make sure that the 2-seconds light flash is always given in its entire duration within 90 seconds.

Repeat a sequence within the trial

In most cases, a sequence of actions is repeated a number of times, or until a condition is met. If you want the two sequences described above to be repeated, you must use the Sub-rule function in Trial Control.

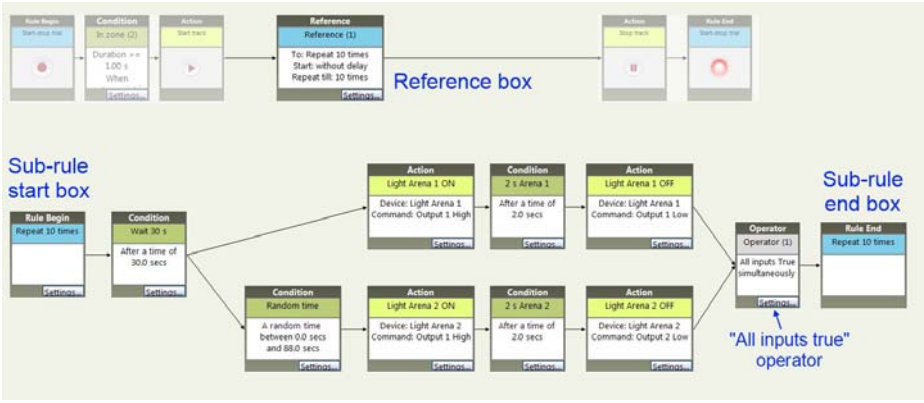


Figure 63 *Setting two protocols to be repeated a number of times. Note that the two branching sequences are connected at their end to the stop box of the sub-rule through an operator. Only when the two sequences are processed, control is passed for a new start of the sub-rule. How repeats start is specified in the Sub-rule Reference settings (click **Settings** in the **Reference** box).*

The Sub-rule settings ensure that the sub-rule is repeated 10 times, and the time between the start of 2 successive repeats is 2 minutes.

For more information on how to use Sub-rules, see Sub-rules on page 54.

ADVANCED CASE: SIX ARENAS

In this use case, we plan to record data in six arenas, with one animal per arena. This use case is considered advanced for several reasons:

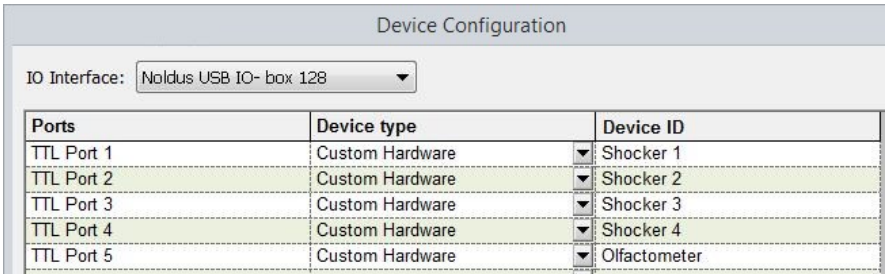
- Each treatment (odor stimulus and a subsequent foot shock) is duplicated in two arenas. This example shows that even when two of a number of arenas share the same protocol, each arena must have its own trial control procedure with *unique* **Device names** defined.
- Together with two treatments that require different protocols, a third group (control) is added that requires no foot shock. Control animals are recorded simultaneously with the other animals.
- All animals share part of the protocol. They all receive an odor stimulus by means of an olfactometer. Therefore, part of the Trial Control procedure must work for all arenas.

Figure 64 *Schematic representation of the treatments in six arenas. Arenas 1-2, 2-3 and 4-5 share the same treatment.*

Experiment Settings

1. Connect all hardware devices to the EthoVision XT computer, via the appropriate port in the Noldus USB-IO box.
2. In the **Experiment Settings**, select the **Number of arenas**.
3. Under **Trial Control Hardware** click **Settings**.
4. Select **Noldus USB-IO box**.

5. In the Device Configuration window, assign a hardware device to a port of the USB IO box (or Mini USB IO box). Here **Device ID** represents a name for each physical device. For example, **Shocker 1** will be installed in Arena 1, **Shocker 2** in Arena 2, etc.



Since the olfactometers work the same way in all arenas, they can be connected to the same port of the USB IO box. Therefore, you need 4 ports for the shock generators (Arenas 1 to 4) and one port for the olfactometer.

Arena Settings

1. In the **Arena Settings**, define and calibrate the arenas as usual. Next, click the **Arena - Hardware Mapping** button in the **Arena Settings** window.
2. In the **Arena - Hardware Mapping** window, click **Add device** two or more times to create multiple rows, one row for each physical device that has to work independently. In this case, 5 rows.
3. For each arena, assign a device in a different row. Make sure that the other cells of the row show **<No device allocated>**.

