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***THE EFFECTS OF UNSIGNALLED AND SIGNALLED DELAYS
OF REINFORCEMENT IN RATS: A BETWEEN-
PROCEDURES COMPARISON***

**EFFECTOS DE LA DEMORA DE REFORZAMIENTO
SEÑALADA Y NO SEÑALADA EN RATAS: UNA
COMPARACIÓN ENTRE PROCEDIMIENTOS**

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Abstract

Using eight experimentally naive Wistar rats, the effects of immediate, unsignaled and signaled delays of reinforcement on lever-pressing response rates were compared with two different procedures. In the Between-Phases procedure, each experimental condition was presented in three thirty-session consecutive phases; whereas, in the Multiple-Schedule procedure, each experimental condition was correlated with one component of a three-component multiple schedule of reinforcement. Regardless of the procedure used, consistent results were observed, that is, differences

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in response rates were found when rats were exposed to a delay of reinforcement condition (Signed or Unsigned) in contrast to the immediate-reinforcement condition. These results suggest that even when rats are exposed to different conditions in the same session, similar results occur to those found when these conditions are arranged between phases. Similarly, they provide some information regarding the usefulness of employing a multiple schedule to assess the effects of different variables (Immediate, Unsigned, and Signed food deliveries) in relatively less time than a between-phases procedure.

Keywords: Signed and unsigned delay of reinforcement, multiple schedule, between phases procedure, comparison, rats.

Resumen

Empleando dos tipos de procedimientos, el presente estudio comparó los efectos de la entrega de comida inmediata y demorada (con o sin señal) sobre la tasa de respuesta. Cuatro sujetos se expusieron al primer procedimiento que consistió en presentar cada una de las condiciones en fases sucesivas (Inmediata, Demora Señalada y Demora no Señalada); para los otros cuatro sujetos, dichas condiciones fueron presentadas a lo largo de la misma sesión experimental empleando un programa de reforzamiento múltiple de tres componentes. Independientemente del procedimiento empleado, fue posible observar diferencias en el responder en función de la entrega demorada de comida. Los resultados sugieren que aun cuando se realiza una manipulación intra-sesión, es posible observar resultados similares a los observados cuando cada una de las condiciones se presentan de manera independiente entre fases. Asimismo, proporcionan información en cuanto a la utilidad de los programas de reforzamiento múltiple para la evaluación de distintas variables en tiempos relativamente cortos, respecto a los requeridos bajo un procedimiento entre fases.

Palabras Clave: Demora señalada y no señalada, programa de reforzamiento múltiple, procedimiento entre fases, comparación, ratas.

Magnitude, quality, rate, and delay are reinforcement parameters that affect the rate at which subjects respond (Kimble, 1961). Delay of reinforcement has been assessed by increasing the temporal interval between a response and the reinforcer. The common result of such a manipulation is a decrease in the rate of response as the temporal interval between the response and reinforcer increases. This effect has

been identified as delay of reinforcement gradient (for recent reviews see Lattal, 2010; Pulido, Lanzagorta, Morán, Reyes, & Rubí, 2004).

Assessing delay of reinforcement is relevant because even when the response-reinforcer contingency is maintained, a disruption in temporal contiguity between both elements affects the rate of responding (Avila & Bruner, 1997; Dews, 1960; Ferster, 1953; Lattal, 1987; Ruiz, Bruner, & Balderrama, 2007; Skinner, 1938; Sosa & Pulido, 2011). When assessing delay of reinforcement, different procedures have been used. For example, most experiments arrange a two-link procedure in which the first link determines when the response will produce the food delivery, and the second link determines the interval between such response and the food delivery (e.g., 0, 5, 10 s). This latter interval has been identified as the nominal delay; therefore, manipulations have focused on that interval. For instance, certain experiments have involved the use of a resetting delay, where responding resets the timer for the delay interval until no responding occurs (Gleeson & Lattal, 1987; Skinner, 1938; van Haaren, 1992), while a nonresetting delay has been studied in others (Avila & Bruner, 1999; Bell, 1999; Lattal, 1984). In the latter, no consequences for responding are programmed, since the latter allows the subject to respond during the delay, thus decreasing the obtained delay. Nevertheless, it has been argued that when using a resetting delay, the decrease in responding due to the delay could be confounded with a reduction in the rate of reinforcement resulting from continued responding during the delay period (Lattal, 2010). Likewise, in any of the above-mentioned procedures, a stimulus change may or may not be programmed during the delay. Such procedures have been identified as signaled and unsignaled, respectively (Chung, 1965; Ferster, 1953; Pierce, Hanford, & Zimmerman, 1972), where tandem or chained schedules of reinforcement arrange food delivery.