Figure 65 *The Arena - Hardware Mapping window. In the first four rows, a physical device is assigned to one arena; for example Shocker 2 in Arena 2. The name "Shocker 2" in the* ***Device name*** *column will be used in the Trial Control instructions.*
In the last row, the olfactometer is selected for all arenas, using the ***Device name*** *"Olfactometer". This name will be used in Trial Control.*

Note that:

- Although Arena 1 and 2 and Arena 3 and 4 share the same treatment, they must be linked to different **Device names**. This to avoid that any command given to for

example the shocker in Arena 3 does not go to Arena 4. In principle we could use the same command (and thereby the same **Device name**) for Arena 1 and 2 because the shock is given at the same time, but to keep this case more general we leave them separate.

- The arenas with no shock treatment (Arenas 5 and 6) have no shocker device selected.
- The olfactometer is used in all arenas, and at the same time. This means that the command to activate/deactivate the olfactometer can be represented by one **Device name** in Trial Control (see the last row in the table above). Therefore, the same **Device name** is assigned to all arenas.

Define the protocols

Make a scheme of the events in each arena. Draw a time line for each arena. On the time line, write down the events that should occur, and their duration. This will help creating the Trial Control protocols.

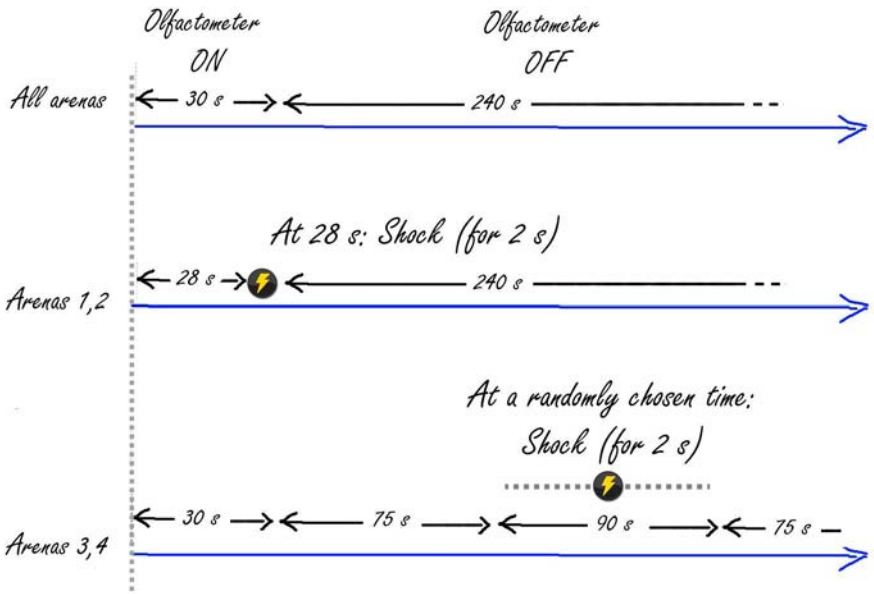


Figure 66 *Make a sketch of your protocols on different time lines.*

Trial Control Settings

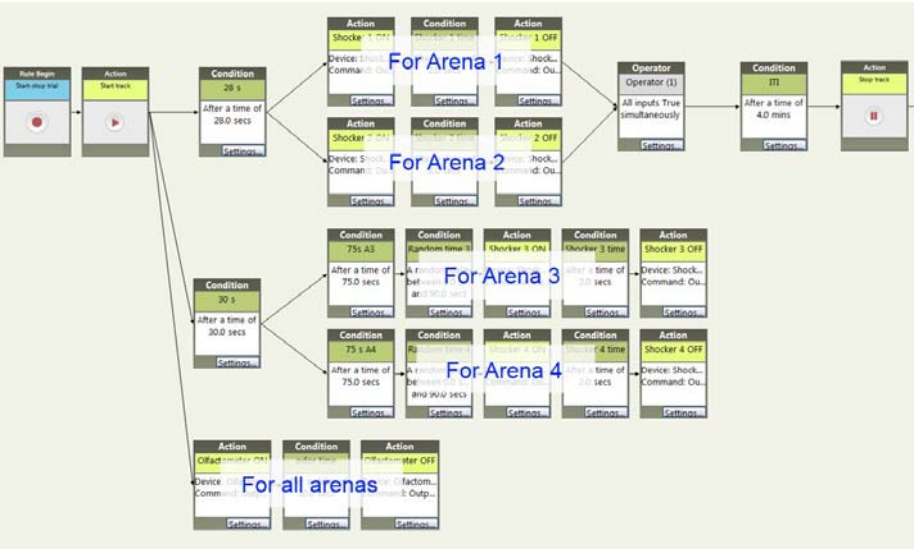


Figure 67 *Overview of the Trial Control Settings. Indicated are the parts of the procedure applied to specific arenas. The total length of the trial is 4 minutes and 30 seconds.*