By signaling the delay, in contrast to an unsignaled delay, a higher rate of responding typically is observed. This result has been explained by means of the conditioned reinforcing function of the signal, where its introduction increases response rates (Azzi, Fix, Keller, & Rocha e Silva, 1964; Lattal, 2010; Pulido et al., 2004). However, Ferster (1953) and Dews (1960) have also explained these results by appealing to mediating behavior occurring during the delay. Thus, a stimulus during delay can develop a two-way function, either as conditioned reinforcer of responding prior to its onset, and as discriminative stimulus of other behavior during its presentation (see Dinsmoor, 1950; Myers, 1958).

Delay of reinforcement has been assessed using different procedures. In one, the delay is systematically changed across different phases (Bruner, Avila, & Gallardo,

1994; Dews, 1960; Lattal, 1984). Another involves using multiple schedules of reinforcement, in which one component is correlated with immediate reinforcement and the other components are correlated with different delay values (Richards, 1973; Ruiz et al., 2007). Other manipulations have assessed the effects of signaled and unsignaled delays across different phases of the experiment (Azzi et al., 1964; Richards, 1981; Schaal & Branch, 1988) or between different components of multiple schedules of reinforcement (Bell, 1999; Podlesnik, Jimenez-Gomez, Ward, & Shahan, 2006; Schaal & Branch, 1990). In one example of the former, Azzi et al. (1964) established lever-pressing in rats using a continuous reinforcement schedule (CRF) where each lever press delivered the programmed reinforcer. Once responding was established, they introduced different delay values in successive phases (i.e. 1, 3, 5, 7.5, 10, 15 and 20 s). Responding decreased as the delay was increased from 0 s in the CRF condition to 20 s in the last phase. In the second part of the experiment, they introduced a stimulus change (blackout) during the 20-s delay. During this condition responding increased in contrast to the condition when no stimulus change was in effect during the delay. Richards (1981) also compared key peck responding of pigeons under different signaled and unsignaled delay values. Like Azzi et al., Richards reported that pigeons responded at higher rates when delays were signaled, and such rates were similar or only slightly below those observed during the immediate reinforcement condition.

Another procedure that has been used to assess the effects of signaled and unsignaled delay of reinforcement is using multiple schedules of reinforcement. For instance, using a two-component multiple schedule, Schaal and Branch (1990) examined the effects of different signal durations imposed during a 27-s delay. In a similar experiment, Bell (1999) assessed the effects of immediate, unsignaled and signaled delay of reinforcement, using a three-component multiple schedule. In both experiments (Bell, 1999; Schaal & Branch, 1990), higher response rates occurred during the signaled delay than during the unsignaled delay component. Nevertheless, opposite to what has been reported in between-phases procedures (Azzi et al., 1964; Richards, 1981), or even to what Schaal and Branch reported using a multiple schedule, Bell found differences between signaled delayed and immediate reinforcement, observing a higher response rate during the former.

Even though in certain experiments (Bell, 1999; Podlesnik et al., 2006; Schaal & Branch, 1990) the effect of delay and reinforcement rate are not confounded, other experiments have reported conflicting findings. For instance, Schaal and Branch's (1990) results are similar to what is commonly reported, that is, higher

response rates with immediate reinforcement in contrast to rates during any of the delay conditions; and higher rates under signaled than unsignaled delay (Azzi et al., 1964; Richards, 1981; Schaal & Branch, 1988). In contrast, subjects in Bell's (1999) experiment responded at higher rates during the fully signaled delay condition than during the immediate-reinforcement condition. Such conflicting results (Bell, 1999) seem difficult to explain since they differ from procedures used for assessing delay. Nevertheless, provided that similar contingencies of reinforcement are arranged, procedural differences should not be accounted for the result, but mostly the fact that when using a multiple schedule other experiments (Podlesnik et al., 2006; Schaal & Branch, 1990) have yielded data that are consistent with most of the delay of reinforcement results when the different delay durations are assessed across phases (Azzi et al., 1964; Bruner et al., 1994; Dews, 1960; Lattal, 1984; Richards, 1981).

The experiments described above suggest that there are two reliable procedures for assessing delay of reinforcement, and both seem to generate the same results. Nevertheless, using a between-phases procedure requires a larger number of sessions to complete; whereas, training subjects using multiple schedules would require less time, since different variables are assessed in each component and thus can contrasted with one another. Such convenience could allow better use of the financial and animal resources available.

Given such considerations, the present experiment was conducted in an attempt to replicate the effects of delays of reinforcement by comparing the effects of immediate, signaled and unsignaled delay of reinforcement across different phases (Azzi et al., 1964; Ferster, 1953; Lattal, 1984), to the effects obtained when those same conditions were imposed as components of a three-component multiple schedule of reinforcement (Bell, 1999; Podlesnik et al., 2006), while keeping the interreinforcer intervals equal regardless of the delay in effect (see Bruner, Pulido, & Escobar, 1999; Flores & Mateos, 2009; Weil, 1984).

Method

Subjects

Eight five-month old, experimentally naïve female Wistar rats were used. Each rat was maintained at approximately 80% of its free-feeding weight throughout the experiment. All subjects were individually housed in a temperature-controlled room under a 12:12 hr. light/dark cycle with free water-access. Subjects care and hous-

ing complied with the animal care guidelines established by the Universidad de Guadalajara, and according to NOM-062-ZOO-1999 (Norma Oficial Mexicana).

Apparatus

Four Med Associates Inc. (ENV-008) operant conditioning chambers were used, all equipped with the following Med Associates Inc®. apparatus. A food dispenser (ENV-203M), which delivered 45-mg grain-based pellets for rodents, was located to the center of the work panel; a stainless-steel, 4.5cm long retractable lever (ENV-112CM) was placed to the left side of the food dispenser at a height of 7cm from the chamber's floor, lever activation required a 0.25 N; a 2-cm diameter translucent light (28-V) was placed above the lever; on the wall opposite the work panel, a white noise amplifier (ENV-225SM) and a speaker producing 80dB of 2000Hz were placed. Each chamber was enclosed in a sound-attenuating box (ENV-022MD) equipped with ventilation fans that provided external-sound masking. A Windows computer coupled to a Med Associates Inc interface (SG-6080D) and the MED-PC IV® software controlled the experimental events and collected data.

Procedure

All rats were exposed to one session daily. Each session started with insertion of the lever 2-minutes after rats were placed in the chamber. Given that two different procedure were used, session durations varied between experimental groups.

Training. All rats were trained to enter the food magazine and to lever press by the differential-reinforcement-of-successive-approximations procedure during three sessions. Each of these sessions terminated after 60 min or once the rat produced 40 reinforcers under a fixed-ratio 1 schedule, whichever occurred first. Once 40 reinforcers were delivered in two consecutive sessions, lever pressing was maintained using a variable interval (VI) schedule, the mean interreinforcer interval of which increased gradually before the final baseline VI schedule value was used. The criterion for ending each session was the same as the previously described. Once all rats reached the final baseline VI value, 2 four-rats groups were formed (a 3-Phase Group and a Multiple-Schedule Group). Rats were assigned to each group by matching final training response rates to the greatest extent.

Between-Phases procedure. During the first phase (Immediate), reinforcers were delivered according to a VI 30-s schedule and no delay was programmed. For

the second, Unsignaled Delay, phase, reinforcers were programmed according to a tandem VI 25-s fixed-time (FT) 5-s schedule, where the second link corresponded to the 5-s delay, during which no stimulus change was programmed. The third phase (Signaled Delay) was the same as the second, except that a 5 s white light above the lever during the delay, converting the schedule to a chained VI 25-s FT 5-s schedule. Each phase was in effect for thirty 30-min sessions.