- **Sequence for Arena 1 and 2 —**

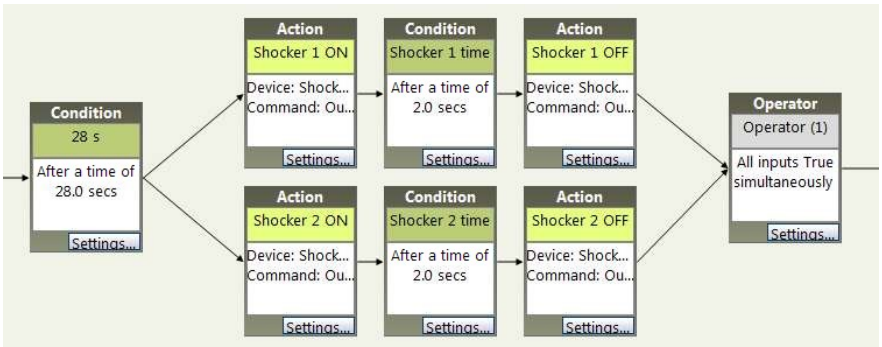


Figure 68 *The trial control sequence for controlling a foot shocker at 28 s from the start, in both Arena 1 and Arena 2. The shock ends at 28+2 = 30 s from the start.*

Note that only the sequences for Arena 1 and 2 are connected to the Stop trial box. There is no need to connect the sequences for Arena 3 and 4 and the sequence for the olfactometers to the Stop Trial box, because these instructions proceed in parallel with those for Arena 1 and 2. The track duration is the same for all arenas.

- **Sequence for Arena 3 and 4 —**

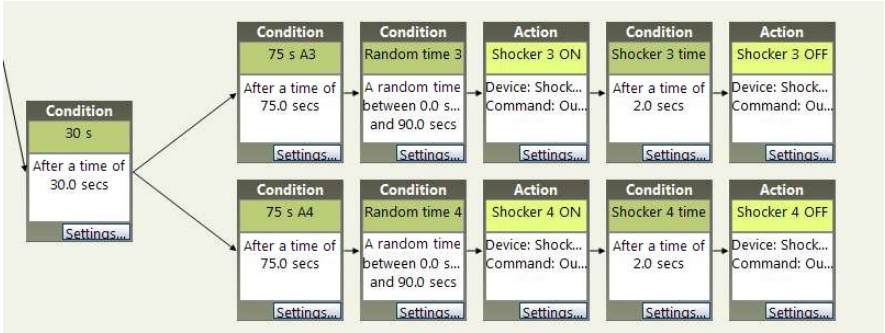


Figure 69 *The trial control sequence for controlling a foot shocker at a time randomly chosen within a 90-s period. In order to center the 90-s period for the random shock in the 4-min period after the offset of the odor (see Figure 66), a 75-s condition has been added (75+90+75 = 240 s = 4 minutes). Note that the 30-s Condition box and the two 75-s conditions could be replaced by one 105-s Condition box, connected to both Random time conditions. In this picture the 105-s time has been kept split in two parts (30 s +75 s) to visually match the sequences for the other arenas.*

Repeat the sequence within the trial

If you want the sequences described above to be repeated a number of times within a trial, you must use the Sub-rule function in Trial Control. Here, the sub-rule is repeated 10 times.

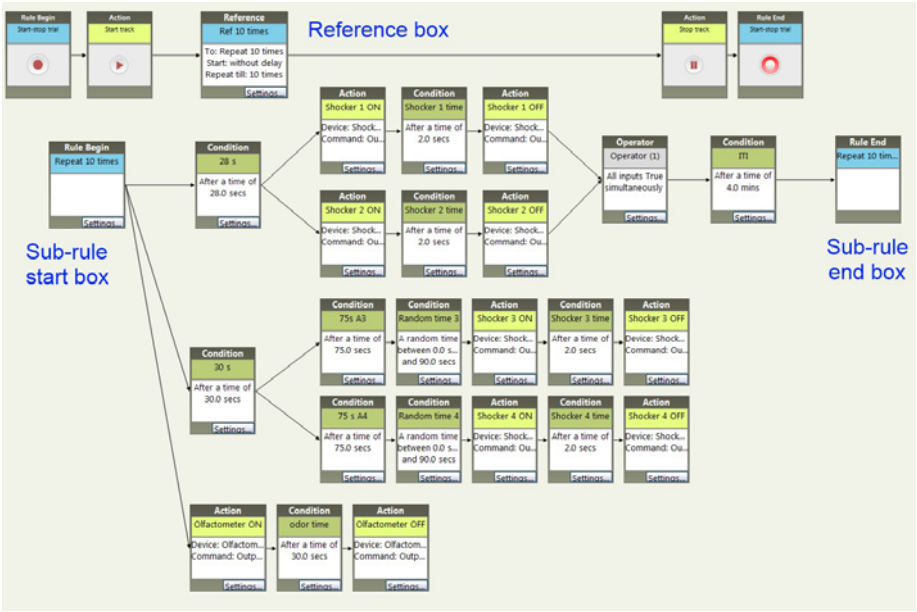


Figure 70 *A sub-rule has been added to repeat the protocol a number of times in the same trial.*

Note that the end box of the sub-rule is connected to the sequence for Arena 1 and 2. This sets the duration of the repeats (30 min. with odor stimulus + 4 minutes), and the trial as a whole.

For more information on how to use Sub-rules, see Sub-rules on page 54.

Index

— B

— C

— D

— I

— J

— L

— M

— N

— O

— P

— Q

— R

— S

— T

— U

— V

— W

— Z