Multiple-Schedule procedure. Rats were exposed to the same conditions as those in the Between-Phase procedure (Immediate, Unsignaled Delay, and Signaled Delay), except that each of these conditions were correlated with different components of a 3-component multiple schedule. Each 2-min component started with the insertion of the lever and was presented 8 times so that during a single session a total of 24 component presentations were arranged. Components occurred quasirandomly, with the restriction that the same component could not be presented more than two consecutive times. No events were programmed during a 30-s intercomponent interval, during which the lever was retracted. Component 1 was correlated with the immediate reinforcer, Components 2 and 3 were correlated with the unsignaled and signaled delays, respectively. During each component, the absence of an added auditory stimulus was correlated with the component arranging immediate reinforcement; whereas for the unsignaled- and signaled-delay components, a fixed or intermittent tone (0.5 s on/off) was used, respectively. Rats were maintained in this condition for 30 sessions.

Results

Rats' mean responses per minute during the last five sessions of each condition are presented in Figure 1. Responses per minute for the Multiple-Schedule and the 3-Phase procedures are shown in the left and right columns, respectively. Responding during the 5-s delay was excluded, thus only mean responses per min during the VI 25-s component are shown.

For all rats, response rates were higher during the Immediate condition in contrast to either of the Delay conditions. Likewise, with the exception of Rat 5, mean responses per minute during the Signaled-Delay condition were higher than those during the Unsignaled-Delay condition, regardless of the procedure.

Figure 2 shows the mean number of responses during the Unsignaled and Signaled Delay conditions calculated from the last five sessions in each condition. Differences in responding during the delays were observed between rats. For rats

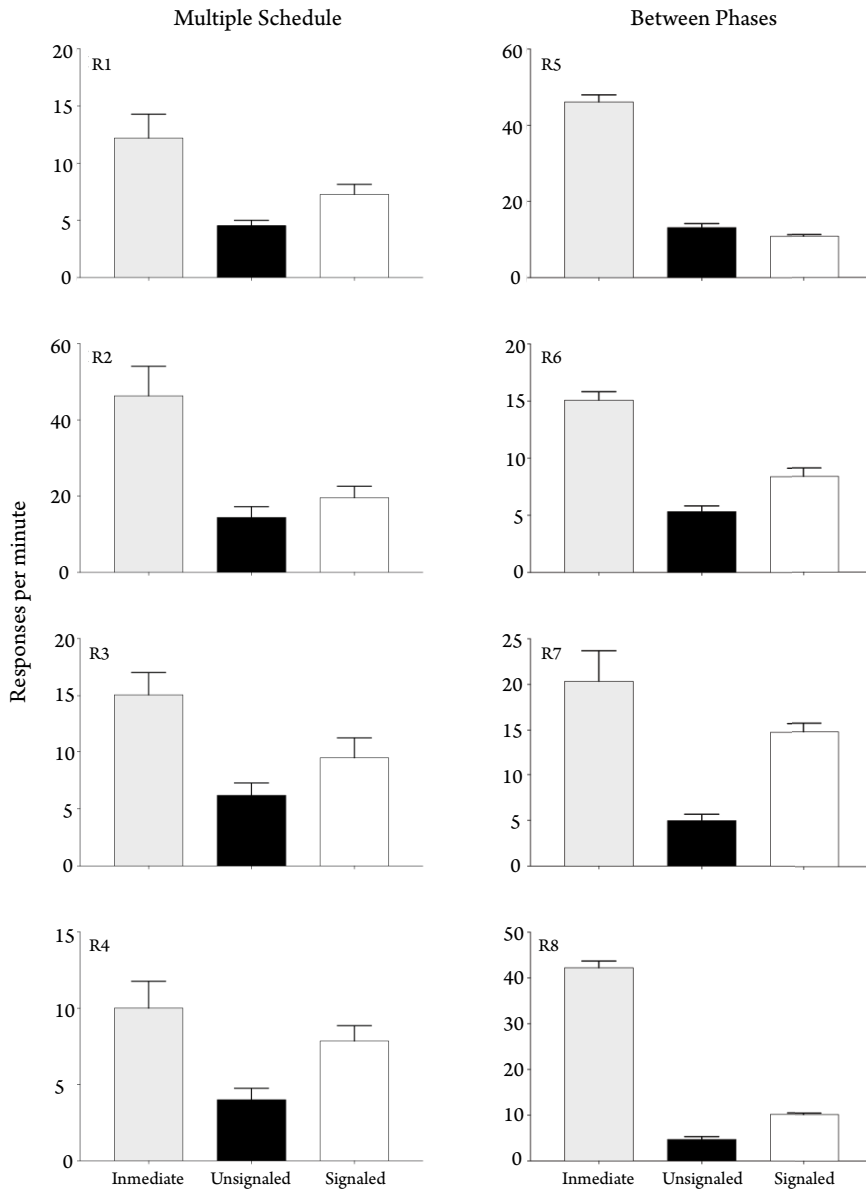


Figure 1. Mean responses per minute of each rat based on the last five sessions of each condition. Rats exposed to the Multiple-Schedule and Between-Phases procedure are shown in the left and right columns, respectively. Error bars represent the standard error of the mean (SEM).

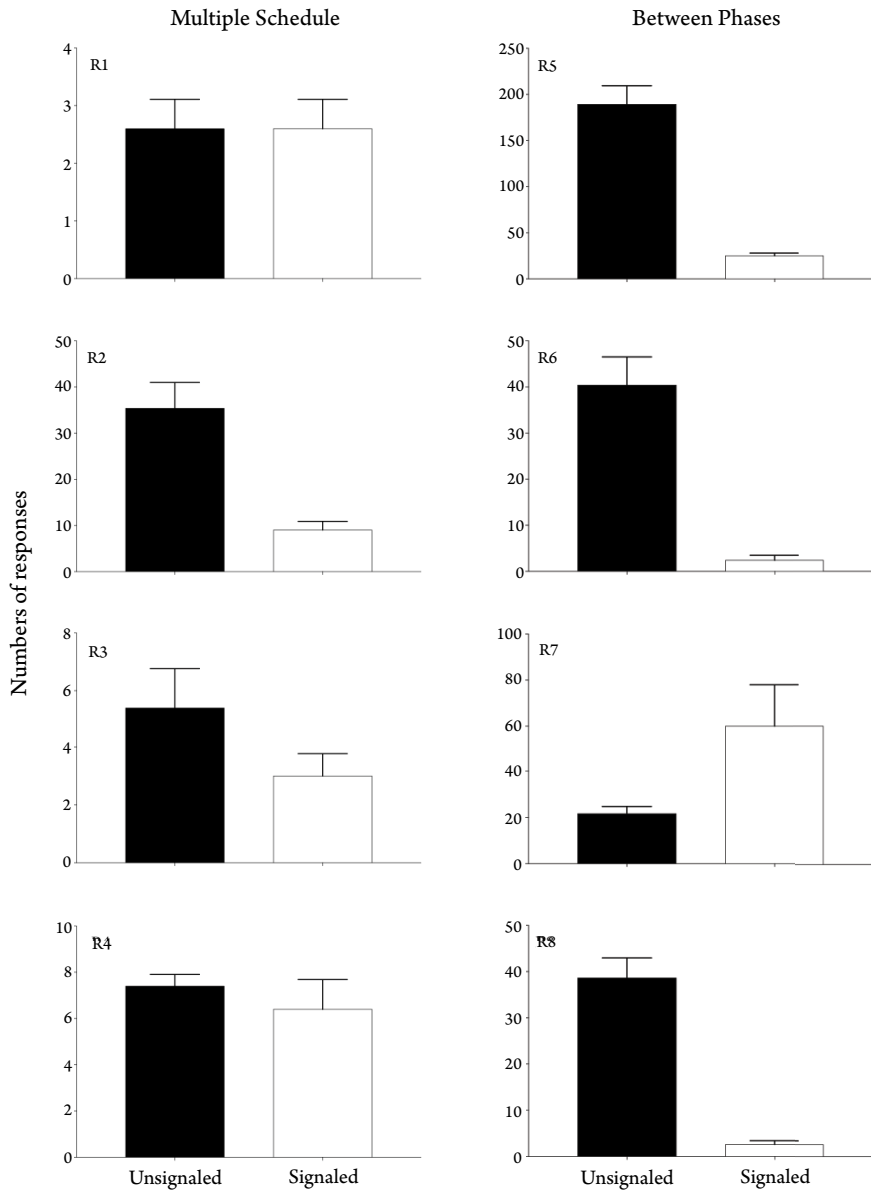


Figure 2. Mean number of responses during the last five sessions of each condition for rats trained using the Multiple-Schedule and Between-Phases procedure are shown in the left and right columns, respectively. Error bars represent the standard error of the mean (SEM).

Table 1. Mean reinforcers per minute (S^R / min) for each rat calculated from the last five sessions of each condition

Subject	Immediate	Unsigned	Signed
	S^R / min	S^R / min	S^R / min
R 1	1.62	1.47	1.57
R 2	1.76	1.52	1.76
R 3	1.52	1.26	1.63
R 4	1.38	1.22	1.42
R 5	1.80	1.70	1.83
R 6	1.73	1.37	1.85
R 7	1.62	1.48	1.99
R 8	1.86	1.44	1.79

Note. Reinforcers per minute for rats trained under the Multiple-Schedule (Rats 1, 2, 3, 4) and the Between-Phases procedure (Rats 5, 6, 7, 8) appear in the upper and lower portion of the table, respectively.

trained under the multiple schedule, response rates were lower during the signaled delay for Rats 2 and 3; whereas for the other two rats, no differences in responding occurred between unsigned and signaled delays. Rats trained under the Between-Phases procedure had higher response rates during the Unsigned Delay condition.

Mean reinforcers per minute (S^R / min), across the different conditions are shown in Table 1. Reinforcers per minute during the last five sessions of each condition were similar for rats trained under the Multiple-Schedule procedure; in contrast to rats trained under the Between- Phases procedure, where for three of the four rats the S^R / min varied between conditions.

Discussion

In the present experiment the effects of the immediate, signaled and unsigned delay of reinforcement on lever-pressing response rates (responses per minute) were compared using two procedures: a three-component Multiple-Schedule and a Between-Phases procedure. As previous experiments (Azzi et al., 1964; Bell, 1999;

Lattal, 1984; Mateos, 2012; Schaal & Branch, 1988), response rates using either procedure were higher when food was delivered immediately when compared to either of the delay conditions. Likewise, with the exception of Rat 5, response rates were higher under signaled than unsignaled delay condition. Thus, overall, consistent results were observed regardless of the procedure used.

The latter finding is consistent with those of several previous experiments (Azzi et al., 1964; Pulido, Rubí, & Backer, 2008; Richards, 1981; Ruiz et al., 2007). In some cases, this result has been explained in terms of a conditioned reinforcement function developed by the signal. Also, as was mentioned, response-dependent signals can develop a dual function; as a conditioned reinforcer when its onset depends upon responding, and as a discriminative stimulus when its presentation controls a different type of responding (Dinsmoor, 1950; Myers, 1958). Some evidence of the latter was observed in the current experiment, where responding during the VI component and during the delay differed between rats. That is, most of the rats responded more when no signal was added during the delay; whereas for others, responding was equal or greater when a stimulus was added. This difference in responding could be accounted for by the discriminative function developed by the signal during its presentation, provided that it controlled a different response topography other than lever-pressing once the latter decreased. An increased operant responding frequency during the delay when a stimulus is added is not at odds with other experiments. For instance, Azzi et al., (1964) reported that most of their rats responded at a higher rate when delay was signaled than when no signal was present; likewise, Lattal (1984) reported a similar result in most of his pigeons. Even though Azzi et al. observed such result in most of their rats, it's quite interesting how some days one of their rats responded less during the signaled delay interval, which in fact is what we observed for most of our rats (*see also* Chung, 1965; Chung & Herrnstein, 1967; Mateos, 2012). The present results during the signaled delay periods are consistent with the variable effects just noted. Likewise, it must be noted that the type of stimulus change during delay has differed across different experiments, which could explain the reported differences in responding during the delay interval (Lattal, 1987). Leaving this variability within and across experiments aside, our results are similar with respect to other comparisons of signaled and unsignaled delays on operant responding maintained by positive reinforcement (Lattal, 1984; Mateos, 2012; Schaal & Branch, 1988, 1990).

Regardless of which procedure was used, the present results were consistent in yielding similar differences between signaled and unsignaled reinforcement delays. These findings could pave the way to future research aimed at the assessment of

multiple variables in a delay of reinforcement experiment, where even when multiple sessions could be required, a broader array of results can be provided by a single phase instead of multiple phases. Most importantly, it provides some support to previous findings using multiple schedules of reinforcement (Bell, 1999; Podlesnik et al., 2006; Ruiz et al., 2007; Schaal & Branch, 1988, 1990) where no direct comparison with between-phases procedures was done.

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